Section: Clinical

Laboratory: Special Pathogens/Biothreat



SP-Guidelines for the Collection and Transport of Specimens for Tuberculosis Testing

Collection	 The Rhode Island State Health Laboratories, like other Public health laboratories throughout the country, performs diagnostic and reference services in support of the National Plan for elimination of TB in the United States. Since the accuracy of laboratory testing for tuberculosis is directly related to the quality of the specimen, we offer these guidelines for specimen collection and transport. Properly collected and handled specimens provide optimal conditions for acid fast bacilli (AFB) recovery.
Abscess: closed or open cellulitis, eye exudate, tissue, skin lesion	 Collection: General: Remove surface exudate by wiping with sterile saline or 70% alcohol. Collect fluid abscess material with Luer tip syringe and/or remove tissue aseptically. For open lesions/abscess, aspirate, if possible, material from under the margin of the lesion/abscess. Remove tissue aseptically. NOTE: Swabs are not recommended for the recovery of Mycobacteria species. Transport: Fluid abscess material should be submitted in a sterile leak-proof container such as a sterile 15 or 50 mL conical tube or sterile urine container. Do not transport the specimen in a commercial swab transport device or in transport gel-based medium. Transport tissue in 2-3 mL sterile nonbacteriostatic saline in a sterile, leak-proof container. Transport time and temperature: Transport as soon as possible at room temperature. If transport is delayed for more than one hour, refrigerate. Do not freeze or preserve tissue. Comments: Tissue (at least 1 g, if possible) or fluid is preferred. A swab is strongly discouraged unless it is the only specimen available; submit swabs in 2 to 3 mL sterile saline. Possible rejection conditions: Swabs submitted in transport medium or a commercial swab transport device are unacceptable. Swabs not submitted in sterile saline will be rejected
Body fluids: abdominal, amniotic, ascites, bile, joint, paracentesis, pericardial, peritoneal, pleural, synovial, thoracentesis	 Collection: Aseptically collect a minimum of 2 mL fluid in a sterile container. Always submit as much fluid as possible. Never submit a swab dipped in fluid. Minimum volume: 2 mL Transport: Fluid should be submitted in a sterile, leak-proof container such as a 50 mL conical tube or sterile urine collection container. Transport time and temperature: Transport as soon as possible at room temperature. If transport is delayed, refrigerate, but do not freeze specimen. Comment: Recommended volume is 15 mL. Bloody specimens may be collected in 10 mL yellow-top (SPS) blood collection tubes. Possible rejection conditions: Swabs of fluids are not acceptable for AFB testing and will be rejected. Volume below the 2 mL minimum may be rejected if unable to inoculate a smear and all culture media.
Bone marrow aspirate	Collection: Prepare the puncture site as for surgical incision. Use a blood collection tube and mix contents of tube after collection.

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CSF Gastric wash or lavage	 Transport: 10 mL yellow-top collection tubes containing SPS are preferred. A sterile, leak-proof container such as a sterile 15 or 50 mL conical tube or sterile urine collection container is also acceptable. Transport time and temperature: Transport as soon as possible at room temperature. If transport is delayed, store specimen at room temperature. Do not refrigerate or freeze. Collection: Aseptically collect 10 mL (optimally) of cerebrospinal fluid. NOTE: Collect separate samples for chemistry and hematology. Minimum volume: 2 mL Transport: Sterile, leak-proof container such as a 15 mL conical tube. Transport time and temperature: Transport as soon as possible at room temperature. If transport is delayed, store specimen at room temperature. Do not refrigerate or freeze the CSF specimen. Possible rejection conditions: Volume below the 2 mL minimum may be rejected if unable to inoculate a smear and all culture media. Collection: Collect in early morning before patients eat and while they are still in bed. Perform lavage with 25 to 50 mL of chilled, sterile, distilled water. Recover sample and place in a leak-proof, sterile container such as a 50 mL conical tube. Transport: Gastric wash or lavage material should be submitted in a sterile, leak-proof container such as a sterile 50 mL conical tube or sterile urine collection container. Transport time and temperature: Transport as soon as possible at room temperature. If transport is delayed for more than four (4) hours from time of collection, neutralize with 100 mg sodium carbonate within 1 hour of collection and transport as soon as possible at room temperature. Comment: Maximum volume of specimen recommended is 15 mL. Limit replicates to 1 per day on 3 consecutive days. Process the specimen promptly as mycobacteria die rapidly in gastric lavage. NOTE: Please contact the Special Pathogens laboratory p
Respiratory (lower): bronchoalveolar lavage,	Collection: Collect washing or aspirate in a sputum trap. Place the brush in a sterile, leak-proof container with up to 5 mL of sterile nonbacteriostatic saline.
brush or wash, endotracheal aspirate, transtracheal aspirate	 Minimum volume: 3 mL Transport: Transport in sterile container such as a sterile 50 mL conical tube or sterile urine collection container.
	 <u>Transport time and temperature</u>: Transport as soon as possible at room temperature. If transport is delayed for more than one hour, refrigerate specimen. <u>Possible rejection conditions</u>: Volume below the 3 mL minimum may be rejected.
Sputum: (expectorated or induced)	• <u>Collection</u> : Instruct the patient on the proper method for sputum collection (i.e., material brought up from the lungs after a productive cough is what is desired, not nasopharyngeal discharge and saliva). Collect 5 to 10 mL of sputum in a sterile container.

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Stool NOTE: Stool cultures for mycobacteria are not encouraged.	Patients who have difficulty producing sputum should undergo sputum induction. Before induction, administering a short-acting bronchodilator to avoid bronchial hyperactivity may be advised. Induction can be performed by inhaling an aerosol of sterile hypertonic saline (3%) or sterile water produced by a nebulizer that causes coughing. Induction should be performed under supervision in areas with adequate environmental controls. Patients should be instructed to <u>not</u> rinse their mouths or brush their teeth with nonsterile water before collection. • Minimum volume: 3 mL • Transport: Sterile container such as a 50 mL conical tube. If induced sputum is collection, label the specimen container "INDUCED". • Transport time and temperature: Transport as soon as possible at room temperature. If transport is delayed for more than one hour, refrigerate specimen. Do not freeze specimens. • Comments: Early morning sputum specimen is preferred. Do not pool specimens. Limit the replicates to 3 specimens collected consecutively in 8- to 24-hour intervals, with at least one being an early morning specimen. • Note: Optimally, specimens should be collected before drug therapy is started, as even a few days of treatment may inhibit growth and prevent isolation of M. tuberculosis complex. • Possible rejection conditions: Volume below the 3 mL minimum may be rejected. Poorly collected specimens (i.e., saliva) or pooled sputum samples will be rejected. • Collection: Pass specimen directly into a sterile, leak-proof container. Do not use holding or transport medium or preservatives. • Minimum volume: 1g • Transport: Sterile, leak-proof container such as a 50 mL conical tube or sterile urine collection container.
encouraged.	 Transport time and temperature: Transport as soon as possible at room temperature. If transport is delayed for more than one hour, refrigerate specimen. Do not freeze specimen.
	 <u>Comment</u>: Do not submit rectal swab for mycobacterial culture or use any preservatives.
	 <u>Possible rejection conditions</u>: Stool specimens in PVA, formalin, or other preservatives and rectal swabs will be rejected. Stool specimens that were frozen will be rejected.
Tissue or lymph node	 Collection: Aseptically collect during surgery or cutaneous biopsy procedure. NOTE: Swabs are <u>not</u> recommended for the recovery of <i>Mycobacterium</i> species. <u>Transport</u>: Sterile, leak-proof container, such as a sterile 50 mL conical tube containing 2-3 mL sterile nonbacteriostatic saline. <u>Transport time and temperature</u>: Transport as soon as possible at room temperature. <u>Comments</u>: Always submit as much tissue as possible. Tissues or lymph nodes
	fixed in formalin or other preservatives are unacceptable. Write the type of tissue on the submitted requisition form.

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	 <u>Possible rejection conditions</u>: Tissue or lymph node material submitted on a dry swab or dry gauze will be rejected. Tissue or lymph node material submitted in formalin or other preservative will be rejected.
Urine: (including collections from a catheter)	 Collection: Collect approximately 40 mL of urine (midstream). A first morning specimen is preferred. Do not pool urine specimens or use preservatives. Use catheterization only if a midstream sample cannot be obtained. Replicate specimens should be limited to 1 specimen per day on 3 consecutive days. Minimum volume: 10 mL Transport: Sterile, leak-proof container, such as a sterile 50 mL conical tube. Transport time and temperature: Transport as soon as possible at room temperature. If transport is delayed for more than one hour, refrigerate specimen. Do not freeze. Possible rejection conditions: Volume below the 10 mL minimum may be rejected
Transport	 Pooled, frozen or specimens submitted in preservatives will be rejected. Specimens for TB testing must be transported to the laboratory as soon as possible after collection. Guidelines developed by the APHL TB Steering Committee recommend that specimens be delivered to the public health laboratory within 24 hours of collection. Every effort should be made to eliminate the risk of exposure during the transport of clinical specimens. The use of triple containment is key to achieving that goal. The primary receptacle should be securely closed and placed within a disposable, leak-proof bag (secondary packaging). Per Department of Transportation regulation, the tertiary packaging must be a rigid outer container with a UN3373 label placed on the outside of the container. The Rhode Island State Health Laboratory provides pre-labeled orange-top transport containers for the submission of AFB specimens.

^{*}Specimens for AFB culture are processed in 50 mL conical tubes. For optimal results, it is recommended that sputum be collected and processed in the same container.

REFERENCE

Clinical Laboratory Standards Institute (CLSI), Laboratory Detection and Identification of Mycobacteria (M48-ED2:2018)