

National Public Health Laboratory Drug Susceptibility Testing Reference Center for *Mycobacterium tuberculosis* Specimen Shipping and Submission Instructions

California Department of Public Health Microbial Diseases Laboratory Branch

Specimen Submission Procedures

- TB DST RC should **always** be notified when an isolate or sediment is shipped. Send shipment notifications to CDPHTBDST@cdph.ca.gov.

Ordering the Test

- Submitting laboratories should complete test order forms on the LabWebPortal (LWP). If the LWP is unavailable, submitters should reach out to TB DST RC for an alternate means of submission. Detailed instructions for accessing the LWP and completing the order form can be found in the [LWP User Guide](#).
- Submitter may order phenotypic DST for First-line (RIF, INH, EMB) and/or Second-Line (injectables, ETA, RFB, MOX) drug panels. Instead of the traditional first-line panel, submitter may select alternative first-line panel (a.k.a First-line DST for 4-month RPT-MOX regimen) that includes RIF, INH, and MOX. Detailed workflow can be found [here](#).
 - PZA is not included in either first-line phenotypic DST or alternative first-line panels because phenotypic DST for PZA is performed on reflex based on whole genome sequencing results. Further details can be found [here](#).

NOTE: The method for sequencing-based DST is determined by the type of material selected in the LWP: Whole Genome Sequencing (WGS) will be performed on submitted isolates, targeted Next Generation Sequencing (tNGS) will be performed on submitted sediments, processed tissues, sterile body fluids, and mixed/nonviable cultures.

Submission of Isolates for Sequencing-Based DST (WGS) and Phenotypic DST

TB DST RC will accept pure cultures with visible biomass on LJ or MGIT broth for WGS and phenotypic DST.

- Minimum Sample Amount:
 - Culture on solid media: visible growth
 - Culture in broth: 0.5 mL; 1.0 mL preferred
 - Biomass equivalent to a 1 µl loopful will ensure timely testing
- Temperature and Stability Requirements:
 - Refrigerated to Room Temperature (approximately 2 – 25°C): up to 12 weeks from subculture date
 - Frozen isolates (0°C and below) must be subcultured prior to submission

NOTE: It is recommended that submitting laboratories keep at least a 1 mL aliquot in their laboratory and send the remainder of growth in the MGIT tube to the TB DST RC.

Submission of Specimens for Sequencing-Based DST (tNGS):

TB DST RC will accept the following specimen types for tNGS:

- Processed specimens (AFB smear positive sediments) from pulmonary and extra-pulmonary sources, including sputum, bronchial alveolar lavage, abscess, and tissues
- Sterile body fluids (abdominal fluid, CSF)
- Mixed or non-viable cultures

- Minimum Sample Amount Required:
 - Sediments: 1.0 mL
 - Culture on solid media: visible growth
 - Culture in broth: 0.5 mL; 1.0 mL is preferred

- Pre-approval via email (cdphtbdst@cdph.ca.gov) or phone call (510-412-3949) is required for:
 - AFB smear-negative specimen
 - Cepheid Xpert® MTB/RIF PCR cycle threshold (Ct) value averaged across probes above 28
 - Sterile body fluids or processed tissues
 - Processed specimen volume below 1.0 mL

- Temperature and Stability Requirements:
 - Room Temp: not acceptable
 - Refrigerated (2 – 8°C): up to 120 days
 - Frozen ($\leq -70^{\circ}\text{C}$): up to 1 year
 - Note: If culture isolation is required, submission at refrigerated temperatures is preferred.

NOTE: Sediments submitted for confirmation of an Xpert MTB/RIF result “RIF resistance detected” may be directly submitted to TB DST RC in lieu of submission to the CDC. Upon confirmation at MDL, samples will be forwarded to the CDC.

Sample Rejection Criteria:

- Samples with insufficient or conflicting labelling
- Leaking samples
- Samples with insufficient quantity
- Samples that are too old
- Samples improperly stored or transported
- Formalin fixed tissue and other specimen/sample types not meeting acceptance criteria

Labeling Specimens or Isolates:

The specimen(s) or isolate(s) must be clearly labeled with patient’s full name and the accession number. Please include the submitting laboratory name on the requisition form.

Packaging and Shipping:

Follow appropriate Federal Regulations for shipping potentially infectious substances under Division 6.2.

- All specimens or isolates should be shipped to the address below:
California Department of Public Health
Microbial Diseases Laboratory ATTN:
TB Drug Susceptibility Testing Unit
850 Marina Bay Parkway
Specimen Receiving Room B106
Richmond, CA 94804
Phone: 510-412-3929

- Shipping conditions:
 - Ship primary specimens (sediments) with cold packs, or if stored frozen, primary specimens can be shipped on dry ice. Note: If culture isolation is required, submission with cold packs is preferred.
 - Cultures may be shipped with cold packs or at ambient temperature. Do not freeze.
 - Please note that inappropriate storage temperatures in transit may affect specimen integrity which can impact the ability to obtain test results. Specimens known to be improperly stored and/or transported may be rejected.
- A print-out of the LWP Order Form should be included for each specimen or isolate that is shipped.
- Provide tracking information to the TB DST RC for the purposes of monitoring shipping times and timely identification of any potential issues.
 - This can either be done using the Lab Web Portal or via email to CDPHTBDST@cdph.ca.gov.
- All specimens should be shipped standard overnight to maximize turnaround time and ensure sample integrity.
- Ensure that culture isolates are sent in media flask or tubes containing a screw-top cap that is additionally sealed with parafilm or tape for additional protection against leaking. Specimens that leak in transit may be rejected. Do not place more than one specimen in a specimen bag to avoid cross-contamination in the event of leakage.
- Packaging and shipping costs will be the responsibility of the submitting laboratory.
- Shipping packages will be returned only with postage paid label or supplied FedEx number through arrangement with submitting laboratories. Damaged containers will not be returned.

NOTE: Please ship samples on Monday through Thursday ONLY. Do not ship specimens on Fridays. MDL is closed on Saturdays and Sundays.

Results Reporting:

The CDPH MDL will report results with patient identifiers to the submitting public health laboratory using the LWP. It is the public health laboratory's responsibility to report results to TB control or the ordering clinician in their jurisdiction. If there are issues with the LWP, results will be sent via secure fax or email.

Points of Contact:

Name		Phone/Fax Number	Function
TB Ref lab	CDPHTBDST@cdph.ca.gov	510.412.3949 (P) 510.412.3704 (F)	DST Submission and Shipping Notifications General Info/Inquiry
Matthew Sylvester	Matthew.Sylvester@cdph.ca.gov	510.412.3929 (P) 510.412.3926 (P)	Technical Information Special Requests
Varvara Kozyreva	Varvara.Kozyreva@cdph.ca.gov	510.412.3715 (P) 510.412.3929 (P)	Technical Information Special Requests (Backup)
Steven Yu	Steven.Yu@cdph.ca.gov	510.412.3949 (P)	General Information

For primary communication, please email CDPHTBDST@cdph.ca.gov or call 510-412-3949.