National Anti-Tuberculosis Drugs Resistant Survey

Standard Operating Procedure

I. Overarching Goal

Characterize the resistance profile to anti-TB drugs to inform treatment guidelines, improve treatment outcomes and help avert transmission of resistant TB genotypes.

II. Objectives

Primary Objective

To determine the prevalence of pulmonary TB patients with multi drug resistant/rifampicin resistant tuberculosis (MDR/RR-TB)

Secondary Objectives

- To determine the proportion of pulmonary TB patients with resistance to other first- and second-line anti-TB drugs, including baseline estimates for new and repurposed drugs
- To describe sociodemographic and clinical characteristics of pulmonary TB patients in Nepal
- To identify factors associated with MDR/RR-pulmonary TB
- To strengthen in-country laboratory capacity, specimen transport, and patient referral systems in view to speed up the transition towards a national system of continuous surveillance of DR-TB.

III. Participants' recruitment criteria

- All newly registered (new and previously treated) pulmonary TB patients bacteriologically confirmed by sputum smear microscopy and/or Xpert MTB/Rif, who are diagnosed and registered in the facility, regardless of their treatment enrolment status will be eligible for the survey. If the PBC cases (new and previously treated) are already enrolled in treatment, those who have received more than seven days of treatment in their current treatment course will not be eligible for the survey.
- Patients detected through any case finding approaches, including active case-finding initiatives where even if the patients' sputum samples were only referred, will also be eligible if the above-mentioned criteria are fulfilled.
- Children (under 15 years) meeting the eligible criteria will also be included in the survey.
- Special settings like diagnostic facilities related to prisons, diplomatic facilities, army/police barracks etc. requiring further administrative clearances will not be included.

IV. Sample Size

A total of **1,914** PBC cases (*1,724* new and *190* previously treated) from selected 50 cluster sites need to be enrolled. All previously treated patients will be enrolled opportunistically in each cluster over the period allocated to each stratum.

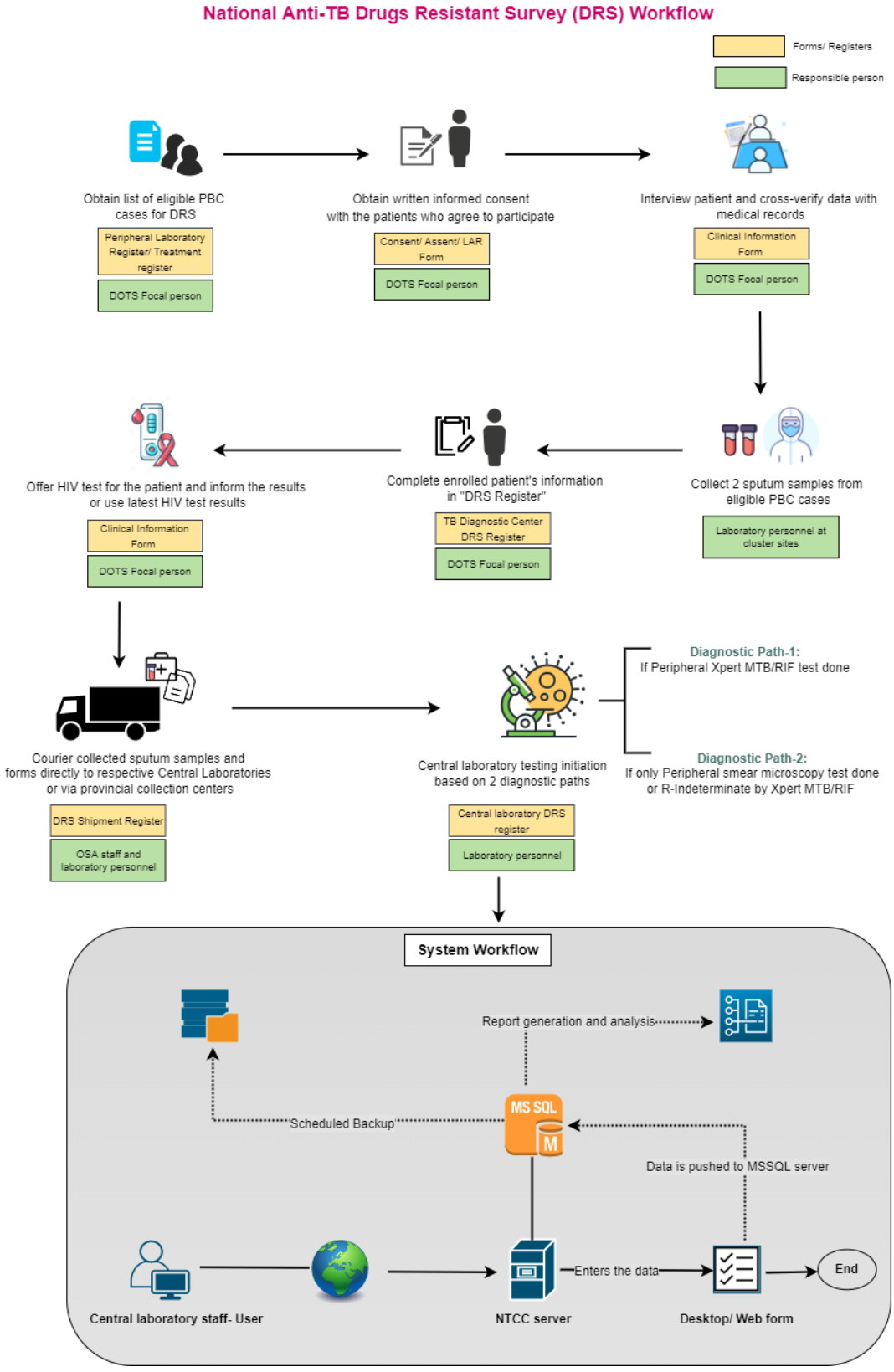
V. Study period

Study period is ~7.4 months (223 days) for data collection and cases diagnosed within this period from Stratum 1, cases diagnosed within ~5.2 months (157 days) from Stratum 2 and cases diagnosed within ~1.7 months (52 days) from Stratum 3, who meet the eligibility criteria, will be enrolled in the study.

Stratum	Intake period	Mo 1	Mo 2	Mo 3	Mo 4	Mo 5	Mo 6	Mo 7	Mo 8
2020 caseload: 6 to 30	223 days *(~7.4 months)*								
2020 caseload: 31 to 250	157 days *(~5.2 months)*								
2020 caseload: > 250	52 days *(~1.7 months)*								

Figure 1. Intake period for each stratum

VI. Study implementation



Step 1:

Obtain list of newly registered patients diagnosed at selected clusters who meet the eligibility criteria and arrange for the interview with eligible patients.

Tools: Peripheral Laboratory registers

Responsible person: DOTS focal person in coordination with laboratory personnel

Step 2:

Obtain written informed consent with the patients who agree to participate in the survey after explaining ethical aspects of the study, including rights to withdraw participation from the survey any time after even providing informed consent initially.

Tools: Informed consent/ Assent / LAR forms (Form no.-1.2.)

Responsible person: DOTS focal person

Step 3:

Two sputum samples (≥3ml and ≤5ml volume each) following bacteriological confirmation of pulmonary TB from peripheral diagnostic sites, will then be taken following routine standard procedures to be tested at central laboratories. Each specimen will be collected into a 50ml centrifugation tube, previously identified with a unique barcoded DRS ID, comprising a unique patient ID plus sample ID (e.g., *KAP/438/01*). One spot and one morning sputum samples are required from each patient. But in case the patient might not be able to return next day with morning sample, two spot samples taken ≥1 hour apart could be taken.

Responsible person: Laboratory personnel

Step 4:

Patient will be interviewed by health workers and patients' medical records will be cross-checked to verify related information provided during the interview.

Tools: Clinical Information form (Form no.-1.3.)

Responsible Person: DOTS focal person

Step 5:

Once the patient is enrolled in the survey, the assigned barcode label with the unique DRS ID will be placed in the "TB Diagnostic Centre DRS Register" and all the necessary details will be filled in the register.

Tools: TB Diagnostic Centre DRS Register (Form no.-1.1.)

Responsible person: DOTS focal person

Step 6:

All patients enrolled in the survey will be offered an HIV test. The latest HIV test result may be used if testing of the patient at the time of enrolment is not feasible (i.e., unforeseen stock out), provided that the latest HIV test result is ≤6 months from the time of enrolment. HIV, sputum smear microscopy and/or Xpert MTB/RIF test results, will be noted in the "Clinical Information Form" (Form no.-RT.1.B)

Tools: Clinical Information form (Form no.-1.3.)

Responsible Person: DOTS focal person

Step 7:

The responsible OSA staff, in coordination with Laboratory personnel and DOTS focal person, will arrange shipment of DRS specimens and forms to the respective central laboratory as soon as possible, and will complete a "DRS Shipment Register" (Form no.-RT.3) for the record in the process.

If the immediate shipment is not possible, the sample will be stored at 2 to 8°C refrigerators. Completing the "DRS Shipment Register" will comprise noting the DRS ID for patients from whom samples are included in the shipment, by attaching a pre-assigned DRS ID barcoded label in the form.

Tools: DRS Shipment Register (Form no.-2.1.)

Responsible persons: OSA staff and laboratory personnel

Step 8:

Each central laboratory will receive two sputum samples from each patient enrolled at respective clusters, along with required forms. Among these, the Clinical Information Form (Form no.-RT.1.B) will contain the initial (peripheral) Xpert MTB/Rif or smear microscopy examination result, based on which, study patients will be stratified into two different diagnostic paths as in *Figure 2*.

If Xpert MTB/RIF was already conducted at peripheral diagnostic sites as informed in CIF, the test will not be repeated at the assigned central laboratory and processing of DRS specimens will follow the "Diagnostic Path 1". When only smear microscopy results are reported from the study sites or when the Xpert MTB/RIF result is available from the peripheral TB diagnostic site, but rifampicin resistance is indeterminate, Xpert MTB/RIF will be performed by the central laboratory and processing of samples will follow the "Diagnostic Path 2".

Diagnosis Path 1	**Diagnosis Path 2**
NALC-NaOH decontamination and centrifugation, after which, the sediment will be reconstituted with 1.0 ml of phosphate-buffer.	
Two LJ slant per sample will be inoculated with 200 µl of reconstituted sediment and one loop full reconstituted sediment will be streaked on slides, fixed, and stained for smear microscopy.	
Inoculation in Solid media shall take place immediately on arrival and latest 72 hours after sample collection (i.e., ≤3 days).	

	500µl of sediment from one sputum sample will be applied to Xpert MTB/RIF according to path 2 as soon as possible.
The remaining each sediment will be transferred into a screw cap 1.5 ml vial, labelled and frozen at -80° C for storage.	
After the Xpert MTB/Rif, pathways 1 and 2 remain identical and it will be processed for LJ culture on double batch.	
The positive culture from all samples, will be tested by MPT 64 for identification of MTBC.	
If MPT 64 is negative, the sample will not be further processed.	
If culture is contaminated, the stored sample is reprocessed on LJ media.	
If MTBC is detected, FL LPA (rifampicin (Rif) and isoniazid (Inh)) and pDST on MGIT (Rif, Inh, plus pDST of levofloxacin (Lfx)) will be performed.	
If rifampicin resistance is detected by any test (i.e., either Xpert MTB/RIF (at the peripheral or the central laboratory), LPA and/or pDST), the positive culture will further be used for DST of the second-line (SL) drugs moxifloxacin (Mfx) (in the concentrations 0.25 and 1.0 mg/L), amikacin (Amk), bedaquiline (Bdq), clofazimine (Cfz), delaminid (Dlm), and linezolid (Lzd).	
A second-line LPA (Genotype MTBDRsl, HAIN-Bruker) will be performed from the Rif-resistant isolate to test for genotypic resistance to fluoroquinolones (FQs) and second line injectables (2Lis).	
Mycobacteria isolates, whether MTBC or NTM, from all positive cultures (i.e., at least two isolates per patient) will be stored frozen in Trypton soya broth at -80°C in the deep-freezer.	
These frozen isolates will be used for later re-testing in case of discrepancies.	
Stored sediments and isolates will also be kept as a back-up in case further tests are needed at the end of the study period.	

Results of the tests will be filled accordingly in Central Laboratory DRS Register (Form no.-RT.4) and will be forwarded for data entry.

Tools: Central Laboratory DRS Register (Form no.-3.1.)

Responsible person: Central Laboratory's focal person for DRS

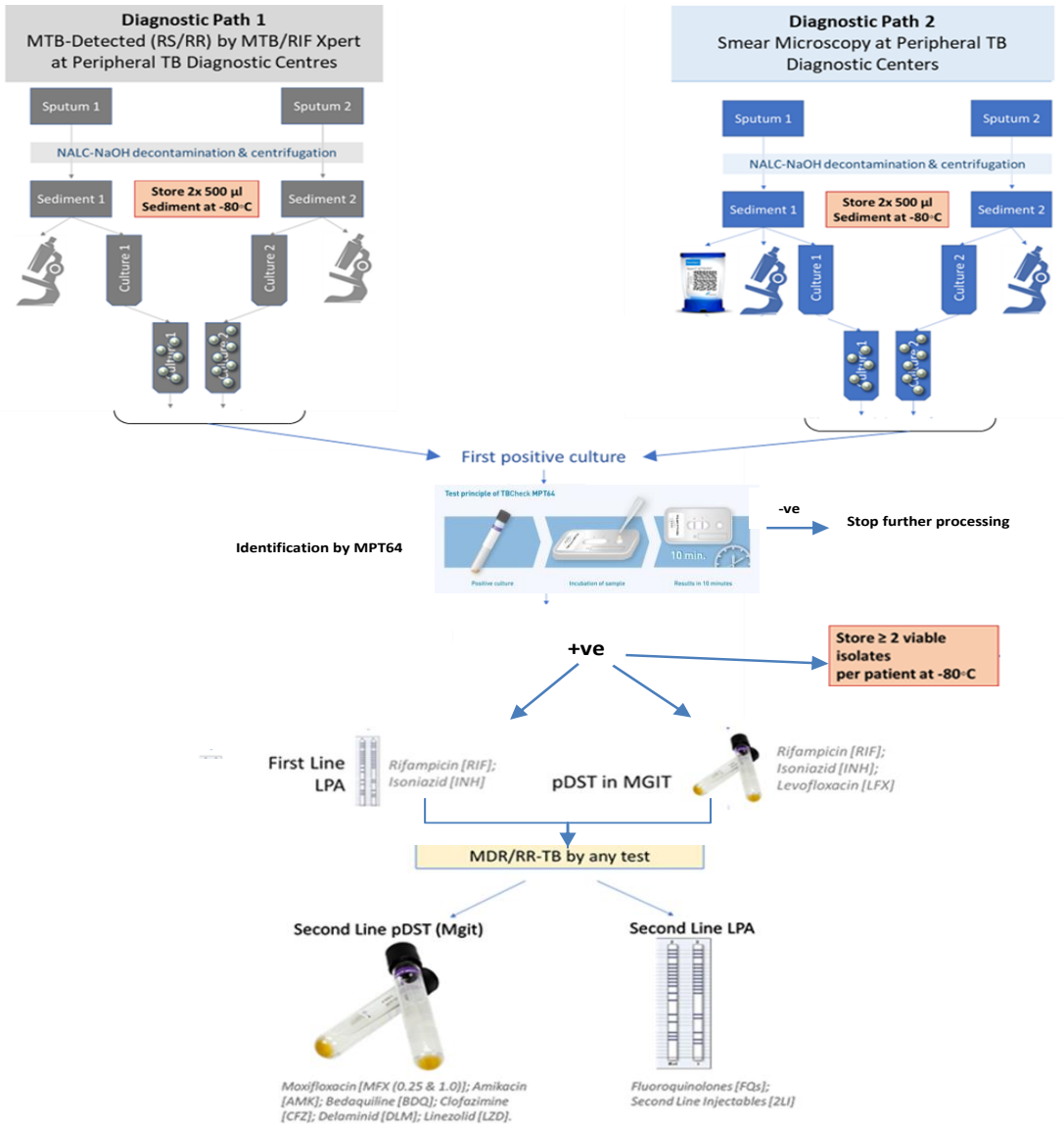


Figure 2. Diagnostic Pathways of National Anti-TB Drugs Resistant Survey 2021

Notes:

- Sample shipment should not be held up by the HIV test.
- DRS data entry clerks in the central operational team can follow-up on missing test results by phone, once the "Clinical Information Form" has been received centrally.
- All patients eligible for bacteriological diagnosis of pulmonary TB, including those directly referred off-site for Xpert MTB/RIF, must be recorded in the TB laboratory register of the cluster. The TB laboratory register will hence contain the master list of patients that are being considered for enrolment, subject to test results and bacteriological confirmation of TB, and will be pivotal to monitor potential under-enrolment and non-consecutive enrolment at each cluster.