

Testing Guidance *Mycobacterium tuberculosis*



Oregon State Public Health Laboratory

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SAMPLE SUBMISSION OVERVIEW

The Oregon State Public Health Laboratory (OSPHL) will test appropriately submitted specimens from patients suspected of having tuberculosis. The OSPHL will 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 specimens from Private Care facilities will be accepted at the request of a State or County public health authorities. In addition to *M. tuberculosis* (MTB) isolates submitted to this laboratory, in compliance with OAR 333-018-0018, the OSPHL will accept unidentified AFB smear positive isolates to rule out MTB if the referring laboratory cannot. Private sector submitters will be charged a fee for this service. Submitters should contact the OSPHL 503-693-4100 for additional information.

AFB SMEAR AND CULTURE

Local Public Health Authorities

Whenever possible, collect and ship specimens early in the week and transport for receipt at the OSPHL Monday through Friday morning. Specimens should be transported to OSPHL as soon as possible after collection. Avoid batch transport of a series of specimens. Holding specimens may compromise specimen integrity and delay testing due to overgrowth of non-tuberculous organisms and inhibit identification of MTB.

The most common specimens submitted for this order are expectorated sputum and urine. Additional specimen sources accepted are provided in Appendix A.

Expectorated sputum

- All required collection and shipping materials are available from OSPHL.
- The best specimen is the first deep sputum obtained in the morning after clearing the saliva from the mouth. Rinsing the mouth with water before collecting the specimen helps prevent contamination. If possible, observe the first sputum collected by the patient to ensure they understand how to collect and refrigerate the sputum
- Collect a series of three to five single, early morning samples in the collection tubes provided. Use a separate tube for each sample, do not pool.
 - A volume of 5 to 10 mL is optimal. However, smaller volumes will be accepted. Results from samples of less than 1.0 mL will be reported with a qualifier.
 - Sample volume greater than 11mL will be split, centrifuged, and then pooled for testing.

- Label the specimen with collection date and at least two unique patient identifiers (e.g., first and last name, date of birth, medical record number).
- Complete and submit a [General Microbiology Test Request Form](#) for each specimen.
- Securely cap tubes to avoid leakage.
- Decontaminate outside of specimen tube with appropriate disinfectant.
- Refrigerate specimens pending shipment to OSPHL, including during transit between the patient's home and the Local Public Health Authority.
- The following resources for sputum collection are provided in multiple languages:
 - Home Sputum Collection: A Step-by-Step Guide, offered by Public Health of Madison and Dane County, WI:
<https://www.youtube.com/playlist?list=PLO9RoNDnObhOcYPpeYMTG3BpPHU5-Le6H>.
 - Instructions for Collecting Sputum Fact Sheet, offered by the Oregon Tuberculosis Program:
<http://www.oregon.gov/oha/PH/DISEASES/CONDITIONS/COMMUNICABLE/DISEASE/TUBERCULOSIS/Pages/factsheets.aspx>

Urine

- Collect a series of three to five single, early morning, mid-stream urine samples. Do not submit pooled specimens.
- Aseptically transfer up to 35 mL of urine to provided tube.
- Secure lid against leakage.
- Label the specimen with collection date and at least two unique patient identifiers (e.g., first and last name, date of birth, medical record number).
- Complete and submit a [General Microbiology Test Request Form](#) for each specimen.
- Store refrigerated pending shipment.

Specimen Shipping

- Ship samples according to current federal regulations. Guidance can be located on OSPHL's [Shipping and Transport webpage](#).
- Improperly packaged or leaking specimens present a hazard and may be rejected.
- OSPHL provides complete kits for packaging and shipping of tuberculosis test specimens.
- Label the specimen with collection date and at least two unique patient identifiers (e.g., first and last name, date of birth, medical record number).
- Complete all sections of the [General Microbiology Test Request Form](#).
- Transfer specimen to 50 mL Falcon tube.
- Secure the lid to prevent leakage during transport.
- Include absorbent material in the specimen transport bag.
- Place the tube sideways into the plastic specimen transport bag.
- Expel the air from the bag.
- Close bag by zipping the top seal.
- Put the General Microbiology Test Request Form into **outer** bag pocket.
- Refrigerate specimen pending shipment to OSPHL.
- Send specimens to the OSPHL as soon as possible after collection. Specimens should be received within 24 hours of collection but no more than 5 days after collection.

The image shows a specimen shipping kit. At the top is a 'GENERAL MICROBIOLOGY REQUEST' form from the Oregon State Public Health Laboratory (OSPHL). The form includes sections for Patient Information (name, date of birth, gender, ethnicity, race, address), Patient Insurance Information (name, policy number, group ID, diagnosis), and Specimen/Isolate Information (date of collection, time of collection, specimen source, and whether it's a new or re-submitted specimen). Below the form is a plastic specimen transport bag with a biohazard symbol and the text 'SPECIMEN ONLY', 'BIOHAZARD PELIGROSO', and 'REFRIGERATE ROOM TEMPERATURE'. A 50 mL Falcon tube is also visible.

Testing and Result Reporting

This order choice includes a primary smear, solid and liquid culture. GeneXpert, AFB PCR identification, and MTB isolate susceptibility testing will be added reflexively when necessary.

- **Primary smear**
 - Determines presence or absence of AFB organisms in primary specimen.
 - Preliminary report issued within 1 working day of receipt.
 - Initial positive results for new patients are called on day of test result. Submitters will also receive a result released on the day of test.
 - Result reports for all submissions are generated and sent within one business day.
 - Submitters should check Copia then contact the OSPHL if a report has not been received within 5 days of specimen receipt.

- **GeneXpert**
 - Determines presence or absence of MTB DNA in primary sputum samples, as well as mutations that confer resistance to Rifampin.
 - Cepheid GeneXpert® testing is FDA cleared for AFB smear positive and smear negative sputa from patients not on anti-tuberculosis therapy (TB-Rx) and who have a presentation consistent with active pulmonary tuberculosis.
 - Reflexively performed on the first positive smear from a previously undiagnosed patient with less than 72 hours of treatment for tuberculosis.
 - Approval may be obtained for testing smear negative specimens from patients that meet established criteria. Contact the OSPHL for additional information.
 - Preliminary report issued within 2 working days of receipt.
 - Initial positive results for new patients are called or released on day of test.
- **Solid media**
 - The OSPHL uses 7H10 solid media to grow and isolate AFB organisms.
 - A preliminary report is generated when acid fast bacilli are isolated. This report will include colony count and organism identification as MTB complex or *M. avium* complex based on detection of nucleic acids by the DNA PCR method or morphology consistent with a previous identification.
 - A final negative report of “AFB not Isolated” is generated after 8 weeks, up to 9 weeks, of incubation.
- **Liquid media**
 - The OSPHL uses MGIT liquid media to grow and isolate AFB organisms.
 - A preliminary report is generated when acid fast bacilli are detected by fluorescence microscopy. This report will include organism identification as MTB complex or *M. avium* complex based on detection of nucleic acids by the DNA PCR method or morphology consistent with a previous identification.
 - A final negative report of “AFB not Isolated” is generated after 6 weeks of incubation.
- **Susceptibility testing**
 - Antibiotic susceptibility testing is reflexively performed on initial MTB culture positive isolates from new cases.
 - Susceptibility testing is repeated at three-month intervals if a patient’s additional samples are MTB culture positive. Intervals are determined based on collection dates of specimens on which susceptibility testing was previously performed.

- “Resistant” susceptibility results are reported and called to the submitter as soon as possible. Results may be accessed via Copia. All hard copy results are released within 24 hours of test completion.
- All antibiotics are tested at the critical concentration for the test method in use (e.g., MGIT or agar proportion) as defined by the Clinical Laboratory Standards Institute (CLSI).
- **MGIT PZA**
 - A liquid based susceptibility method that evaluates growth of MTB in the presence of PZA at the critical concentration of 100 ug/mL
 - Resistant results will be confirmed by repeat testing.
 - Results are typically available within two to three weeks following identification of MTB.
- **MGIT IRE**
 - A liquid based susceptibility method that evaluates growth of MTB in the presence of Isoniazid (critical concentration 0.1 ug/mL), Ethambutol (critical concentration 5.0 ug/mL), and Rifampin (critical concentration 1.0 ug/mL)
 - Resistant results will be confirmed by agar proportion at CDC.
 - Results are typically available within two to three weeks following identification of MTB.
- **Agar Proportion**
 - A solid agar-based susceptibility method that quantifies the proportion of resistant mutants within a population. When the percentage of resistant mutants is greater than 1% of the population, the isolate is categorized as “resistant.”
 - Ofloxacin, a second line drug, is evaluated at the critical concentration of 2.0 ug/mL.
 - Results are typically available four to five weeks following organism identification.

Private Care Laboratories

Please contact the OSPHL (503) 693-4100 for approval and additional information about requesting AFB Smear/Culture

AFB ISOLATE IDENTIFICATION

Local Public Health Authorities

LPHAs should submit primary specimens for smear and culture as indicated above. LPHAs do not order this test.

Private Care Laboratories

Specimen Collection

- Primary specimens should be collected and processed according to internal laboratory protocols and CLSI standards. AFB smear positive isolates may be submitted for the purpose of ruling in or out *M. tuberculosis* and *M. avium*.
- Submitters should retain subcultures of all submissions.
- 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.
- AFB smear positive liquid media from automated TB systems, such as MGIT or BTA, will be accepted for testing.
 - To submit specimens from BTA systems, do not submit in BacT bottle.
 - Remove 5mL of material from the BacT bottle, spin for 15 minutes at 4150 rpm. Decant the supernatant, leaving approximately 0.5mL. Resuspend and transfer to a microcentrifuge tube, sealed with parafilm, for submission.
 - Specimens that cannot be spun may be submitted in conical tubes sealed with parafilm if needed.
 - If a liquid culture is submitted, evaluate DNA PCR results with caution. Results should be correlated with smear and culture morphology before releasing patient results to charting system.

Specimen Shipping

- Ship samples according to current federal regulations. Guidance can be located on OSPHL’s [Shipping and Transport webpage](#).
- Specimens should be packaged for secure transport
 - Parafilm primary specimen container.
 - Place primary specimen container in secondary containment (biohazard bag, 15 mL conical tube, etc.)
 - Place appropriately contained specimen into a biohazard bag with absorbent material prior to shipping.

Testing and Result Reporting

○ AFB PCR

- A fluorescent smear will be performed prior to testing to confirm the presence of AFB bacteria before testing is initiated. If the concentration of AFB bacteria is less than a standardized inoculum, the test will be reported as UNSATISFACTORY.
- Specimens are generally tested on Wednesdays and Fridays.
- Indeterminate results are repeated once before reporting.
- Results will be called to submitters at the conclusion of testing.
- If *Mycobacterium tuberculosis* is not detected for AFB positive submissions, no further testing will be performed at the OSPHL.
 - If further identification will be performed by a reference laboratory, it is recommended that a new specimen be collected if possible. A list of laboratories that may provide further testing is provided at the end of this document.
 - If a new specimen cannot be collected, the OSPHL may be able to forward the existing isolate or specimen to the submitting facility or provider at their expense.
 - If a facility that did not submit the specimen needs the specimen sent from the OSPHL to their facility, a patient authorization must be obtained following the OSPHL procedures. This should be the last option utilized whenever possible.

○ Susceptibility testing

- Antibiotic susceptibility testing is reflexively performed on initial MTB culture positive isolates from new cases.
- Susceptibility testing is repeated at three-month intervals if a patient's additional samples are MTB culture positive. Intervals are determined based on collection dates of specimens on which susceptibility testing was previously performed.
- "Resistant" susceptibility results are reported and called to the submitter as soon as possible. Results may be accessed via Copia. All hard copy results are released within 24 hours of test completion.
- All antibiotics are tested at the critical concentration for the test method in use (e.g., MGIT or agar proportion) as defined by the Clinical Laboratory Standards Institute (CLSI).

○ MGIT PZA

- A liquid based susceptibility method that evaluates growth of MTB in the presence of PZA at the critical concentration of 100 ug/mL
- Resistant results will be confirmed by repeat testing.
- Results are typically available within two to three weeks following identification of MTB.

- **MGIT IRE**
 - A liquid based susceptibility method that evaluates growth of MTB in the presence of Isoniazid (critical concentration 0.1 ug/mL), Ethambutol (critical concentration 5.0 ug/mL), and Rifampin (critical concentration 1.0 ug/mL)
 - Resistant results will be confirmed by agar proportion at CDC.
 - Results are typically available within two to three weeks following identification of MTB.
- **Agar Proportion**
 - A solid agar-based susceptibility method that quantifies the proportion of resistant mutants within a population. When the percentage of resistant mutants is greater than 1% of the population, the isolate is categorized as “resistant.”
 - Ofloxacin, a second line drug, is evaluated at the critical concentration of 2.0 ug/mL.
 - Results are typically available four to five weeks following organism identification.

M. TUBERCULOSIS ISOLATE SUSCEPTIBILITY

Local Public Health Authorities

LPHAs should submit primary specimens for smear and culture as indicated above. LPHAs do not order this test.

Private Care Laboratories

Specimen Collection

- Primary specimens should be collected and processed according to internal laboratory protocols and CLSI standards.
- Isolates that have been identified by the submitting laboratory as M. tuberculosis may be submitted for susceptibility testing only.
- Submitters should retain subcultures of all submissions.
- Submit AFB culture isolates on Lowenstein-Jensen, 7H10, or other comparable solid AFB media. Liquid culture that has been positively identified as containing M. tuberculosis DNA may also be submitted.
- Failure to submit “pure” cultures may delay or adversely affect the identification process.

Specimen Shipping

- Ship samples according to current federal regulations. Guidance can be located on OSPHL’s [Shipping and Transport webpage](#).
- Specimens should be packaged for secure transport
 - Parafilm primary specimen container.

- Place primary specimen container in secondary containment (biohazard bag, 15 mL conical tube, etc.)
- Place appropriately contained specimen into a biohazard bag with absorbent material prior to shipping.

Testing and Result Reporting

○ Susceptibility testing

- Susceptibility testing is repeated at three-month intervals if a patient's additional samples are MTB culture positive. Intervals are determined based on collection dates of specimens on which susceptibility testing was previously performed.
- "Resistant" susceptibility results are reported and called to the submitter as soon as possible. Results may be accessed via Copia. All hard copy results are released within 24 hours of test completion.
- All antibiotics are tested at the critical concentration for the test method in use (e.g., MGIT or agar proportion) as defined by the Clinical Laboratory Standards Institute (CLSI).
- **MGIT PZA**
 - A liquid based susceptibility method that evaluates growth of MTB in the presence of PZA at the critical concentration of 100 ug/mL
 - Resistant results will be confirmed by repeat testing.
 - Results are typically available within two to three weeks following identification of MTB.
- **MGIT IRE**
 - A liquid based susceptibility method that evaluates growth of MTB in the presence of Isoniazid (critical concentration 0.1 ug/mL), Ethambutol (critical concentration 5.0 ug/mL), and Rifampin (critical concentration 1.0 ug/mL)
 - Resistant results will be confirmed by agar proportion at CDC.
 - Results are typically available within two to three weeks following identification of MTB.
- **Agar Proportion**
 - A solid agar-based susceptibility method that quantifies the proportion of resistant mutants within a population. When the percentage of resistant mutants is greater than 1% of the population, the isolate is categorized as "resistant."
 - Ofloxacin, a second line drug, is evaluated at the critical concentration of 2.0 ug/mL.
 - Results are typically available four to five weeks following organism identification.

GENEXPERT

Local Public Health Authorities

Specimen Collection

- Pre-approval is required for testing smear negative samples. Please contact the OSPHL for additional instructions (503) 693-4100.
- GeneXpert is performed reflexively on AFB smear/culture orders on the first positive smear from a previously undiagnosed patient with less than 72 hours of treatment for tuberculosis.
 - Testing may only be performed for patients not on anti-tuberculosis therapy (TB-Rx) and who have a presentation consistent with active pulmonary tuberculosis. Patients with less than 72 hours of TB-Rx or who have not had TB-Rx in the previous 12 months may be tested.
- All required collection and shipping materials are available from the OSPHL.
- The best specimen is the first deep sputum obtained in the morning after clearing the saliva from the mouth. Rinsing the mouth with water before collecting the specimen helps prevent contamination.
- Label the specimen with collection date and at least two unique patient identifiers (e.g., first and last name, date of birth, medical record number).
- Complete and submit a [General Microbiology Test Request Form](#).
- Securely cap tubes to avoid leakage.
- Decontaminate outside of specimen tube with appropriate disinfectant.
- Refrigerate specimens pending shipment to OSPHL, including during transit between the patient's home and the Local Public Health Authority.
- The following resources for sputum collection are provided in multiple languages:
 - Home Sputum Collection: A Step-by-Step Guide, offered by Public Health of Madison and Dane County, WI:
<https://www.youtube.com/playlist?list=PLO9RoNDnObhOcYPpeYMTG3BpPHU5-Le6H>.
 - Instructions for Collecting Sputum Fact Sheet, offered by the Oregon Tuberculosis Program:
<http://www.oregon.gov/oha/PH/DISEASES/CONDITIONS/COMMUNICABLEDISEASE/TUBERCULOSIS/Pages/factsheets.aspx>

Specimen Shipping

- Ship samples according to current federal regulations. Guidance can be located on OSPHL's [Shipping and Transport webpage](#).
- Improperly packaged or leaking specimens present a hazard and may be rejected.
- OSPHL provides complete kits for packaging and shipping of tuberculosis test specimens.
- Label the specimen with collection date and at least two unique patient identifiers (e.g., first and last name, date of birth, medical record number).

- Complete all sections of the [General Microbiology Test Request Form](#).
- Transfer specimen to 50 mL Falcon tube.
- Secure the lid to prevent leakage during transport.
- Include absorbent material in the specimen transport bag.
- Place the tube sideways into the plastic Biohazard bag.
- Expel the air from the bag.
- Close bag by zipping the top seal.
- Put the General Microbiology Test Request Form into **outer** bag pocket.
- Refrigerate specimen pending shipment to OSPHL.
- Send specimens to the OSPHL as soon as possible after collection. Specimens should be received within 24 hours of collection but no more than 5 days after collection.



Testing and Result Reporting

- **GeneXpert**
 - Determines presence or absence of MTB DNA in primary sputum samples, as well as mutations that confer resistance to Rifampin.
 - Cepheid GeneXpert® testing is FDA cleared for AFB smear positive and smear negative sputa from patients not on anti-tuberculosis therapy (TB-Rx) and who have a presentation consistent with active pulmonary tuberculosis.
 - Preliminary report issued within 2 working days of receipt.
 - Initial positive results for new patients are called and released on day of test.

Private Care Laboratories

Specimen Collection

- 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. Collect sputum according to internal laboratory protocol and CLSI guidelines.
- Process sputum using CDC NALC-NaOH decontamination methodology.

Specimen Shipping

- A minimum of 1mL 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. Friday shipments must be received by noon. Ship samples according to current federal regulations. Guidance can be located on OSPHL's [Shipping and Transport webpage](#).

Testing and Result Reporting

- GeneXpert
 - Determines presence or absence of MTB DNA in primary sputum samples, as well as mutations that confer resistance to Rifampin.
 - Cepheid GeneXpert® testing is FDA cleared for AFB smear positive and smear negative sputa from patients not on anti-tuberculosis therapy (TB-Rx) and who have a presentation consistent with active pulmonary tuberculosis.
 - Preliminary report issued within 2 working days of receipt.
 - Initial positive results for new patients are called and released on day of test.

REFERENCES

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http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm?s_cid=mm5801a3_e
4. State of Oregon Guide to NAAT testing:
<http://public.health.oregon.gov/DiseasesConditions/CommunicableDisease/Tuberculosis/Documents/tools/NAATGuide.pdf>
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APPENDIX A: AFB Smear/Culture – Additional Specimen Sources Accepted

The most common specimens submitted for this order are expectorated sputum and urine. Additional specimen sources accepted are provided below.

- Gastric lavage
 - 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 pHydrion buffer capsules or tablets.
 - Secure lid against leakage and label specimen tube with two patient identifiers, and collection date.
 - Complete and submit a General Microbiology Test Request Form for each specimen.
 - Store refrigerated pending shipment.
- Body Fluids (cerebrospinal fluid, thoracentesis, pleural fluid, synovial fluid, etc.)
 - Aseptically transfer specimen to plastic specimen tube. There is no minimum volume.
 - Secure lid and label collection tube with two patient identifiers, and collection date.
 - Complete and submit a [General Microbiology Test Request Form](#) for each specimen.
 - Store refrigerated pending shipment.
- Tissue
 - Aseptically transfer specimen, along with a minimal amount of sterile saline as needed to prevent desiccation, to sterile plastic tube.
 - Secure lid against leakage and label tube with two patient identifiers, specimen source, and date of collection.
 - Complete and submit a [General Microbiology Test Request Form](#) for each specimen.
 - Store refrigerated pending shipment.
 - Transport to OSPHL on wet ice or ice packs as soon as possible. Call the OSPHL for additional packaging and shipping instructions as needed.
- Wounds and lesion
 - Aseptically obtain purulent material with a sterile swab or by aspiration or washing.
 - Aseptically transfer specimen to a sterile tube. Add a small amount of 7H9, 7H11 or sterile isotonic saline to prevent specimen dehydration.
 - Secure lid against leakage and label specimen tube with two patient identifiers, specimen source and collection date.
 - Complete and submit a [General Microbiology Test Request Form](#) for each specimen.
 - Store refrigerated pending shipment.

APPENDIX B: Reference Labs with Testing for Nontuberculous Mycobacteria

This list is provided for reference and information only to medical providers. The Oregon State Public Health Laboratory (OSPHL) and TB Program, Oregon Health Authority do not endorse the services of reference laboratories on this list.

If a new specimen can be collected, please do so. If another specimen cannot be collected, the OSPHL may ship the specimen to the facility or provider that submitted the specimen. Payment of testing, specimen transport, and coordination of shipping specimens from OSPHL is the responsibility of the medical provider or facility, not local or state public health.

ARUP Laboratories

Client Services: 800-522-2787

Lab Test Directory: <http://www.aruplab.com/testing>

Quest Diagnostics

Customer Service: 866-697-8378

Test Center: <http://www.questdiagnostics.com/testcenter/TestCenterHome.action>

National Jewish Health Laboratories

Main Customer Service: 200-550-6227, Option 6

Mycobacteriology (TB & NTM) Customer Service: 800-550-6227, Option 3

Test Catalog: <https://www.nationaljewish.org/professionals/clinical-services/diagnostics/adx/search-adx-tests>