

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 COLLECTION AND PACKAGING OF SPECIMENS FOR MYCOBACTERIAL AND FUNGAL CULTURE, VDH KIT #6

PLEASE READ INSTRUCTIONS CAREFULLY BEFORE COLLECTING SPECIMEN

To ensure personnel safety and maintain integrity of the specimen, 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 and cardboard outer mailing container with a mailing label
- Requisition form (MICRO 220) and these 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 50 mL conical 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 distilled or purified water before collecting sputum specimen. Cough deeply to obtain the thick, mucous secretion from the lungs.
- Refrigerate the specimen until it can be transported to the Vermont Department of Health Laboratory.

See <https://www.aphl.org/aboutAPHL/publications/Documents/ID-2018Apr-Guidelines-TB-Sputum-Submission.pdf> for more information

NOTE: American Thoracic Society Guidelines recommend 2 positive sputum cultures (of the same NTM species or subspecies of *M. abscessus*) or a single positive bronchial culture to establish laboratory indication of nontuberculous mycobacterial lung disease.

Sputum Specimens for RAPID TB TESTING using Cepheid GeneXpert MTB/RIF Assay

This nucleic acid amplification test is used for the direct detection of MTB complex (with or without rifampin resistance) from raw sputum samples and from concentrated sputum sediments. The test is intended for use with patients showing signs and symptoms consistent with active pulmonary tuberculosis who have had less than 3 days of anti-tuberculosis therapy or have not received such therapy.

Sputum sediment: Store resuspended sediments at 2–8 °C for up to seven days. One mL needed.

Raw sputum: Transport and store specimens at 2–8 °C before processing whenever possible. If necessary, sputum specimens can be stored at a maximum of 35 °C for up to three days and then at 2–8 °C for an additional seven days.

Performance of NAATs with clinical specimens other than sputum specimens has not been established by the manufacturer or the Vermont Department of Health Laboratory.

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.

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Extra pulmonary specimens:

Abscess: Closed-remove surface exudates by wiping with sterile saline or 70% alcohol. Collect fluid abscess material with Luer tip syringe and or remove tissue aseptically. Aspirate material from under the lesion or abscess margin. Submit in sterile 50 mL conical tube provided or sterile urine cup. Transport as soon as possible at room temperature or refrigerate at 2-8°C if transport delayed for more than 1 hour.

Blood: Collect 10mL whole blood in yellow top (SPS) or green top (heparin) collection tube. Do **not** collect in red top, purple EDTA or a yellow top ACD. Transport as soon as possible. Store and ship at room temperature. Minimum volume: Adult-5mL, child-1 mL

Body fluids: Volume >2 mL required, 15mL or more recommended. Aseptically transfer fluid into the sterile plastic centrifuge tube. Store refrigerated at 2-8°C until the specimen can be shipped at room temperature.

Bone marrow aspirate: Prepare puncture site as for surgical incision. Use blood collection tube and mix contents after collection. Preferred container is 10 mL yellow top SPS tube. Transport as soon as possible. Store and ship at room temperature.

CSF: > 2 mL needed, 10mL recommended. Collect separate samples for chemistry and hematology. Ship in collection vessel or transfer into sterile plastic centrifuge tube. Transport as soon as possible. Store and ship at room temperature.

Feces: Not optimal for recovery of mycobacteria. Minimum amount 1 gram, sent in sterile container without holding or transport medium or preservatives. Store refrigerated until specimen can be shipped at room temperature.

Gastric lavage/wash: submit only neutralized specimen

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: minimum 10-15 mL, 40 mL recommended. Collect first morning, midstream specimen. Send catheterization specimen if unable to collect midstream specimen. Do not pool specimens. 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. Mark tube with patient name and DOB.
- 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.
- 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 container
- Older than 5 days when received
- No requisition form included or otherwise lacking patient identification
- 24-hour pooled urine or sputum specimen

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- Submitted in formalin or frozen
- Abscess material submitted on dry swab
- Swabs submitted in commercial swab transport or transport gel based medium

THE LABORATORY WILL DECONTAMINATE AND DISCARD ANY SPECIMEN NOT PROPERLY PACKAGED THAT PRESENTS 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.

Appropriately packaged suspect *Candida auris* isolates or other drug-resistant *Candida* species isolates are accepted for identification.

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.