

Guidelines for Microbiology Specimen Collection and Submission



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These guidelines are designed to provide detailed instructions for submission of samples that will be analyzed in the IU Health Pathology Laboratory. Tests that are not listed may require special preparation or advance notice. Please notify the laboratory in advance for tests not listed in this manual. Please call the laboratory manager to obtain additional information.

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Specimen Collection & Transport General Guidelines

Introduction

Specimen collection and transport are preanalytical steps key to organism recovery. Quality specimens must be collected to ensure most clinically relevant microbiologic data for our patients. This manual aims at providing healthcare professionals, details regarding appropriate collection and submission of clinical specimens for microbiologic tests offered in-house. Instructions for testing sent to reference laboratories may differ based on test ordered and/or the platform they use. In such instances always contact the laboratory prior to specimen collection.

Specimen Collection

- 1. Collect specimen before administering antibiotics or antivirals when possible.
- 2. Universal precautions must be used for all specimens collected. Collect specimen with as little skin contamination as possible to ensure that the sample collected represents the infected site.
- 3. Utilize appropriate collection devices. Use sterile equipment and aseptic technique to collect specimens to prevent introduction of microorganisms during invasive procedures.
- 4. Collect fluid specimens in sturdy, sterile, screw cap, leak proof containers with lids that do not create an aerosol when opened.

- 5. Ensure a sufficient amount of specimen is collected. Insufficient samples may result in false-negative outcomes. A larger specimen quantity enhances the detection of low levels of microorganisms.
- 6. Tissue and aspirates are considered/recommended specimens of choice. Specimens submitted for fungal or mycobacterial culture should **NEVER** be submitted on swabs, unless there is no other means to collect the specimen. Reports for such specimens will include a note indicating the use of a suboptimal collection device, which may lead to false negative results.
- 7. Any Microbiology and Virology specimens collected in formalin are **UNACCEPTABLE** for culture!
- 8. Clearly label each specimen primary container with:
 - a. Full name of patient
 - b. Identifying number: Inpatients Medical Record Number (MRN); Outpatient/Outreach MRN, Date of Birth, or Social Security Number.
 - c. Time and date of collection
 - d. Specimen type or source when not apparent
 - e. Identification of collecting and labeling personnel

Specimen Transport

The following are the minimum specimen packaging standards that should be followed when submitting specimens.

- 1. Ensure that all specimen container caps and lids are properly tightened to prevent leakage.
- 2. Requisition forms need to contain the following information:
 - a. Full name of patient.
 - b. Date of request.
 - c. Ordering party with authorized signature.

- d. Identification of who(s) and where(address/location) the report is to be sent.
- e. Who is to be charged for the test(s) /services.
- f. A completed ABN should accompany specimen if appropriate.
- g. Request forms should also include pertinent clinical information:
 - Tissue source,
 - Collection site(s) of each specimen specimens should be numbered if multiple.
 - Appropriate related patient history
- h. Some tests may have special requirements for information see the on-line directory of services at <u>IU Health Lab Test Directory | search</u> for test specific details.
- 3. Collect the specimen(s) and transfer to a proper transport container, if needed. Double check the specimen container to ensure that the device is not beyond its stated expiration date.
- 4. The specimen transport biohazard bag has two pouches.
 - a. Place the specimen container(s) in the front pocket.
 - b. Insert the requisition into the rear pocket with the bar code visible in the bottom corner of the bag



- 5. Frozen specimens should be transported in plastic-screw-cap containers only. Frozen specimens must be placed in a separate specimen bag along with a separate test requisition.
- 6. If the specimen has been classified as an "infectious substance," transport in a box designed in accordance with the federal regulation covering the requirements is the Federal Department of Transportation (DOT); 49CFR 170-185. For more information consult with your local shipping and receiving section or commercial carriers (e.g., Postal Service, UPS, Fed-Ex, etc.).

NOTE: The information contained in this document cannot be used as proof of training for that purpose.

7. All specimens must be promptly transported to the laboratory, preferably within 2 hours of collection. If transport is delayed the specimen can be stored under the conditions specified in the specimen collection table

Policies

Specimen Rejection Policy

The Microbiology Laboratory follows the following general criteria for specimen rejection:

- Improperly labeled with patient identifiers (e.g., specimen unlabeled or mislabeled).
- Improper collection container, improper preservative
- Incomplete test requisition (i.e., required information e.g., source, type not present).
- Specimen volume inadequate for analysis.
- Excessive delay in specimen transport and/or improper transport conditions (e.g., frozen swabs submitted for bacterial culture)
- Specimen container grossly contaminated with biological material, nonsterile or leaking.

If a specimen is determined to be unacceptable, Client Services at IUHPL will inform the care provider of the problem and that a new specimen will be required. This fact will be noted in the LIS system. The online work card will also contain the time and date of the telephone contact.

Should it be determined that the requested testing be carried out on the specimen, the <u>Microbiology</u> <u>Supervisor/Technical Specialist/Medical Director shall give approval</u> for such testing and the original problem will be noted along with any results obtained.

Verbal Test Request Policy

Microbiology laboratory does not accept verbal requests for testing.

Table 1 - SPECIMEN COLLECTION AND TRANSPORT BASED ON SOURCE

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
	Clean catch midstream urine	Skin preparation a. Female While holding labia apart, cleanse urethral opening and vaginal vestibule area with soapy water from front to back. Rinse the area well.	Sterile screw cap container – (1 ml) BD Vacutainer Plus C&S Preservative Tube (gray-topped urine culture tube) – (3 ml min)	Sterile container or speci-cath white top tube: Transport at RT within 2 hours. >2 hrs - refrigerate at 4°C within 24 hrs.
		b. Male Cleanse the penis, retract the foreskin (if not circumcised), and wash with soapy water. Rinse the area well.	Speci-cath urine collection tubes (white top) – (1 ml)	BD Vacutainer Preservative tube: Transport at RT, within 24 hrs. Preferred for clients using courier services for transport. Capped syringe:
		2. Collect voided urine directly into a sterile container without halting the stream, preferably moving the container into the path of the already voiding urine, after several milliliters have passed.	The state of the s	Transport at RT within 2 hrs. ATM: Transport at RT within 24 hrs; Preferred anaerobic medium for clients
Urine culture	Straight Catheterization	Skin preparation as under CCMS Discard initial 15-30 ml; collect the next flow of urine	As above, for indwelling catheter	using courier services for transport.
	Indwelling catheter	 Clean the catheter port with 70% alcohol swab Aspirate urine using a syringe from the catheter port and place it in a sterile container. Alternatively, can use the BD SureStep™ urine sampling kit. 	Sterile screw cap container – (1 ml) BD Vacutainer Plus C&S Preservative Tube (gray-topped urine culture tube) – (3 ml)	ADDITIONAL REMARKS: - Patients with chronic indwelling catheters always have bacteria in their bladders. Do not test these patients unless they are symptomatic.
	lleal conduit	 Cleanse the stoma with 70% alcohol. Insert a catheter to a depth beyond the fascia and collect the urine by aspirating back on the syringe. 	As above, for indwelling catheter	-Indicate on requisition the source appropriately as specimens are processed differently based on source.
	Suprapubic aspirate, Cystoscopy, Nephrostomy, Urostomy, Prostate massage.	Collected by clinician or authorized personnel. Basic collection guidelines in Appendix A.	Suprapubic aspirate: Capped syringe or Anaerobic transport media (ATM) [P] – (1 ml) Rest: Sterile screwcap container– (1ml)	

SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Closed Deep Wounds (soft tissue abscess, visceral abscess e.g.: – lung, liver)	Aspirate: 1. Remove surface exudates and decontaminate area with 70% alcohol. Allow surface to dry. 2. Aspirate abscess fluid/area of maximum inflammation with needle and syringe. 3. If the initial aspiration fails to obtain material, inject sterile non-bacteriostatic saline subcutaneously and reattempt aspiration.	Aspirate – Sterile screw cap container (preferred only if >5 ml), capped syringe, red vacutainer tube, ATM vial [P]. (5 ml)	Transport all collection devices at RT within 24 hrs. Anaerobic culture work up is done for deep wounds, bite wound aspirates, IF specimens are collected/transported in appropriate medium: - Capped syringe: at RT within 2 hrs. - Sterile container: at RT within 2 hrs.
Open Deep Wounds (Draining/open abscess, open infected surgical sites, severe burns, traumatic injuries)	Aspirate: 1.As above; preferred collection method Swab 1. Remove surface exudates and decontaminate area with 70% alcohol. Allow surface to dry. 2. Pass swab deep into lesion and sample the lesions advancing edge.	Aspirate – As above. Swab - Copan ESwab®	- ESwab: at RT within 24 hrs ATM: at RT within 24 hrs; preferred for clients using courier services for transport.
Superficial wound (abrasion/cut/laceration, ulcer, cellulitis/folliculitis/impetigo)	Aspirate: 1.As above; preferred collection method Swab (swab for culture ONLY when there is not enough pus or fluid to aspirate): 1. Culture only when non-healing, chronic drainage, or new purulence 2. Roll swab over the surface of the wound x5 times; focus where there is pus or inflamed tissue.	Aspirate – Sterile screw cap container (preferred only if >5ml), capped syringe. (5 ml) Swab - Copan ESwab®	Sterile container ATM vial ESwab Red vacutainer tube
Bite wounds	Same as Abscess, above		ADDITIONAL REMARKS: - Swabs from skin provide little clinical information. Aspirates or tissue biopsy
Diabetic foot ulcer/pressure ulcer	Same as superficial wound, as above		samples are specimen of choice.
	Closed Deep Wounds (soft tissue abscess, visceral abscess e.g.: – lung, liver) Open Deep Wounds (Draining/open abscess, open infected surgical sites, severe burns, traumatic injuries) Superficial wound (abrasion/cut/laceration, ulcer, cellulitis/folliculitis/impetigo) Bite wounds Diabetic foot ulcer/pressure ulcer	Closed Deep Wounds (soft tissue abscess, visceral abscess e.g.: – lung, liver) Open Deep Wounds (Draining/open abscess, open infected surgical sites, severe burns, traumatic injuries) Superficial wound (abrasion/cut/laceration, ulcer, cellulitis/folliculitis/impetigo) Superficial wounds Suab (Swab for culture ONLY when there is not enough pus or fluid to aspirate): 1. Culture only when non-healing, chronic drainage, or new purulence 2. Roll swab over the surface of the wound x5 times; focus where there is pus or inflamed tissue. Bite wounds Diabetic foot ulcer/pressure Aspirate: 1. Remove surface exudates and decontaminate area with 70% alcohol. Allow surface to dry. 2. Pass swab deep into lesion and sample the lesions advancing edge. Swab (swab for culture ONLY when there is not enough pus or fluid to aspirate): 1. Culture only when non-healing, chronic drainage, or new purulence 2. Roll swab over the surface of the wound x5 times; focus where there is pus or inflamed tissue. Same as Abscess, above	Closed Deep Wounds (soft tissue abscess, visceral abscess e.g.: – lung, liver) Open Deep Wounds (Draining/open abscess, open infected surgical sites, severe burns, traumatic injuries) Superficial wound (abrasion/cut/laceration, ulcer, cellulitis/folliculitis/impetigo) Superficial wound (Braining/open abscess, open infected surgical sites, severe burns, traumatic injuries) Superficial wound (Braining/open abscess, open infected surgical sites, severe burns, traumatic injuries) Superficial wound (abrasion/cut/laceration, ulcer, cellulitis/folliculitis/impetigo) Bite wounds Same as Abscess, above Aspirate - Sterile screw cap container (preferred only if >5 ml), capped syringe, red vacutainer tube, ATM vial [P]. (5 ml) Aspirate - As above. Aspirate - As above. Aspirate - As above. Swab Swab Lesions advancing edge. Aspirate - As above. Swab - Copan ESwab Swab - Copan ESwab

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Tissue Culture	Surgically obtained biopsy/tissue	1.Collected by clinician or authorized personnel 2. Sample should be collected from areas within and adjacent to area of infection. 3. Container type: a. Sterile container: For small samples, add several drops of sterile non-bacteriostatic saline to keep sample moist. b. Anaerobic transport medium (ATM) jar: Unscrew the cap and place specimen onto the surface of the gel. Do not inject into or mix the specimen with the gel. Immediately recap the jar.	Sterile screw cap container, ATM jar [P] (0.5-1 cu cm) ATM jar Sterile container	Transport <u>all collection devices</u> at RT within 24 hrs. Anaerobic culture work up is done if the following collection/transportation conditions are met: - Capped syringe: at RT within 2 hrs. - Sterile container: at RT within 2 hrs. - ATM: at RT within 24 hrs; preferred for clients using courier services for transport.
	Fine needle aspirate	1.Collected by clinician or authorized personnel 2. Tissue must be sampled from various directions. 3. Transfer/transport specimen in an appropriate collection device for anaerobic work up to be done. (ATM inoculation instructions, as above)	Sterile screw cap container, capped syringe, ATM vial ATM vial	ADDITIONAL REMARKS: -Do not allow tissue to dry outDo not submit in formalin -Portion of the surgical specimen submitted for culture should be separated from all other tests ordered, into separate containers.

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
OR Aerobic + Anaerobic Culture + Stain	Surgically obtained Fluid and/or Tissue	1.Collected by clinician or authorized personnel 2.For inoculation instructions of tissue in ATM jar, refer to Tissue Culture. 3.For inoculation instructions of fluid in ATM vial, refer to Body Fluid Culture + Stain	Sterile screw cap container Capped syringe ATM vial or jar [P]	Transport all collection devices at RT within 24 hrs. Anaerobic work up is done if the following collection/transportation conditions are met: - Capped syringe: at RT within 2 hrs. - Sterile container: at RT within 2 hrs. - ATM: at RT within 24 hrs; preferred for clients using courier services for transport.
Orthopedics culture	Surgically obtained Joint Fluid and/or Joint Tissue	1.Collected by clinician or authorized personnel. 2. Collect 3-5 tissue samples should be collected and sent to the micro lab in separate collection devices. 3.For inoculation instructions of tissue in ATM jar, refer to Tissue Culture. 4.For inoculation instructions of fluid in ATM vial, refer to Body Fluid Culture + Stain	Eswab (LEAST PREFFERED) Volume: Tissue: 0.5-1 cu cm Fluid: Preferred vol 5 ml; min 2 ml	ADDITIONAL REMARKS: -Orthopedic cultures will be held for 14 days to optimize recovery of Cutibacterium acnes

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
	External ear	Swab: 1.Remove crusted debris using swab moistened with non-bacteriostatic, sterile saline. 2. Insert swab in the ear canal until resistance is met and firmly rotate swab to collect fluid.	Swab - Copan ESwab ®	Transport <u>all collection devices</u> at RT within 24 hrs. Anaerobic work up is done on tympanocentesis fluid if the following
Ear Culture + Stain	Middle ear	Tympanocentesis fluid: 1. Clean external ear with 70% alcohol and then aspirate fluid from the eardrum using a syringe. Ruptured ear drum 1. Collect exudate by inserting a sterile swab through an auditory speculum.	Sterile screw cap container (preferred only if >5 ml), ATM vial [P]. (5 ml) Capped syringe (5 ml) Swab - Copan ESwab ®	collection/transportation conditions are met: - <u>Capped syringe</u> : at RT within 2 hrs. - <u>Sterile container</u> : at RT within 2 hrs. - <u>ESwab</u> : at RT within 24 hrs. - <u>ATM</u> : at RT within 24 hrs; preferred for clients using courier services for transport.

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Eye Culture + Stain	Conjunctiva	 Moisten swab with sterile saline. Firmly roll over the conjunctiva, avoiding the lashes and eye lid margin. Sample both eyes (even if one is infected), using two separate swabs May inoculate media at the time of collection 	Swab - Copan ESwab® Media – BAP, CHOC	Plates: Transport at RT within 24 hrs. ESwab: Transport at RT within 24 hrs ADDITIONAL REMARKS:
	Corneal scrapings	1. Instill anesthetic drops. 2. Using a sterile spatula, scrape ulcers or lesions from the leading edge using short, firm strokes in one direction. Inoculate directly onto media in a C shaped formation. 3. Apply remaining material in a circular motion to 2 clean slides for staining.	Media – Blood agar plate, Chocolate agar plate; Thayer Martin agar if <i>Neisseria gonorrhea</i> is suspected.	-When only one eye is infected, sampling both can help distinguish indigenous microflora from true pathogens

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
		Sterile container 1. Pass stool in a clean, dry hat mounted on the toilet. 2. Use the provided wooden spatula, transfer a quantity of the stool specimen (Choose bloody, slimy, watery area of stool if present)	Sterile container (5 ml liquid stool, 1 gm of material or a walnut sized portion of stool)	Sterile container: Transport at RT within 2 hrs. Para-Pak® C&S orange cap: Transport at RT within 24 hrs - preferred for clients using courier services for transport.
Stool Culture	Feces	Para-Pak® C&S orange cap 1. Pass stool in a clean, dry hat mounted on the toilet. 2. Carefully remove the lid of the Para-Pak vial. 3. Use the spoon on the lid to add enough to displace fluid to the fill line on the side of the vial. DO NOT FILL VIAL ABOVE THIS LINE! Refer to Appendix B for further details.	Para-Pak® C&S orange cap (up to the fill line) [P].	Additional remarks: - Screened for the presence of Campylobacter, Shigella, Salmonella sp. & E. Coli 0157:H7Test includes E. coli shiga-like toxin EIA - Provider MUST notify the laboratory if infection due to Yersinia, Aeromonas, Plesiomonas or Vibrio spp is suspected
Gastrointestinal Panel by PCR	Feces	Same as stool culture, above		Sterile container and Para-Pak® C&S orange cap: Transport at RT within 24 hrs. Additional remarks: Only one GPP per hospital admission Not recommended for patients hospitalized > 72 hours Assay is not intended for use as a test of cure.
C. difficile Testing Algorithm	Feces	Sterile container: Collection instructions, same as stool culture	Sterile container (min 1 ml)	Sterile container: Transport at RT within 24 hrs. Additional remarks: -GDH antigen and toxin EIA with reflex to PCR - Formed or hard stool is rejected.

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
VRE Screen Culture	Rectum or Feces	Para-Pak® C&S orange cap 1. Pass stool in a clean, dry hat mounted on the toilet. 2. Carefully remove the lid of the Para-Pak vial. 3. Use the spoon on the lid to add enough to displace fluid to the fill line on the side of the vial. DO NOT FILL VIAL ABOVE THIS LINE!	Para-Pak® C&S orange cap (up to the fill line)	
		Swab 1. Insert swab an inch beyond the anal sphincter, rotate 2-3 times, and remove. 2. Swab must show fecal material.	BD CultureSwab® Cary- Blair Agar Single Swab	
Fluoroquinolone Resistant Enteric Screen		Swab 1. Insert swab an inch beyond the anal sphincter, rotate 2-3 times, and remove. 2. Swab must show fecal material.	BD CultureSwab® Cary-Blair Agar Single Swab	Transport <u>all collection devices</u> at RT within 24 hrs.
Carbapenem resistant Enterobacterales Rectal screen	Rectum		BD CultureSwab® Liquid Stuart's Double Swab	

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Blood Culture	Blood	 Skin preparation: Disinfect site with 2% chlorhexidine gluconate or 2% iodine tincture in adults and children over 2 months. Requires exposure time of at least 30 seconds. BACTEC bottle preparation: Visually inspect all bottles for contamination, cracks, or other signs of deterioration. Do not use bottles that appear turbid or damaged. Mark bottle at desired fill level. Remove flip-off caps from culture bottle. Wipe top of each vial with a single alcohol swab and 	BACTEC Aerobic Plus Gray Cap (10 ml) BACTEC Anaerobic Lytic Purple cap (10 ml) BACTEC Pediatric Plus Pink cap (refer to Appendix C for weight- based volume	Specimen transport/storage: For optimum recovery and best patient care, send bottles immediately to the clinical microbiology lab. If delayed transport is unavoidable, store BD BACTEC bottles at room temperature and send within 18-24 hours. Do not refrigerate or incubate above room temperature.
Blood Fungus and AFB culture		allow to dry completely, usually 60 seconds. 3. Blood collection: a) Perform venipuncture per protocol. Inoculate first the aerobic bottle and then the anaerobic bottle b) Gently invert the bottle to mix contents and avoid clotting.	BD BACTEC ™ Myco/F Lytic (3-5 mL)	Additional remarks: Please notify lab if Brucellosis, Tularemia, or other unusual pathogen/infection is suspected.
Bone Marrow Culture + Stain	Bone Marrow	Collected by a physician or authorized personnel.	Green vacutainer tube (containing heparin) - (3-5 ml)	Transport at RT within 24 hrs.

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICE and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
		Sterile tube, Sterile container, Red vacutainer tube 1. Disinfect overlying skin with chlorhexidine 2. Obtain specimen via percutaneous needle aspiration or surgical procedure.	Sterile tube (5 mL)	
		3. Aspirate with utmost precaution to avoid introducing microorganisms and avoid contamination of the specimen. 4. Transfer into a collection device	Sterile container (5 mL)	Transport all collection devices at RT within 24 hrs.
	Peritoneal/Ascitic fluid, Pericardial fluid, Pleural	IF ANAEROBIC WORK UP IS NEEDED, SPECIMEN MUST BE SENT IN: <u>Anaerobic Transport Medium (ATM) vial</u> 1. Disinfect the rubber septum with an alcohol	Red vacutainer tube (5 mL)	IF ANAEROBIC WORK UP IS NEEDED: - Sterile container/tube, capped syringe: at RT within 2 hrs ATM and Blood culture bottles: at RT within 24 hrs - preferred for clients
Body Fluid Culture + Stain	fluid, Pericardial fluid, Synovial fluid, Amniotic fluid, Aqueous/Vitreous fluid, Bile, Drain	wipe, wait 15-30 seconds. 2. Using aseptic technique, aspirate specimen with a sterile syringe and needle. Expel air from syringe and inject the fluid into the tube at a slow rate.	Capped syringe (5 mL)	using courier services for transport. Additional remarks:
		Syringe May be submitted to the lab by first removing the needle and capping the syringe.	ATM vial [P] (5 mL)	- Synovial fluid aspirate collected using saline lavage are accepted though please note consensus guidelines recommend against it due to decreased sensitivity and increased false negative rates.
		BACTEC Blood Culture bottles: Sterile body fluids may be inoculated directly into blood culture bottles at the bedside. Submit a separate specimen container if Gram Stain is needed.	BACTEC blood culture bottles (8-10 mL)	

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Cerebrospinal fluid (CSF) Culture + Stain	CSF collected from Lumbar Puncture (LP) or Ventricular Shunt/Drain/Ommaya reservoir	LP: 1. Disinfect the puncture to prevent specimen contamination and introduction of infection. 2. Insert a needle with stylet at the L3-L4, L4-L5, or L5-S1 interspace. When the subarachnoid space is reached, remove the stylet; spinal fluid will appear in the needle hub. 3. Sequentially collect CSF into four calibrated sterile tubes labeled #1 to #4. Ventricular device: 1. Clean the reservoir site with antiseptic solution and alcohol prior to prevent introduction of infection. 2. Remove fluid by aspiration of CSF from the Ommaya reservoir or by collection from the ventricular drain or shunt. Sequentially collect CSF as stated above (under LP).	Sterile tubes For volumes, refer to Appendix D	Transport at RT as soon as possible; within 24 hrs. Additional remarks: Specimens should be collected during the acute phase of an infection prior to initiating antimicrobial or antifungal medications.
Meningitis/ Encephalitis panel	CSF collected from LP	Same as LP, under CSF Culture + Stain, above	Sterile tube (#2 – refer to Appendix D)	Transport at RT as soon as possible; within 24 hrs.

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Respiratory Culture + Stain	Sputum (expectorated, induced) Aspirate (Endotracheal/Tracheosto -my/Transtracheal/Lung) Bronchoscopy Specimens [Bronchial wash/Bronchial Alveolar Lavage (BAL)/Bronchial brushings]	Expectorated sputum: 1. Rinse mouth and gargle with sterile water prior to collection. 2. Cough deeply and expectorate in a sterile container. Ensure not to expectorate saliva or post-nasal discharge Induced sputum: 1. Using a wet toothbrush, brush the buccal mucosa, tongue, and gums prior to the procedure. 2. Rinse mouth thoroughly with sterile water. 3. Using a nebulizer, have the patient inhale 3 - 10% NaCl (max neb time ~ 10 mins). 4. Have the patient cough vigorously & collect the induced sputum in a sterile screw cap container. Bronchoscopically collected specimens: 1. Avoid injection of topical anesthetic to prevent contamination; aerosol application preferred. 2. Obtain bronchial wash and BAL specimens before brushing or biopsy specimens to prevent contamination with blood 3. Avoid suctioning working channel before retrieving specimen to prevent contamination. 4. For bronchoscopy brush specimens — place brush in sterile vial containing 1 ml of non-bacteriostatic saline. Aspirate: Endotracheal/tracheostomy: Aspirate into a sputum trap. Transtracheal/Lung: Send aspirated specimen in anaerobic transport medium.	Sterile container (1 mL, 5 mL if BAL) Luken's trap (1 mL, 5 mL if BAL) ATM vial	Sterile container or Luken's trap: Transport at RT within 2 hours. >2 hrs - refrigerate at 4°C – stable for 24 hrs on ice/refrigerated - preferred for clients using courier services for transport. ATM: Transport at RT within 24 hrs; Preferred anaerobic medium for clients using courier services for transport. Additional remarks: - Early morning specimen is optimum. - Quantitative bacteriology cultures performed on the bronchial washing and bronchial brush specimens - Specimen source and site are required for appropriate work up. Anaerobic media is set up by default for transtracheal and lung aspirates if sent in appropriate transport medium

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Cystic Fibrosis Respiratory Culture + Stain	Pharynx Other respiratory sources, under respiratory culture + stain above	Swab: 1. Depress tongue gently with tongue depressor. 2. Extend swab between the tonsillar pillars and behind the uvula; Avoid touching the cheeks, tongue, uvula, or lips. 3. Sweep the swab back and forth across the posterior pharynx, tonsillar areas and any inflamed or ulcerated areas. Sputum, bronchoscopy specimens, aspirates: Same as respiratory culture + stain, above	Swab - Copan ESwab ®	Transport at RT within 24 hrs.
<i>Legionella</i> Culture	Pleural fluid Tissue Other respiratory sources, under respiratory culture + stain above	Pleural fluid: As under, body fluid culture + stain Tissue: As under, tissue culture Sputum, bronchoscopy specimens, aspirates: Same as respiratory culture + stain, above		
Streptococcus pneumoniae antigen Legionella pneumophila antigen	Urine	Random urine collection	Sterile screw cap container – (3 ml) BD Vacutainer Plus C&S Preservative Tube (gray-top) – (3 ml)	Transport at RT within 24 hrs. Additional remarks: -This assay detects Legionella pneumophila serogroup 1 antigen ONLY

[[]P] – preferred collection device; RT: room temperature

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
MRSA PCR	Nares	 Insert swab (both swabs at once) approximately one-fourth inch into the anterior nares. Swab in a circular motion; and repeat in second nostril, using the same two swabs. 		Transport at RT within 24 hrs.
Group A Streptococcus rapid antigen	Pharynx	Collect specimen from both tonsillar areas, posterior pharynx, and any areas of inflammation.		Transport refrigerated at 2-8 °C, within 24 hrs. Additional remarks: -All negative tests get reflex Group A Streptococcus PCR test
Group B Streptococcus, rapid PCR	Vagina + Rectum	Collect specimen from the distal vagina. Using the same swab, collect specimen from	Bill. ** Colorente and ** Addit by	Transport at RT within 24 hrs. Additional remarks: -GBS rapid PCR is for intrapartum testing.
Group B Streptococcus, screen	<u>-</u>	anal crypts just inside anal ring. Avoid fecal contamination as much as possible		-GBS screen is for antepartum testing and involves incubation in an enrichment broth before PCR testing.

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Cath tip culture	Intra-vascular catheter	 Perform skin antisepsis per protocol, prior to catheter removal. Allow enough time to dry. Hold exposed end of the catheter and carefully remove it from the patient with a sterile instrument, taking care to avoid contact with exposed skin. Hold the distal end over a sterile container, cut the tip with sterile scissors, dropping 2 inches of the distal tip into the catheter. Avoid drying by sealing the container and submitting to the lab as soon as possible 	Sterile screw cap container	Transport at RT as soon as possible; within 24 hrs. Additional remarks: -Not acceptable for culture: foley, peritoneal drain tips, chest tube tips
Foreign body culture	Cardiac implantable electronic devices (CIED), CNS shunts, vascular grafts, joint prosthesis/hardware	1.Collect explanted device in a sterile airtight container. 2. Contamination during specimen collection and transport should be avoided		

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
	Respiratory: Sputum, BAL fluid	Same as Respiratory Culture + Stain, above Collect first early morning sputum sample, from a deep cough.	Sterile screw cap container	If >1 hr delay: Transport <u>CSF & bone marrow</u> at RT
	CSF	Same as <u>CSF Culture + Stain</u> , above	Capped syringe	within 24 hrs. Transport rest refrigerated at 2-8 °C, within 24 hrs; preferred for clients using courier services for transport.
	Urine	Same as Urine culture, above Submit <u>early morning</u> clean voided urine specimen.	Green vacutainer	Additional remarks: - Specimens in ATM cannot be processed for AFB cultures. Specimens for bacterial culture
AFB Culture + Stain	Tissue	Same as <u>Tissue culture</u> , above Pieces of tissue must be kept moist with a small amount of sterile saline. Do not wrap in gauze.	tube [For Bone marrow only] work sepa are r	work up sent in ATM must also be sent separately in sterile container, if AFB cultures are needed. - AFB Culture + Stain for Urine samples are sent
	Wound (abscess)	Same as Aspirate, under Wound Culture + Stain, above. SWABS ARE NOT ACCEPTABLE SPECIMEN TYPE, unless pus/exudate cannot be aspirated.	Volumes:	to Mayo Clinic; samples accepted only Mondays-Thursdays. - Xpert MTB/RIF PCR is done ONLY on smear positive respiratory specimens
	Body fluid	Same as <u>Body Fluid Culture + Stain</u> , above	Respiratory samples: 5-10 ml CSF: Refer to Appendix D Urine: 20-50 mL Tissue: 0.5 cu cm Wound: 5 mL	- All MTBC isolates are sent out for susceptibility testing. - Mycobacterium abscessus, Mycobacterium fortuitum, Mycobacterium chelonae, Mycobacterium avium complex isolates from
	Bone Marrow	Same as <u>Bone Marrow Culture + Stain</u> , above	Body fluid: 5-10 mL Bone marrow: 3-5 mL	sterile sources are sent out for susceptibility testing. - NTM isolates from non-sterile sites are sent ONLY on request.

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Fungal Culture + Stain	Respiratory: Sputum, BAL fluid	Same as <u>Respiratory Culture + Stain</u> , above	Sterile screw cap	Transport all at RT within 2 hrs.
	CSF	Same as <u>CSF Culture + Stain</u> , above	Capped syringe	If >2 hr delay: Transport sterile (tissue, body fluids, bone marrow) and cutaneous specimens at RT within 24 hrs. Transport non-sterile (gastrointestinal, genitourinary, respiratory sites)
	Urine	Same as <u>Urine Culture</u> , above Submit <u>early morning</u> clean voided urine specimen.		
	Tissue	Same as <u>Tissue culture</u> , above Pieces of tissue must be kept moist with a small amount of sterile saline.	Capped syringe	specimens refrigerated at 2-8 °C, within 24 hrs; preferred for clients using courier services for transport.
	Wound	Same as Aspirate, under Wound Culture + Stain, above. SWABS ARE LEAST PREFERRED SPECIMEN TYPE unless pus/exudate cannot be aspirated.	only] for Fungal cultures. Specimens for culture work up sent in ATM must sent separately in sterile contain fungal cultures are needed.	- Specimens in ATM cannot be processed for Fungal cultures. Specimens for bacterial
	Body fluid	Same as <u>Body Fluid Culture + Stain</u> , above		culture work up sent in ATM must also be sent separately in sterile container, if fungal cultures are needed.
	Bone Marrow	Same as <u>Bone Marrow Culture + Stain</u> , above	Volumes: Respiratory samples: 5-10 mL CSF: Refer to Appendix D	- Refrigeration can decrease viability of

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
	Nail, Hair/Scalp and Skin scrapings	1.HAIR: - Cleanse scalp area with 70% alcohol Using sterile forceps, pluck broken/diseased hair Send at least 10 hair with some including insertion points If diseased hair stubs are not apparent, scrape the edges of the scalp lesion with a sterile scalpel. 2.SKIN: - Cleanse skin lesions with 70% alcohol Scrape from the outer edges of skin lesions. 3.NAILS: - Cleanse area to be sampled with 70% alcohol Scrape away the superficial portions with the side of a surgical blade Scrape or clip off portions of abnormal appearing nails. Also collect material under the nails. Place all specimens in a sterile container. DO NOT add saline to the container.	Urine: 1-2 mL Tissue: 0.5 cu cm Wound: 5 mL Body fluid: 5-10 mL Bone marrow: 3-5 mL	certain fungi, for example. Histoplasma capsulatum. -Notify the lab if Coccidioides spp, Paracoccidioides spp, Talaromyces marneffei, Cladophialophora bantiana, Rhinocladiella mackenziei, Verruconis gallopava, are suspected.

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Candida auris screen	Skin folds	Axilla and Groin: 1. Firmly rub the soft end of the swab over the left and then the right axilla, targeting the crease in the skin where the arm meets the body (i.e. swiping back and forth ~5 times per armpit). 2. With the same swab used on the axilla, rub both sides of the swab tip over the left groin skin, targeting the inguinal crease in the skin where the leg meets the pelvic region and repeat with the right side (i.e., swab the skin of both hip creases swiping back and forth ~5 times per hip crease). Swab right and left axillae & groin with a single swab	BD CultureSwab® Liquid Stuart's Double Swab	Transport refrigerated at 2-8 °C, within 24 hrs. Additional remarks: The skin (specifically axilla and groin) appears to be the highest yield sites to swab to identify patients colonized with C. auris. However, C. auris has also been isolated from swabs taken from the nares, oropharynx, external ear canal, vagina, and rectum. These sites can also be considered for sampling.
Pneumocystis jirovecii stain	Bronchoscopically collected respiratory specimen	Same as Respiratory Culture + Stain, above	Sterile screw cap container (1 mL)	Transport refrigerated at 2-8 °C, within 24 hrs. Additional remarks: - Note, PJP stain is NOT a STAT test

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Arthropod and Worm Identification	Worm and worm segments	Submit in a sterile container with liquid medium including 10% buffered formalin or 70-95% laboratory-grade ethanol.		
	Arthropods (bugs, ticks etc)	Submit in a sterile container with 70-95% laboratory-grade ethanol.		Transport at RT within 24 hrs.
	Skin scrapings	Skin scrapings for SCABIES examination: 1. Scrapings are best performed at the end of the burrows in non-excoriated and non-inflamed areas. 2. Add a small amount of mineral oil to the suspected area or distal end of a burrow, that allows adherence of the mite to the scalpel. 3. Scrape the leading edge of the lesion with a sterile scalpel at a 90° angle to the skin while holding the skin taut until superficial skin tissue is removed. 4. Alternately, if a scalpel is unavailable, place two drops of mineral oil on a clean slide and gently tip the slide to make the mineral oil flow to the long edge of the slide. Use this slide to obtain skin scrapings, as explained above. 5. Place two drops of mineral oil, side by side, on a microscope slide. Suspend scraped skin in the mineral oil. Place a single coverslip on each suspension and seal the coverslip with clear nail polish along the edges.	Sterile screw cap container	Additional remarks: - Worms and Arthropods are macroscopically examined Skin scrapings for scabies involve microscopic examination.

[[]P] – preferred collection device; RT: room temperature

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Cryptosporidium and Giardia antigen	Feces	Same as Stool Culture, above	Para-Pak® C&S vial (orange cap)- (up to the fill line) [P].	Transport at RT within 24 hrs. Additional remarks: - Any sterile container that is not pictured can also be used.

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION and PREFFERED VOL		TRANSPORT INSTRUCTIONS
Ova and Parasite exam, stool	Feces	Same as Stool Culture, above	Para-Pak® EcoFix vial (green cap) (up to the fill line) [P].	The soft	Additional remarks: Not accepted for patients that are inpatient for >3 days Any sterile container that is not pictured can also be used. Microscopic exam for ova, cysts and trophozoites, is performed ONLY IF the patient meets the following criteria: Immunocompromised host. Persistent symptoms with appropriate travel history. C. Origin from endemic country. ** Optimal collection for microscopic exam is three separate stool specimens within a 7-10-day period
Ova and Parasite exam, other	Respiratory (expectorated sputum, bronchoscopy specimen)	Same as Respiratory Culture + Stain, above. Early morning sputum specimen is optimum.	Sterile screw cap container (all specimen types)		Sterile container: Transport at RT within 2 hrs >2 hrs: refrigerated at 2-8 °C and transport within 24 hrs - preferred for clients using courier services for transport.
	Aspirates (cyst, duodenal, bile,	Same as <u>Aspirate</u> , under <u>Wound Culture + Stain</u> , above.			
	Tissue (sigmoidoscopy material, liver, spleen, lymph node, muscle)	Same as <u>Tissue culture</u> , above.			
	Urine	Collect <u>15-30 mL</u> in a sterile container. For Schistosoma haematobium collect at midday. (noon to 3 pm) or 24-hour collection in a preservative free container.			

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Pinworm examination	Peri-anal region	Refer to <u>Appendix F.</u>	Pinworm paddle	Transport at RT within 24 hrs.
Blood and Tissue Parasite Exam	Blood	- 4 mL whole blood in EDTA tube Non-AAHC facilities and outreach clients must send air dried, unfixed thick and thin smears to the laboratory.	Lavender vacutainer tube	Blood: Transport at RT within 1 hour of collection Additional remarks: -Specimens are screened 24/7 by core lab, followed by confirmation and speciation by microbiology laboratory - If Loaisis is suspected, recommended specimen collection time is mid-day (10 am-2pm) - If lymphatic filariasis is suspected, recommended specimen collection time is in the evening (after 8 pm).

TEST/ ORDERABLE	SOURCE	COLLECTION GUIDELINES	ACCEPTED COLLECTION DEVICES and PREFFERED VOLUMES	TRANSPORT INSTRUCTIONS
Influenza/RSV/ SARS-CoV-2 PCR	Upper respiratory	 Nares: Insert a swab 1-2cm into one of the anterior nares. Rotate the swab against the nasal mucosa for about 3 seconds and withdraw. Repeat with the other anterior nare using the same swab. Nasopharynx: Tilt the patient's head back slightly (about 70°) Gently insert the swab into one nostril, parallel to the palate, straight back to the nasopharynx until resistance is met. Rotate swab up to 5 times and hold it in place for 5-10 seconds to absorb secretions. Slowly withdraw the swab while rotating it and repeat the same on other side. 	Viral/Universal Transport Medium	Transport at 2-8 °C within 24 hrs.
Respiratory viral pathogen 2 PCR (RVP2)	Nasopharynx	Same as Upper Respiratory under, Influenza/RSV/SARS-CoV2-PCR above.		

²

APPENDIX A

URINE COLLECTION GUIDELINES FOR MICROBIOLOGIC TESTING - PATIENTS

Materials supplied

Urine culture collection device (sterile container) Biohazard transport bags.

Collection instructions:

A. Clean catch midstream urine (CCMS):

- 1) Verify there is a packet of castile soap towelettes and either a BD Vacutainer® urine collection cup or other sterile urine collection container.
- 2) Do not touch the inside of the cup. Remove the cap and place it on the counter with the straw facing upwards. Do not touch the inside of the cap or straw. Do not remove the yellow label on top of the cup.
- 3) Open the towelettes and set aside.
- 4) Wash hands thoroughly with soap and water.
- 5) SKIN PREPARATION and COLLECTION:

FEMALE INSTRUCTIONS:

- -- Stand in a squatting position over the toilet. Separate the folds of skin around the urinary opening. Cleanse the area around the opening with the first towelette. Repeat using the second towelette.
- -- Void the first portion of the urine into the toilet.
- -- While continuing to void, place the collection cup into the midstream to collect the urine specimen. Do not touch the inside or tip of the cup with the hands or any other part of the body. Void remainder of the urine into the toilet.
- -- Replace the cap on the cup touching only the outside surfaces of the cap and cup. Screw the lid on tightly.

MALE INSTRUCTIONS:

- -- Cleanse the end of the penis with the first soap towelette beginning at the urethral opening and working away from it (the foreskin of an uncircumcised male must first be retracted). Repeat using the second clean towelette.
- -- Void the first portion of the urine into the toilet.
- -- While continuing to void, place the collection cup into the midstream to collect the urine specimen. Do not touch the inside or lip of the cup with the hands or any other part of the body. Void remainder of the urine into the toilet.
- -- Replace the cap on the cup touching only the outside surfaces of the cap and cup. Screw the lid on tightly.

NOTE: Never collect a urine specimen from a bedpan or urinal.



URINE COLLECTION GUIDELINES FOR MICROBIOLOGIC TESTING - HEALTHCARE PROFESSIONALS

A. In and Out catheterization (CIO):

- 1) Skin preparation:
- a. Female

While holding labia apart, cleanse urethral opening and vaginal vestibule area with soapy water from front to back. Rinse the area well.

b. Male

Cleanse the penis, retract the foreskin (if not circumcised), and wash with soapy water. Rinse the area well

2) Discard initial 15-30 ml; collect the next flow of urine.

B. Indwelling catheter:

- 1) Do not send urine collected from a catheter bag.
- 2) If necessary, clamp the catheter tubing to collect urine in the tube but do not allow the clamp to remain for more than 30 minutes.
- 3) Clean the catheter port with 70% alcohol swab.
- 4) Aspirate urine using a syringe from the catheter port and place it in a sterile container. Alternatively, can use the BD SureStep™ urine sampling kit (allows collection from the catheter port directly into a Vacutainer tube using a holder and needle − BD sure step urine sampling kit package insert



C. Ileal conduit

- 1) Cleanse the stoma with 70% alcohol.
- 2) Insert a catheter to a depth beyond the fascia and collect the urine by aspirating back on the syringe

D. Suprapubic aspirate

- 1) Performed mostly in pediatric population where CCMS collection is difficult
- 2) Bladder should be full and palpable before aspiration.
- 3) Shave and disinfect the skin over the bladder with 70% alcohol. Make a small lancet wound through the epidermis above the pubic symphysis. Aspirate using a needle and syringe.
- 4) Aspirated urine must be transported in either the capped syringe without a needle or in an anaerobic transport system.

E. Cystoscopy

- 1) Urine collected from the bladder using a cystoscope must be placed in a sterile container labeled CB (catheterized bladder).
- 2) After the bladder is washed using non-bacteriostatic 0.85% NaCl and emptied, the fluid must be placed in a sterile container labeled WB (washed bladder).
- 3) Urine collected after ureteral catheters are placed must be collected in a sterile container labeled LK (left kidney) or RK (right kidney) 1, 2, 3, to signal timed sequential collections.

F. Nephrostomy tube

- 1) Clean nephrostomy tube and collecting bag junction with 70% alcohol.
- 2) Detach nephrostomy tube from its existing collecting bag. Collect urine in a preservative tube (preferred) or

sterile container by placing it at the tip of the nephrostomy tube and allowing gravity to provide fresh urine.

G. Urostomy bag

- 1) Remove the external device and discard urine within device.
- 2) Gently cleanse the stoma and insert a catheter to collect urine by aspirating back on the syringe. Discard an initial 15 to 30 ml of urine. Transfer urine to a Vacutainer tube (preferred) or sterile container.

H. Prostate massage

- 1) Skin preparation same as CIO in men
- 2) First 10 mL of voided urine (VB1) should be collected in sterile specimen container.
- 3) 10 mL midstream urine sample (VB2) should be collected in a sterile specimen container after the patient has voided approximately 100 to 150 ml of urine.
- 4) The physician then performs a prostate massage for approximately 1 minute and expressed prostatic secretions (EPS) that emerge from the urethra within two to three minutes of the prostate massage should be collected in a sterile specimen container
- 5) Finally, the first 10 mL of urine (VB3) following prostate massage should be collected in a sterile specimen container.

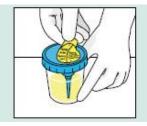
SPECIMEN COLLECTED FROM THE AFOREMENTIONED APPROACHES CAN BE SENT TO THE LAB IN:

- A sterile container (ORANGE or WHITE cap).

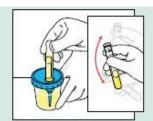


- BLUE cap container as part of the BD SureStep urine collection kit MUST not be sent to the lab. **Urine MUST be transferred** to vacutainer tubes (as below), which should be sent to the lab.

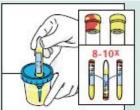




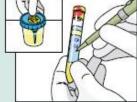
- Peel back protective sticker to expose rubber-covered cannula.



- Push C&S preservative tube (gray top) into the integrated transfer port.
- Hold in position until flow stops.
- Remove tube.
- Shake tube vigorously.



- Push UA Preservative Tube (cherry red/yellow top) pr plain UA tube (yellow top) into integrated transfer port.
- Remove tube.
- Invert UA Preservative Tube 8-10 times to mix the sample.



- Place protective sticker back over the integrated transfer port.
- Label both filled tubes with patient's information required as per policy.



- Remove lid from cup and dispose in a sharp's container
- Dispose urine
- Dispose collection cup as biohazard

APPENDIX B

STOOL COLLECTION GUIDELINES FOR MICROBIOLOGIC TESTING

Materials supplied

Stool culture collection device (Sterile container or Para Pak ® vial) Wooden spatula Biohazard transport bags

Collection instructions:

*** DO NOT USE ANTIBIOTICS, LAXATIVES, ANTI-DIARRHEA COMPOUNDS, BARIUM, BISMUTH, MINERAL OIL BEFORE COLLECTING STOOL SPECIMEN ***

• Pass stool in a clean, dry hat mounted on the toilet (left), if provided. Or on a clean plastic bag/wrap placed over the toiled seat opening (right). **DO NOT USE TOILET PAPER** to collect stool. Toilet paper may be impregnated with barium salts, which are inhibitory for some fecal pathogens.





• <u>Diaper dependent patients</u>: Line the back half of the diaper with plastic wrap to collect the stool. An alternative is to put a disposable diaper on the child inside out and remove it immediately after the bowel movement. The skin should be cleansed of any lotions or powders. **DIAPERS ARE NOT ACCEPTABLE.**

Sterile Container

Test: Stool Culture \square Cdiff testing \square

- Carefully remove the lid.
- Use the provided wooden spatula to transfer a quantity of the stool specimen (choose bloody, slimy, watery area of stool if present)
- Replace the lid on the container and tighten securely.
- Place specimen inside the biohazard transport bag (one specimen per bag).
- Wash and dry hands thoroughly.
- The paperwork (requisition or doctor's orders) should be placed in the rear pocket of the bag.
- Transport to the Lab within 2 HOURS at room temperature or if >2 hours refrigerate and deliver within 24 hours.



Para Pak ® C&S vial (orange top)

Test: Stool Culture □ GI Pathogen Panel □ Giardia/Cryptosporidium Ag □

- Carefully remove the lid.
- Use the spoon on the lid to add enough to displace fluid to the fill line on the side of the vial. DO **NOT** FILL VIAL ABOVE THIS LINE! (choose bloody, slimy, watery area of stool if present)
- Replace the lid on the container and tighten securely.
- Shake until well mixed.
- Place specimen inside the biohazard transport bag (one specimen per bag).
- Wash and dry hands thoroughly.
- The paperwork (requisition or doctor's orders) should be placed in the rear pocket of the bag.
- Transport to the Lab within 24 HOURS at room temperature.







Para Pak ® EcoFix vials (green top)

Test: Ova & Parasite complete □

- Carefully remove the lid.
- Use the spoon on the lid to add enough to displace fluid to the fill line on the side of the vial. DO **NOT** FILL VIAL ABOVE THIS LINE! (choose bloody, slimy, watery area of stool if present)
- Replace the lid on the container and tighten securely.
- Shake until well mixed.
- Place specimen inside the biohazard transport bag (one specimen per bag).
- Wash and dry hands thoroughly.
- The paperwork (requisition or doctor's orders) should be placed in the rear pocket of the bag.
- Transport to the Lab within 24 HOURS at room temperature.



APPENDIX C

BLOOD CULTURE COLLECTION GUIDELINES

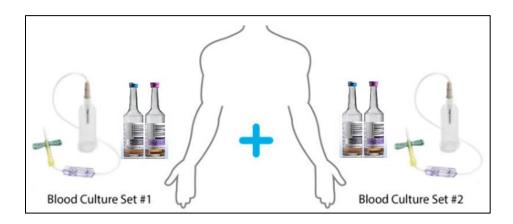
Important terms to know:

- Blood culture set:

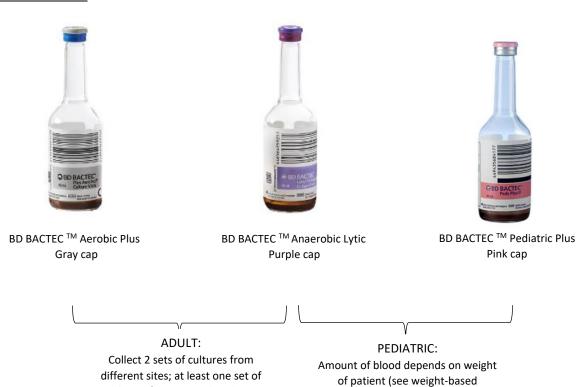
Refers to bottles inoculated from the same venipuncture site. Includes one aerobic and one anaerobic bottle.

- Blood culture order:

Consists of two sets of blood culture bottles from **two different** venipuncture sites.



Collection device:



minimums below)

cultures from a peripheral site

Collection instructions:

1. BACTEC bottle preparation:

- Visually inspect all bottles for contamination, cracks, or other signs of deterioration. Do not use bottles that appear turbid or damaged.
- Mark fluid volume in bottle and then mark volume of blood to be drawn using the hatch marks on bottle (each hatch is a 5-mL increment). Refer to tables below for recommended volumes.
- Wash hands with soap and water with friction for 15 seconds or use alcohol-based hand rub per policy.
- Remove flip-off caps from culture bottle. Wipe top of each vial with a single alcohol swab and allow to dry completely, usually 60 seconds.

2. Skin preparation:

- Apply gloves and tourniquet then select puncture sites.
- Do not draw blood cultures from lines unless there is absolutely no alternative site
- Remove tourniquet for skin preparation.
- If palpation of site prior to puncture is anticipated, wear STERILE gloves; if palpation of site prior to puncture is not anticipated, wear unsterile gloves
- If skin is soiled, clean with 70% isopropyl alcohol before disinfecting. Requires exposure time of at least 30 seconds.
- Disinfect site with 2% chlorhexidine gluconate in adults and children over 2 months. Cleanse using a back-and-forth motion for 30 seconds.
- Allow it to air dry.
- Do not blow at the site after cleansing the skin.

3. Blood collection:

- Perform venipuncture per protocol.
- Use a vacutainer butterfly needle with hub to minimize chances of contamination. Make certain that the needle does not touch anything before entering the skin.
- Inoculate first the aerobic bottle and then the anaerobic bottle
- Gently invert the bottle to mix contents and avoid clotting.
- NOTE: Draw cultures before the initiation of antibiotic therapy if possible

4. Bottle labelling:

- Label each bottle with patient's name and medical record number and site of draw.



Do not cover the window with the ruled lines! (Lab needs to be able to ensure proper blood amounts are in the bottles.)

Specimen volumes:

with the label.

Optimal blood fill volumes critically determine the diagnostic yield of blood cultures. Each mL of blood, up to 10 mL, can increase the sensitivity of the blood culture by 3-5%. While overfilling of bottles may cause false positivity, underfilling reduces the sensitivity of blood cultures.

Adults:

Recommended blood culture volume		Total volume to be drawn	BD Bactec Aerobic Plus F	BD Bactec Lytic/Anaerobic
			Blood Volume	
Adult Collection or Pediatric collection > 26 kg	Ideal Volume	20 ml	10 ml	10 ml
	Acceptable Volume	8-20 ml	4-10 ml	4-10 ml
	Difficult Collection	< 7 ml	7 ml	

Pediatric:

Patient Weight (kg)	Minimum Blood Volume to Collect (mL)	Media Type and Minimum Inoculation Volume (mL)	
		BD Bactec Peds Aerobic Plus	BD Bactec Lytic/Anaerobic
1 kg or less	2	1	1
* 1.1 kg – 1.5 kg	2	1	1*
1.6 kg – 3.9 kg	2	1	1
4 - 7.9 kg	4	2	2
8 - 13.9 kg	6	3	3
14 - 18.9 kg	10	5	5
	•	BD Bactec Peds Aerobic Plus	BD Bactec Lytic/Anaerobic
19-25.9 kg	16	8	8
More than 26 kg	20	10	10

^{*}Anaerobic cultures (1 ml of blood goes into the anaerobic vial and 1 ml of blood goes into the aerobic vial) should be considered for neonates \leq 1.5 kg if the mother had chorioamnionitis, premature rupture of membranes, a bowel perforation, or any other injury/condition that could introduce anaerobes into the womb, or if the neonate has necrotizing enterocolitis.

Fungal and AFB blood cultures

Optimal Volume for fungal and AFB cultures: 3-5 ml (minimum 1 ml, maximum 5 ml)



BD BACTEC TM Myco/F Lytic

Specimen transport/storage:

For optimum recovery and best patient care, send bottles immediately to the clinical microbiology lab. If delayed transport is unavoidable, store BD BACTEC bottles at room

temperature and send within 18-24 hours. Do not refrigerate or incubate above room temperature.

Additional remarks:

Please notify lab if Brucella (Brucellosis), Francisella (Tularemia), or other unusual pathogen/infection is suspected.

Cultures are continuously monitored and incubated for 5 days. Cultures will be incubated for 14 days if Brucella or Francisella is suspected, and the lab notified.

APPENDIX D

Cerebrospinal fluid specimens

Spinal fluid tubes must be properly labeled with the patient's name and medical record number and identified with sequential numbers: #1, #2, #3, and #4.

Default testing for each tube is as follows:

#1 Chemistry (CSF Glucose, Protein, etc.)

#2 Microbiology (bacterial, fungal, AFB, etc.)

#3 Hematology (CSF cell count, appearance, color, RBC, nucleated cells)

#4 Other (cytology, CSF freeze store for add on testing like VDRL, viral PCRs, etc.)

<u>Optimal specimen submission</u>: Three to four tubes containing CSF should be submitted to provide adequate specimen volume for performance of multiple tests, repeat testing if needed, and/or for addon test requests. The preferred total collection volume for microbiology work up is 5 ml. Refer to the table below for minimum volumes required to perform specific tests.

<u>Minimum specimen submission</u>: If a less-than-ideal volume of CSF is submitted, include test priority and provider contact information in the comment field or include a note with specimens.

Depending on volume submitted, the laboratory cannot guarantee performance of every ordered test – in such instances, tests ordered for which there is not enough CSF will be resulted as "quantity not sufficient for analysis" (QNS).

Test	Minimum Volume*	LP Tube Number
CSF Glucose, Protein (Chemistry)	1.0 mL	1
CSF Culture & Stain	5 mL	2, 3 or 4 (most turbid
Fungal Culture & Stain	(same 5 ml covers both tests)	tube preferred)
AFB Culture & Stain	0.5 mL	2, 3, or 4
CSF Pathogen Panel, PCR (lumbar puncture only)	0.5 mL	2, 3, or 4
Cryptococcal antigen (lumbar puncture only)	0.5 mL	2, 3, or 4
CSF cell count (Hematology)	1.0 mL	3

Note: Additional volume will be needed if additional tests are ordered (for example, West Nile Virus or Varicella Zoster PCR). Required volumes for specific tests can be found in the IU Health Lab Test Directory

^{*}Greater volumes than recommended above increase the chance of organism recovery.

APPENDIX E

SPUTUM COLLECTION GUIDELINES

Materials supplied

Respiratory culture collection device (Sterile container)

Biohazard transport bags

Collection instructions:

- 1. It is best to collect sputum early in the morning; because secretions have accumulated overnight, however samples taken at other times of the day are acceptable.
- 2. Wash and dry hands thoroughly.
- 3. Remove dentures if present.
- 4. Rinse mouth and gargle with **sterile water** prior to collection. Do NOT use tap water.
- 5. Open the lid of the sample container.
- 6. Take several deep breaths and exhale.
- 7. Cough deeply from the diaphragm and expectorate sputum in a sterile container.
- 8. Ensure specimen in thick mucus not saliva or post-nasal discharge
- 9. Screw cap on tightly being careful not to touch the inside of the container or lid.
- 10. Place specimen inside the biohazard transport bag. Put the completed requisition in the side pouch of the bag.
- 11. Deliver to the microbiology laboratory at room temperature within 2 hours; or store specimen in the fridge (do not freeze) and deliver within 24 hours of collection.

NOTE: For AFB sputum cultures, **three** specimens at 8–24-hour intervals (24 hours when possible) with at least one first-morning specimen must be collected.

APPENDIX F

PINWORM EXAMINATION COLLECTION GUIDELINES

Materials supplied
Pinworm paddle
Biohazard transport bags

Collection instructions:

- 1. Time of collection is best immediately upon waking up in the morning.
- 2. Do 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.
- 3. Pinworm eggs are very infectious. Wear gloves when collecting sample and wash hands immediately after collection.
- 4. Unwrap the paddle from its package prior to use. Hold the paddle by the cap and remove it from the tube. Set aside the tube.
- 5. To collect the specimen, use the "sticky side" of the paddle, which is labeled on the far end of the paddle near the cap. If you are not sure which side is the "sticky side," feel the end of the paddle with the fingertip to feel the stickiness.
- 6. In obtaining the sample, spread the buttocks and press the tacky surface of the paddle firmly against several areas (the right and left) of the peri-anal region. Do not press into the anus.
- 7. Return the paddle in the original tube for transport to the laboratory.
- 8. Place specimen inside the biohazard transport bag. Put the completed requisition in the side pouch of the bag.
- 9. Deliver to the microbiology laboratory at room temperature within 24 hours.

