

THE VERMONT DEPARTMENT OF HEALTH LABORATORY

Physical: 359 South Park Drive, Colchester, VT 05446

Mailing: PO Box 1125, Burlington, VT 05402-1125

1-800-660-9997 (VT only) or 1-802-338-4724

INSTRUCTIONS FOR THE COLLECTION AND PACKAGING OF SPECIMENS FOR THE ISOLATION OF MYCOBACTERIA AND YEAST VDH KIT #6

PLEASE READ INSTRUCTIONS CAREFULLY BEFORE COLLECTING SPECIMEN

In order to comply with postal regulations and ensure the safety of laboratory personnel and others involved in transport of specimens, and maintain integrity of the sample, the Vermont Department of Health Laboratory will accept for testing only those specimens that are packaged according to the instructions below.

The kit should contain:

- A sterile plastic centrifuge tube
- A metal inner container
- A cardboard outer mailing container with a mailing label
- Requisition form (MICRO 220)
- Instructions (MICRO 418)

INSTRUCTIONS FOR COLLECTION OF SPECIMENS

Pulmonary specimens:

Respiratory, lower bronchoalveolar lavage, brush or wash, endotracheal aspirate, transtracheal aspirate: Minimum volume is 3 mL. Place the brush in sterile plastic centrifuge tube with up to 5 mL of sterile saline.

Sputum, expectorated or induced: A series of 3 sputa (not saliva or postnasal fluid), collected at **8-24 hour intervals where at least one specimen is collected early morning** is optimal. Collect at least 3 - 10 ml of specimen into the sterile plastic centrifuge tube. Specimens from one morning may be pooled, but **DO NOT POOL SAMPLES FROM DIFFERENT DAYS.**

- Rinse mouth with water before collecting sputum specimen. Cough deeply in order to obtain the thick, mucous secretion from the lungs.
- Refrigerate the specimen until it can be transported to the Vermont Department of Health Laboratory.

NOTE: 2007 American Thoracic Society Guidelines recommend 2 positive sputum cultures or a single positive bronchial culture to establish laboratory indication of nontuberculous mycobacterial lung disease.

DIGESTED/DECONTAMINATED PULMONARY SPECIMENS FOR RAPID TB TESTING using Nucleic Acid Amplification TESTING (NAAT) ONLY

The Xpert MTB/RIF Assay is used for the detection of MTB-complex and RIF resistance from raw sputum samples and from concentrated sputum sediments.

The HOLOGIC® AMPLIFIED™ Mycobacterium Tuberculosis Direct (MTD) Test detects *M. tuberculosis* complex rRNA in bronchial washings and bronchoalveolar lavages.

Both NAATS are intended for use only with specimens from patients showing signs and symptoms consistent with active pulmonary tuberculosis. Patients suspected of having pulmonary TB who have had less than 3 days of anti-tuberculosis therapy, or have not received such therapy may be evaluated.

- Respiratory sediments must be stored at 2° to 8°C and tested within 3 days of digestion/decontamination
- 1 mL of sediment is needed for testing
- Specimens that are grossly bloody should not be tested with a NAAT
- Performance of NAATs with clinical specimens other than respiratory specimens has not been established by the manufacturers or the Vermont Department of Health Laboratory.

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GeneXpert Analyzed Specimens Flagged as Rifampin Resistant

- ❖ Please contact the laboratory to arrange for referral to CDC for gene sequencing. Sequencing allows characterization of mutations and recognition of silent mutations flagged as rifampin resistant

Extra pulmonary:

Abscess: Remove surface exudates by wiping with sterile saline or 70% alcohol. Collect fluid abscess material with Luer tip syringe and or remove tissue aseptically. For open lesions or abscesses aspirate material from under the margin. Submit in the sterile 50 mL conical tube provided or sterile urine cup. Transport as soon as possible at room temperature or refrigerate at 2-8°C.

Blood: Aseptically collect 5 - 10mL in yellow top container containing SPS or green top collector with heparin. Do not use EDTA (purple top) or a yellow top collector containing ACD. Vacutainers may be shipped or the blood may be transferred into the sterile plastic centrifuge tube. Store and ship at room temperature.

Bone marrow aspirate: Ship in collection vessel (such as 10mL yellow top container containing SPS) or transfer into sterile plastic centrifuge tube. Ship at room temperature.

CSF: > 2 mL needed. Ship in collection vessel or transfer into sterile plastic centrifuge tube. Store and ship at room temperature.

Feces: Stool cultures are not optimal for the recovery of mycobacteria. Routine screening of gastrointestinal specimens to predict disseminated *M. avium* complex infection is not recommended by the American Thoracic Society.

Pleural fluid, ascetic fluid, synovial fluid, paracentesis fluid, and thoracentesis fluid: A volume of more than 5 ml is required. Aseptically transfer the fluid into the sterile plastic centrifuge tube. Store refrigerated at 2-8°C until the specimen can be shipped at room temperature.

Tissue/lymph node (any site, indicate source): Aseptically place into sterile centrifuge container with 2-3 mL sterile saline to prevent desiccation. Store and ship at room temperature.

Urine: Collect a minimum of 40 mL from a first morning specimen, clean catch or catheterization. Transfer into the sterile plastic centrifuge tube. Store refrigerated until specimen can be shipped at room temperature.

- Screw the cap tightly on the centrifuge tube.
- Place the centrifuge tube containing the specimen in the metal container provided. Screw cap on tightly.
- Fill out the requisition form (MICRO 220) fully. Include patient information, health care provider or laboratory information, date of collection, reason for test, specimen type and source. Do not forget to check the appropriate test requested. Please note if anti-fungal treatment is intended for any recovered yeast from specimens for mycology testing. Omitting any of the above information may result in a delay in testing.
- Fold the completed requisition around the **outside** of the metal container.
- Place the requisition and the metal container inside the cardboard outer mailer. Screw the cap on securely.
- Mail or deliver the specimen to Vermont Department of Health Laboratory as soon as possible after collection (optimally within 24 hours of collection). Pulmonary and urine specimens should be refrigerated until mailed or delivered. Avoid freezing specimens. All specimens may be shipped at room temperature.

SPECIMENS MAY BE REJECTED IF:

- Submitted in leaking or broken specimen containers
- Older than 5 days when received

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- No requisition form included or otherwise lacking patient identification
- 24 hour pooled urine or sputum specimens
- Submitted in formalin or frozen

THE LABORATORY WILL DECONTAMINATE AND DISCARD ANY SPECIMEN WHICH IS IMPROPERLY PACKAGED AND MAY PRESENT A RISK TO PERSONNEL.

Specimens for mycobacterial and mycological examination are not preserved. Although specimens submitted for testing may be accepted 48 hours post-collection, best results will be obtained with specimens received less than 48 hours after collection. Specimens received after 48 hours of collection may be heavily contaminated with normal flora and test results may be compromised.

If you have any comments or questions regarding these instructions, please call one of the numbers listed at the front of this form. Thank you.