

Tuberculosis (*Mycobacterium tuberculosis*)

The Oregon State Public Health Laboratory (OSPHL) will test appropriately submitted specimens from patients suspected of having TB. The OSPHL will also test specimens submitted to help monitor therapy of previously diagnosed TB patients or to aid in contact investigations.

The OSPHL accepts primary specimens from County Health Programs and their affiliates. Primary samples from Private Care facilities will also be accepted at the request of a State or County TB Public Health program.

In addition to *M. tuberculosis* isolates submitted to this laboratory, in compliance with OAR 333-018-0018, the OSPHL will also accept unidentified AFB smear positive isolates to rule out *M. tuberculosis*. Susceptibility testing will be performed on all confirmed *M. tuberculosis* isolates.

Private Laboratories may also submit NALC-NaOH processed sputum pellets for nucleic acid amplification testing (NAAT). This is a fee for service test for private labs. Submitters should contact the OSPHL 503-693-4100 for additional billing information.

Primary Specimens (Local Health Departments)

An AFB smear and culture is routinely performed on all submitted primary specimens. Additionally an NAAT will be performed on at least one respiratory sample from any previously undiagnosed patient. The NAAT performed by the OSPHL is the GenProbe MTD®. GenProbe MTD® testing is FDA approved for AFB smear positive and smear negative respiratory samples from patients not on anti-tuberculosis therapy (TB-Rx) and who have a presentation consistent with active pulmonary tuberculosis. Patients with less than 7 days of TB-Rx or who have not had TB-Rx in the previous 12 months may also be tested.

Testing is always performed in conjunction with AFB smear and culture. If NAAT is not desired, this should be indicated on the requisition slip. Repeat testing of additional samples from a specific patient occurs in the following instances:

- a. The smear is negative and the MTD is positive
- b. The smear is positive and the MTD is negative
- c. The initial smear and MTD are negative but a later specimen on same patient is smear positive.

For Guides to Use and Interpretation of MTD, see References 3, 4.

Submitting Samples

Three to five AFB negative specimens, collected on consecutive days, may be required to assure a negative diagnosis. However one AFB positive specimen may be sufficient to confirm a suspect diagnosis. **Do not pool specimens.**

Collection Instructions

All required collection and shipping materials are available from the OSPHL.

1. Expectorated sputum

- a. The best specimen is the first deep sputum obtained in the morning after clearing the saliva from the mouth.
- b. Collect a series of three to five single, early morning samples in the collection tubes provided. Use a separate tube for each sample.
 - A volume of 5 to 10 ml is adequate for each sample. However, smaller volumes will be accepted.
 - Results for samples of less than 1.0 ml will be reported with a qualifier.
- c. Securely cap all tubes to avoid leakage.
- d. Decontaminate outside of specimen tube with appropriate disinfectant.
- e. Label tube with patient name and collection date.
- f. Complete a test requisition form for each specimen submitted.
- g. Refrigerate specimens pending ship-

ment to the OSPHL.

2. Bronchial wash/induced sputums

There is no minimum volume for this specimen type. Follow same guidelines as for expectorated sputum.

3. Gastric lavage

- a. Specimens which cannot be processed within four hours of collection must be neutralized. For each 35 – 50 ml of specimen, use 1.5 ml of sterile 40% anhydrous disodium phosphate (Na_2HPO_4) or two pH 7.4 pHydration buffer capsules or tablets.
- b. Secure lid against leakage and label specimen tube with patient name and collection date.
- c. Complete a test requisition form for each specimen submitted.
- d. Store refrigerated pending shipment.

4. Urine

A three-to-five day series of single, early morning, mid-stream urines should be submitted. Do not submit pooled specimens.

- a. Aseptically transfer up to 35 ml of urine to provided tube.
- b. Secure lid against leakage and label collection tube with patient name and collection date.
- c. Complete a test requisition form for each specimen submitted.
- d. Store refrigerated pending shipment.

5. Body fluids (CSF, thoroacentesis, pleural fluid, synovial fluid, etc.)

- a. Aseptically transfer specimen to plastic specimen tube. There is no minimum volume.
- b. Secure lid against leakage and label collection tube with patient name, specimen source and collection date.
- c. Complete a test requisition form for each specimen submitted.
- d. Store refrigerated pending shipment.

6. Tissue

- a. Aseptically transfer specimen, along with a minimal amount of sterile saline as needed to prevent desiccation, to sterile plastic tube.
- b. Secure lid against leakage and label tube with patient name, specimen source and date of collection.
- c. Complete a test requisition form for each specimen submitted.
- d. Store refrigerated pending shipment.
- e. Transport to OSPHL on wet ice or ice packs as soon as possible. Call the OSPHL for additional packaging and shipping instructions as needed.

7. Wounds and lesions

- a. Aseptically obtain purulent material with a sterile swab or by aspiration or washing.
- b. Aseptically transfer specimen to a sterile tube. Add a small amount of 7H9, 7H11 or sterile isotonic saline to prevent specimen dehydration.

- c. Secure lid against leakage and label specimen tube with patient name, specimen source and collection date.
- d. Complete a test requisition form for each specimen submitted.
- e. Store refrigerated pending shipment.

Precautions

1. All specimens should be kept refrigerated pending shipment.
2. Do not pool specimens. Transport each specimen as soon as possible after collection.
3. When possible, collect and ship specimens early in the week.
4. Ship samples according to current federal regulations. Refer to the Shipping and Transport section of this Guide to Services

Improperly packaged or leaky specimens present a hazard to laboratory personnel and may be rejected.

Note: Isolates of *M. tuberculosis* are classified as Category A.

Secondary Specimens

Private Care Laboratories

AFB Isolates submitted for identification/confirmation of organism ID and/ or TB susceptibility testing

1. Submit AFB culture isolates on Lowenstein-Jensen, 7H10, or other comparable solid AFB media. Failure to submit “pure” cultures may delay or adversely affect the identification process.

2. AFB “Positive” liquid media tubes from automated TB systems, such as MGIT or BTA, will also be accepted for testing. Note: these liquid media culture results can be adversely affected by inadequate organism load (potential false negatives) or blood in the media (potential false positives). Cultures with mixed AFB organisms or cultures with bacterial contamination may also yield misleading results
Evaluate DNA probe results from liquid culture with caution. Results should be correlated with specimen, smear/culture morphology, and available patient data, before releasing patient results to charting system.
3. Submitters should retain subcultures of all submissions as a backup precaution.

Private Care Laboratories

Processed concentrates of primary sputum pellets for NAAT testing

1. Submitters must process samples using CDC NALC-NaOH decontamination methodology
2. A minimum of 0.5 ml of the processed/concentrated pellet (stored and shipped at 2-8°C) should be submitted to the OSPHL for receipt within 2 days of the processing date.
3. Ship according to Category B regulations
4. Private care facilities will be assessed a fee for this test. Please contact the

OSPHL for billing information or if additional information or guidance is required, (503)693-4100.

OSPHL Testing and Reporting

Primary samples, Local Health Departments

First Preliminary Report (AFB smear report):

- a. Acid Fast Stains are performed the day following receipt of specimen.
- b. Initial positive results for new patients are called or faxed on day of test.
- c. Written reports for all submissions are generated and sent within one business day.
- d. Submitters should check WebRad or call the OSPHL if a written report has not been received within 5 days of specimen receipt.

Second Preliminary Report (MTD, NAAT, tested primary samples)

1. Positive MTD results are called or faxed on day of test. Routine MTD runs will be Monday and Thursday.
 - a. Written reports for MTDs are generated and sent within one business day of testing.
 - b. Submitters should check WebRad or call the OSPHL if a written report is not received within 5 days of MTD testing.

Cultures are monitored for up to 8 weeks before reporting as culture negative for AFB.

Additional Preliminary Report (Culture Results)

Additional preliminary reports are generated for any culture with growth of acid fast bacilli. This report will include organism identification by the Gen-Probe DNA-Probe (Accuprobe) test for *M. tuberculosis* complex and *M. avium* complex.

No additional ID testing is performed on AFB organisms testing negative for *M. tuberculosis* complex or *M. avium* complex.

If further identification is needed, contact the OSPHL. The organism may be sent to a reference laboratory having full ID capacity.

Private care laboratories may submit isolates for DNA-probe ID as described in the isolate submission section.

All results of *M. tuberculosis* complex from new patients will be called to submitter on day of test.

Final Culture Report

Cultures with no growth at eight weeks are reported as “AFB (acid fast bacilli) NOT ISOLATED”.

Final reports for positive cultures will include colony count information if available.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing is performed on initial TB isolates from new patient cases.

Susceptibility testing is also repeated if a patient’s additional samples are still TB culture positive 3 months or more after the original susceptibility testing was performed.

Additional susceptibility tests will be done on request. Antibiotic susceptibility testing of *M. tuberculosis* isolates is available to all submitters.

Preliminary Susceptibility results of MGIT S.I.R.E. and final MGIT PZA testing are typically available eight days to two weeks following culture identification of *M. tuberculosis*.

Final, Indirect susceptibility results are usually available four to five weeks following organism identification.

“Resistant” susceptibility results are called as soon as possible after completion of test. All hard copy results are mailed within 48 hours of test completion. Results may also be accessed via WebRad.

Specimen Packaging and Shipping

OSPHL provides complete kits for packaging and shipping of tuberculosis test specimens.

1. Have patient name and specimen collection date on the container label.

Complete all sections of the requisition form & attach the OSPHL barcoded label to the specimen.

2. Transfer appropriate specimen to collection tube. Secure the lid with adhesive tape to prevent leakage during transport.

3. Place the tube with absorbent into the plastic zip-top bag.

a. Place the tube sideways into the plastic bag.

b. Expel the air from the bag with your hands.

c. Close the bag by zipping the top seal.

d. Put the lab slip in the outer bag pocket.

e. Wrap bag around tube.

4. Place the sealed bag and slip into the inner metal tube which must bear a biohazard label.

a. Screw the lid onto the container.

b. **Make sure the lid is secure** to ensure a leak-proof seal.

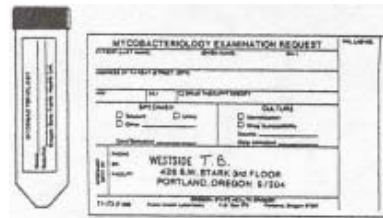
5. Place the metal tube into the outer mailing container.

a. Make sure the lid is closed securely.

b. Refrigerate specimen pending shipment to OSPHL.

Send specimens as soon as possible after collection - OSPHL must receive no later than 5 days after collection to avoid overgrowth contamination.

1.



MYCOBACTERIOLOGY EXAMINATION REQUEST

OSPHL
3150 NW 229th Ave, Suite 100
Hillsboro, OR 97124

DATE: _____ TIME: _____

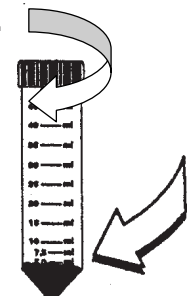
PATIENT NAME: _____

TEST REQUESTED: Sputum Urine Smear Culture MDR Xpert GeneXpert

LABORATORY: _____

WESTSIDE T.B.
428 S.W. STARK 3RD FLOOR
PORTLAND, OREGON 97204

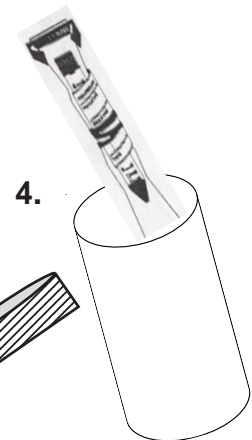
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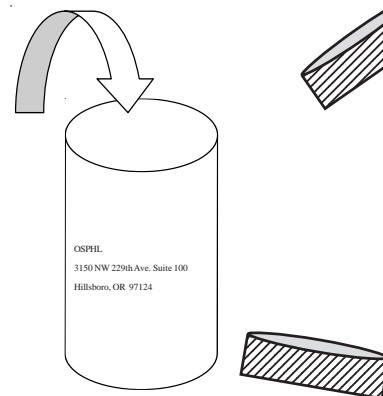
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4.



5.



OSPHL
3150 NW 229th Ave, Suite 100
Hillsboro, OR 97124
503 693-4100

References

1. U.S. Dept of Health, Education, and Welfare/Public Health Service/Centers for Disease Control. Public Health Mycobacteriology, A Guide for the Level III Laboratory. 1985.
2. U. S. Dept of Health, Education and Welfare/Public Health Service/Centers for Disease Control. Procedures for the Isolation and Identification of Mycobacteria. 1975. HEW Publication No. (CDC) 77-8230.
3. Centers for Disease Control and Prevention. 2009. United States Morbid. and Mortal. Weekly Rep. January 16, 2009 /8(01);7-10 <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm>
4. State of Oregon Guide to NAAT testing: <http://www.oregon.gov/DHS/ph/tb/tools/NAATguide.pdf>