The Office of Laboratory Services (OLS) provides primary isolation and identification of *Mycobacterium* species in human diagnostic specimens. Reference specimens of AFB isolates are identified for other laboratories.

The DNA probe test is used to identify *M. tb* complex after the culture shows sufficient growth. *Mycobacterium tuberculosis* complex consists of *M. tuberculosis*, *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. microti*, and *M. canetti*. A positive DNA test means the organism is one of these six. In West Virginia, it is rare to find any organisms other than *M. tuberculosis*.

Treatment may be started based on the positive results of the *M. tb* complex probe. Drug susceptibility testing (DST) for *Mycobacterium tuberculosis* complex is performed on isolates from a new patient, any continued positive after three months treatment and at special request of the physician. Due to low incidence of Mycobacterium *tuberculosis* in WV, DSTs are sent to CDC in Atlanta. First and second line drugs are tested and reported. After the initial *M. tb* complex ID (DNA probe), a niacin is done on subsequent cultures for three months. Drug susceptibility testing is performed only on *M. tuberculosis*.

A Mycobacterium other than tuberculosis (*MOTT*) is identified by DNA probe for *M. avium* complex, *M. gordonae*, and *M. kansasii*. Biochemical tests are used to identify other *MOTT* isolates. Results may take from one to seven weeks to identify. Neither this laboratory nor CDC performs susceptibility testing for *M. avium* complex. CDC no longer offers susceptibilities on rapid growers (January 2005).

**SPECIMEN REQUIRED:**

*Sputum, urine, body fluid or tissue*. The source of specimen is required. (Also see Table 1)

Collection of early morning specimens of urine and sputum on each of 3 consecutive days is optimum. Include information about prior anti-tuberculosis therapy.

**UNRELIABLE SPECIMENS**

- < 5 ML,
- Date of collection unknown.
- Resembles saliva.

RESEMBLES SALIVA accepted in special cases (prison inmates, very young, nursing home residents, and elderly patients) and will be marked under COMMENTS on report as “unreliable, resembles saliva”.

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**MYCOBACTERIOLOGY SERVICE MANUAL**
Specimens unable to be recollected (surgical specimens) will be tested and reported as unreliable.

**UNSATISFACTORY SPECIMENS**

please recollect:

- DRY MATERIAL
- IN TRANSIT 5 DAYS
- INSUFFICIENT AMOUNT
- LAB ERROR
- LEAKED IN TRANSIT
- MATERIAL COLLECTED & TRANSPORTED ON SWABS
- MIXED CULTURE FOR ID; RESUBMIT PURE ISOLATE
- MULTIPLE SPECIMENS COLLECTED ON SAME DAY FROM SAME SITE
- NONSERTILE CONTAINERS
- NO PROVIDER
- NO SPECIMEN RECEIVED
- RESEMBLES SALIVA
- UNLABELED/ NO IDENTIFICATION

As of 12-1-2007

UNLABELED specimens from any source will not be accepted, due to CLIA regulations.

Label blue top tubes when kits are given to patient!

**ATTENTION: PROVIDERS**

**CLIA** requires laboratories to have a system that ensures positive identification of patient’s specimen.

Fill out request forms for your patients with the following:

1. Patient name
2. Patient address
3. Date of birth and sex
4. Specimen type
5. Current TB medications, if any
6. Provider’s name and address
7. Any unique patient identifier number (optional).

On outer cardboard box, fill in provider’s return address

**ATTENTION: PATIENT**

Fill in date of collection to provide reliable results for your test and mail on collection day.
TABLE 1

Specimen requirements for mycobacterial isolation and acid-fast stains

<table>
<thead>
<tr>
<th>SPECIMEN TYPE</th>
<th>SPECIMEN REQUIREMENTS</th>
<th>SPECIAL INSTRUCTIONS</th>
<th>UNACCEPTABLE SPECIMENS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abscess contents, aspirated fluid</td>
<td>As much as possible in syringe with Luer tip cap or sterile container</td>
<td>Cleanse skin with alcohol before aspirating sample. Collect specimen on swab and place in transport medium only if volume is insufficient for aspiration by needle and syringe. Laboratory may provide 7H9 broth for transport of small volumes of aspirates.</td>
<td>Dry swab.</td>
</tr>
<tr>
<td>Body fluids (pleural, pericardial, peritoneal, etc.)</td>
<td>As much as possible (10 ml minimum) in sterile container.</td>
<td>Disinfect site with alcohol if collecting by needle and syringe. Conical polypropylene bottles (200 ml) for centrifugation of large volumes are commercially available.</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>Bone in sterile container without fixative or preservative</td>
<td>Collect aseptically.</td>
<td>Specimen submitted in formalin.</td>
</tr>
<tr>
<td>Bone marrow, Blood</td>
<td>As much as possible in 7H9 broth tube or 1.5 ml in pediatric Isolator tube.</td>
<td>Collect aseptically. Call before sending</td>
<td></td>
</tr>
<tr>
<td>Bronchoalveolar lavage or bronchial washings</td>
<td>Equal to or greater than 5 ml in sterile container.</td>
<td>Avoid contaminating bronchoscope with tap water. Saprophytic mycobacteria may produce false-positive culture or smear results.</td>
<td></td>
</tr>
<tr>
<td>Bronchial brushings</td>
<td>Sterile container or Middlebrook 7H9 broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>Equal to or greater than 2 ml in sterile container.</td>
<td>Use maximum volume attainable. Plant 0.5 ml on LJ slant, incubate overnight before sending</td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>Node or portion in sterile container without fixative or preservative.</td>
<td>Collect aseptically and avoid indigenous microbiota. Select caseous portion if available. Do not immerse in saline or other fluid or wrap in gauze. Freezing decreases yield.</td>
<td>Specimen submitted in formalin.</td>
</tr>
<tr>
<td>Skin lesion material</td>
<td>Submit biopsy specimen in sterile container without fixative or preservative.</td>
<td>Swabs in transport medium (Amies or Stuarts) are acceptable only if biopsy sample or aspirate is not obtainable. For cutaneous ulcer, collect biopsy sample from periphery of lesion, or aspirate material from under margin of lesion. If infection was acquired in Africa, Australia, Mexico, South America, Indonesia, New Guinea, or Malaysia, note on request because Mycobacterium ulcerans may require prolonged incubation for primary isolation.</td>
<td>Dry swab</td>
</tr>
<tr>
<td>Smear on slide</td>
<td>Smear specimen over</td>
<td>Heat fix smear. Transport in slide</td>
<td></td>
</tr>
<tr>
<td>(All sources)</td>
<td>1.5 x 1.5 cm area of clear slide</td>
<td>container taped closed and labeled BIOHAZARD</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Sputum</strong></td>
<td>5-10 ml in sterile, wax-free, disposable container. Collect an early morning specimen from deep, productive cough on at least 3 consecutive days. <strong>Do not pool specimens</strong>. For follow-up of patients on therapy, collect at weekly intervals beginning 3 weeks after initiation of therapy.</td>
<td>For expectorated sputum, instruct patient on how to produce sputum specimen as distinct from saliva or nasopharyngeal discharge. Have patient rinse mouth with water before collecting sputum to minimize contaminating specimen with food particles, mouthwash, or oral drugs, which may inhibit the growth of mycobacteria. For induced sputum, use sterile hypertonic saline. Avoid sputum contamination with nebulizer reservoir water. Saprophytic mycobacteria in tap water may produce false-positive culture or smear results. Indicate on request if specimen is induced sputum as these watery specimens resemble saliva and risk rejection as inadequate.</td>
<td>Saliva (secretions from the mouth; spit) Insufficient specimen. Unlabeled specimen. Broken or leaky specimen. Specimen too old.</td>
</tr>
<tr>
<td><strong>Tissue biopsy sample</strong></td>
<td>1 gm of tissue, if possible, in sterile container without fixative or preservative.</td>
<td>Collect aseptically and avoid indigenous microbiota. Select caseous portion if available. Do not immerse in saline or other fluid or wrap in gauze. Freezing decreases yield.</td>
<td>Specimen submitted in formalin.</td>
</tr>
<tr>
<td><strong>Transtracheal aspirate</strong></td>
<td>As much as possible in syringe with Luer tip cap or other sterile container.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td>As much as possible (minimum, 40 ml) of first morning specimen obtained by catheterization or of midstream clean catch in sterile container. For suprapubic tap, as much as possible in syringe with Luer tip cap or other sterile container.</td>
<td>Collect first morning specimen on 3 consecutive days. Accept only one specimen/day. Organisms accumulate in bladder overnight so first morning void provides best yield. Specimens collected at other times are dilute and are not optimal. Centrifuge for 15 minutes. Remove supernatant by pouring off or using a pipette and send in 50 ml conical tube.</td>
<td>24 hour pooled specimens. Urine from catheter bag Specimens of less than 40 ml unless larger volume is not obtainable.</td>
</tr>
<tr>
<td><strong>Wound material</strong></td>
<td>See biopsy or aspirate.</td>
<td>Swabs are acceptable only if biopsy or aspirate is not obtainable. If used, they must be placed in transport medium (Amies or Stuarts). Negative results are not reliable.</td>
<td></td>
</tr>
</tbody>
</table>
### Turnaround Times (General Guide)

<table>
<thead>
<tr>
<th>Event</th>
<th>Description</th>
<th>Average Time</th>
<th>Exceptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receipt of specimen to stain report</td>
<td>&lt;24 hours Monday-Friday. Reported on day performed by fax or hard copy/mailed. Delivery method determined by provider.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receipt of raw specimen which does not grow mycobacterium</td>
<td>Liquid media, no growth 6 weeks, solid media, 7 weeks</td>
<td>Contaminated cultures are reported when found.</td>
<td></td>
</tr>
<tr>
<td>Receipt of raw specimen to detection of growth</td>
<td>21 days</td>
<td>Range of 4 days to 7 weeks, depending on type of organism recovered</td>
<td></td>
</tr>
<tr>
<td>Receipt of isolate for TB screen</td>
<td>up to 5 working days</td>
<td>If culture is mixed with other flora requiring decontamination procedure, a significant delay could occur.</td>
<td></td>
</tr>
<tr>
<td>Identification of Mycobacterium other than tuberculosis</td>
<td>2 days to 5 weeks</td>
<td>Slowly growing mycobacterium may require additional time for identification. If the culture is mixed with other flora requiring decontamination procedure, a significant delay could appear.</td>
<td></td>
</tr>
<tr>
<td>Susceptibility testing performed at CDC and results reported</td>
<td>Unknown, time dependant on CDC TAT</td>
<td>Slow growing isolates.</td>
<td></td>
</tr>
</tbody>
</table>
REFERRED CULTURES OF MYCOBACTERIA

The shipment of any referred culture must be prepared by a certified person from the facility following federal regulations (49 CFR) for mailing infectious substances. (Shippers declaration link)

Fill out consolidated micro request form. Keep a subculture in the event the organism is not viable after shipping, in which case an additional culture will have to be sent.

Solid Media

Isolates on tube media (Lowenstein-Jensen, 7H10, etc.) must be pure cultures.

Liquid Media

The OLS will accept for confirmation and identification and/or drug susceptibility test, cultures which contain acid fast bacilli.

a. It is recommended to centrifuge contents of any bottle in a 50 ml blue cap centrifuge tube (use the OLS TB collection kit for this shipment).

b. Remove supernatant liquid by pouring off or using a pipette. Send at least 1 ml of broth. Use safety precautions and disinfect lip and outside of tube if necessary.

c. Label the tube with the patient’s name and mail in the proper mailing container.

d. Larger amounts of liquid media may be shipped by ground if it is packaged correctly and the private courier agrees to transport.

Refer to WV Reportable Infectious Disease Code 16-3-1; 64 CSR 7 which requires that one isolate per case of M. tb be submitted to WVOLS. Susceptibility testing will be performed if needed and the isolate will be held. Fingerprinting is available through the CDC to aid epidemiology in determining outbreaks or clusters. The TB Unit will fax a confirmation receipt when specimens arrive at OLS to complete the shipping cycle.
Mycobacterium Other Than Tuberculosis (MOTT) for Health Providers

Potential Clinical Significance
1. Present only as pathogens
2. Present usually as pathogens
3. Present commonly as nonpathogens
4. Present usually as nonpathogens

Mycobacterium species Potential Clinical Significance
Non photochromogens-Non pigmented
- M. avium complex 2
- M. gastri 4
- M. malmoense 1
- M. haemophilum 1
- M. nonchromogenicum 4
- M. terrae complex 4
- M. triviale 4

Photochromogens-produce pigment in light only
- M. marium 2
- M. kansasii 2
- M. simiae 3-2

Scotochromogens-produce pigment in light and dark
- M. scrofulaceum 3-2
- M. szulgai 1
- M. gordonae 4
- M. flavescens 4
- M. xenopi 3

Rapid growers-grow in 7 days or less
- M. fortuitum complex 4-3
- M. fortuitum group 4-3
- M. chelonae 4-3
- Other rapid grower 4

The significance of the isolation of Mycobacterium Other Than Tuberculosis (MOTT) may be difficult to assess since many species are opportunistic pathogens. The following criteria are useful in establishing a role for MOTT in the disease process:

1. Repeated isolation of a large number of colonies of the same species from the same anatomical site over an extended period;
2. Clinical or radiographic evidence of disease;
3. Histopathologic evidence of the presence of mycobacteria in tissue;
4. Increasing numbers of mycobacteria in sequential specimens;
5. Isolation from a normally sterile site;
6. Predilection of the species to cause disease at that site;
7. Presence of predisposing conditions in the patient;
8. Absence of other identifiable causes of disease.