Common Protocol Phase I

Cohort A: Participants with Active Pulmonary TB

Provisional Inclusion Criteria

1. Presents with one of the following:
   a. Presumed DS TB case: Signs or symptoms consistent with active pulmonary TB that include, but are not limited to: persistent cough, hemoptysis, fever, unintended weight loss or failure to thrive (children), fatigue or lethargy, night sweats, or pleuritic chest pain (see the MOP for definitions); or
   b. Presumed MDR/XDR TB: Individuals already diagnosed with pulmonary TB, but not responding to treatment with first-line standard anti-TB drugs, e.g., persistent symptoms or persistently positive microbiology results, and are thus suspected to have MDR or XDR TB.
2. Has documentation of one of the following:
   a. CXR findings consistent with TB;
   b. Sputum smear-positive by microscopy; or
   c. Mtb detection by rapid diagnostic test, such as GeneXpert.
3. Has documentation of, or willingness to be tested for HIV infection. Adults (≥18 years of age) and children born to an HIV-positive mother must provide documentation of HIV status (confirmed positive test any time in the past or last negative test within 90 days prior to the Screening Visit) or be willing to be tested as part of the study.

Exclusion Criteria

To be considered eligible for enrollment, an individual must not meet any of the following criteria:

1. Plans to move from his/her current residence, which would interfere with the participant's ability to complete all study visits (through the 6-Month Post-Treatment Visit).
2. Has an active psychiatric condition, or alcohol or drug dependence that, in the opinion of the site investigator or designee, might interfere with the ability to give true informed consent and to adhere to the study requirements.
3. Is currently imprisoned.
4. Additional Exclusion Criteria for Presumed DS TB:
   a. Received >1 week (daily or intermittent doses) of any drugs with anti-TB activity within 30 days prior to provisional enrollment, including:
      i. First line anti-TB drugs in any combination: isoniazid (INH), rifampicin, pyrazinamide, ethambutol, and streptomycin;
      ii. Fluoroquinolones: e.g., ofloxacin, ciprofloxin, levofloxacin, moxifloxacin, nalidixic acid, sparfloxacin, and gatifloxacin;
      iii. Injectable second line anti-TB drugs: e.g., kanamycin, amikacin, and capreomycin;
      iv. Oral second line anti-TB drugs: e.g., p-aminosalicylic acid, cycloserine, terizidone, ethionamide, prothionamide, thiocetazone; or
      v. Other bacteriostatic second-line anti-TB drugs: e.g., clofazamine, linezolid amoxicillin/clavulanate, imipenem/cilastin, meropenam, clarithromycin, bedaquiline, and delamanid.
5. Additional Exclusion Criteria for Presumed MDR TB:

a. Received >1 week (daily or intermittent doses) of drugs with anti-TB activity, other than first-line anti-TB drugs, within 30 days prior to provisional enrollment. These include:
   i. Fluoroquinolones: e.g., ofloxacin, ciprofloxin, levofloxacin, moxifloxacin, nalidixic acid, sparfloxacin, and gatifloxacin;
   ii. Injectable second line anti-TB drugs: e.g., kanamycin, amikacin, and capreomycin;
   iii. Oral second line anti-TB drugs: e.g., p-aminosalicylic acid, cycloserine, terizidone, ethionamide, prothionamide, thiocetazone; or
   iv. Other bacteriostatic second-line anti-TB drugs: e.g., clofazamine, linezolid amoxicillin/clavulanate, imipenem/cilastin, meropenam, clarithromycin, bedaquiline, and delamanid.

6. Additional Exclusion Criteria for Presumed Pre-XDR and XDR TB:
   a. Received >1 week (daily or intermittent doses) of current anti-Pre-XDR or XDR TB regimen within 30 days prior to provisional enrollment.

Confirmatory Inclusion Criteria

Final confirmation must be documented within 6 months after provisional enrollment into the study. Provisionally enrolled participants must meet disease confirmation criteria to remain study-eligible, as defined below:

1. Culture-confirmed pulmonary TB (regardless of age or initial smear results):
   a. Mtb identified by culture of expectorated or induced sputum (a positive culture of NP aspirates or GAs may also be accepted in lieu of sputum culture for children ≤14 years of age).
   b. Mtb identified by culture results from respiratory secretions obtained by bronchoalveolar lavage or bronchial wash may not be used to determine study eligibility.
   c. Mtb identified from either liquid or solid culture is acceptable and may be from either clinical or study-related specimens.
   d. Those who have extra-pulmonary manifestations of TB in addition to pulmonary TB may also be enrolled.
2. Clinically-documented pulmonary TB is allowed for children (≤14 years of age)[7, 8] who are culture-negative or culture-unknown and meet the following criteria[2]:
   a. Signs or symptoms consistent with TB;
   b. Culture-negative after two attempts at sputum collection using expectorated or induced sputum, NP aspirates, or GAs (or has documented reason for lack of specimen for culture);
   c. A CXR that is consistent with intrathoracic disease due to TB; and
   d. There is at least one of the following:
      i. A positive clinical response to standard multidrug anti-TB therapy;
      ii. Documented exposure to a case of active TB (active TB case has documented or verbal report of smear-positive, culture-positive, or TB treatment); or
      iii. Immunological evidence of Mtb infection (e.g., reactive tuberculin skin test [TST] or positive Interferon-Gamma Release Assay [IGRA]).
3. Additional Confirmatory Inclusion Criteria for MDR/XDR TB:

a. DST or other appropriate molecular testing (see Laboratory Manual) to confirm MDR/XDR status*

*MDR/XDR suspected participants who are not confirmed to have MDR or XDR TB may remain on study as long as all criteria for DS participants were met, otherwise, they will be discontinued from the study.

Participants who fail to meet the confirmatory inclusion criteria noted above will be discontinued from the study. However, specimens that were previously collected from the participant may be retained for use as control specimens.

Table 1.Schedule of Events for Cohort A – Active Pulmonary TB

			TREATMENT PHASE				
Activities / **Visit**	**SCREENING**	**BASELINE**	**VISIT 1**	**VISIT 2**	**END OF TX (-4 wks/+6 wks**	**6-M POST-TX (-4 weeks/+6 wks)[h]**	**TX F/R/W**
Informed consent	X						
Eligibility assessment	X	X					
Eligibility confirmation[f]			**X**	**X**	**X**		**X**
Demographic, medical history, and clinical data		X					
Participant status[i]			X	X	X	X	X
CXR[a]		X		X[a]	X[a]		X[a]
HIV test[b]		X					X
CD4 count if HIV-infected[b]		X					X
CBC and lymphocyte count		X					
HbA1c		X					
Sputum smear & culture[c, d]		X	X	X	X		X
Sputum DST[c, d]		X					X
Mtb isolate subculture for storage		X					X
Whole blood (PAXgene) for storage		X[e]	X	X	X		X
Whole blood (PBMC **and plasma**) for storage		X[e]	X	X	X		X
Whole blood for storage (genetic analyses)		X[e]		**X**	**X**		**X**
Saliva for storage[g] (genetic analyses)		X			X		X
Urine for storage		X	X	X	X		X
Sputum for storage[d]		X	X	X			X

[a] CXR at baseline, unless done within 4 weeks prior to the Baseline Visit as part of standard of care or through the Parent Protocol. Data from Visit 2, End of Treatment, and TX F/R/W Visits will be collected if CXRs were conducted as part of standard of care or as part of the Parent Protocol. Pregnant women are not required to have a CXR.

[b] HIV testing to be performed per national guidelines. HIV testing not required if there is documentation of a confirmed positive test at any time in the past or the last negative HIV test was obtained ≤90 days prior to the study visit; or the participant is a child <18 years of age who was not born to an HIV positive mother; abstract the data from the participant's medical chart or research record; CD4 count will only be performed on participants who are HIV-positive and who have not had a CD4 count performed in the preceding 6 months.

[c] For children whose diagnosis was made on the basis of an NP aspirate, GA, or clinical criteria, subsequent NP/GA inductions will not be required.

[d] Children diagnosed clinically and who are unable to expectorate will not be expected to provide these specimens.

[e] If blood volume in combination with other clinical or protocol blood collection requirements exceeds the allowable volume by the ICMR or IRB/IEC guidelines, the participant will be requested to return at the earliest possible time point to collect the baseline specimen, as long as the minimum specimen collection criterion is met before more than 1 week of anti-TB therapy was received (see protocol section 6).

[f] Eligibility confirmation, when possible, if not done previously.

[g] CRUs may choose not to collect saliva specimens for participants <5 years of age.

[h] If TB relapse is suspected at the 6-Month Post-Treatment Visit, complete evaluations required for the TX F/R/W Visit.

Outcome Measures for Cohort A

Data for Cohort A will be collected to support several key outcome measures as defined below:

1. Bacteriologic Outcomes for Participants who Initially have Bacteriologically-Confirmed, DS TB[5]
    a. DS bacteriologic cure: Completion of standard, first-line multidrug TB therapy and the participant has documentation of a negative culture result in the last month of treatment and on at least one previous occasion.
    b. DS treatment completed/bacteriologic status indeterminate: Completion of standard, first-line multidrug TB therapy without evidence of bacteriologic failure but without documentation of a negative culture result in the last month of treatment and on at least one previous occasion, either because tests were not done or because results are unavailable.
    c. DS bacteriologic failure: A participant whose sputum culture is positive at Month 5 or later during treatment and the culture has not been determined to be a falsepositive culture.
    d. DS bacteriologic relapse***:** Participant was declared *cured* or *treatment completed/bacteriologic status indeterminate* at the end of his/her most recent course of treatment, and is then diagnosed with a recurrent episode of TB confirmed by a clinical specimen collected from any anatomical site during the follow-up phase that is culture-positive for Mtb, when the culture has not been determined to be a false-positive culture.

*The term “relapse” is used per the World Health Organization (WHO) definition. It is recognized that without genotype results, a status between relapse and **reinfection** cannot be discriminated.

e. DS emerging resistance**:** A participant who has a change in baseline drug sensitivity before DS bacteriologic failure can be determined (i.e., after the Baseline Visit, but before Month 5 of treatment).

2. Bacteriologic Outcomes for Participants who Initially have Bacteriologically-Confirmed, MDR or XDR TB

a. DR bacteriologic cure: Completion of MDR or XDR TB treatment as recommended by National TB policy and 3 or more negative consecutive cultures taken at least 30 days apart after the intensive phase and no evidence of bacteriologic or clinical failure between the last negative culture and end of treatment.

b. DR treatment completed/bacteriologic status indeterminate: Completion of MDR or XDR TB treatment, as recommended by National TB policy, without documentation of 3 or more negative consecutive cultures as defined under bacteriologic cure, but no evidence of bacteriologic or clinical failure.

c. DR bacteriologic failure: Treatment terminated or need for permanent regimen change of at least two anti-TB drugs because of:

i. Lack of culture conversion to negative status by the end of the intensive phase; or

ii. Culture reversion to positive status in the continuation phase after conversion to negative during the intensive phase; or

iii. Evidence of additional acquired drug-resistance to fluoroquinolones or secondline injectable drugs.

d. DR bacteriologic relapse*: Participants who were declared *cured* or *treatment completed* at the end of their most recent course of MDR or XDR treatment, and are then diagnosed with a recurrent episode of TB confirmed by a clinical specimen collected from any anatomical site during the follow-up phase that is culturepositive for Mtb, when the culture has not been determined to be a false-positive culture.
*The term “relapse” is used per the WHO definition. It is recognized that without genotype results, a status between relapse and reinfection cannot be discriminated.

e. DR emerging resistance: A participant who has a change in baseline drug sensitivity before DR bacteriologic failure can be determined (i.e., after the Baseline Visit, but before the end of the intensive phase of treatment).

3. **Clinical Outcomes**

Note: Participants who achieve a bacteriologic outcome will not be assigned a clinical outcome.

Participants ≤14 years of age who did not have culture results or were culture-negative at the beginning will be assigned a clinical outcome. In addition, some adults or children with initial bacteriologically-confirmed TB may be assigned a clinical outcome (i.e., not bacteriologically-confirmed), as defined below. These outcomes are based on signs and symptoms of TB without accompanying bacteriologic confirmation.

a. **Clinical response:** Completion of TB treatment as recommended by National TB policy in a child participant ≤14 years of age who has resolution, by the end of therapy,

of signs and symptoms attributable to TB. Participants older than 14 years of age cannot be assigned a clinical response outcome.

b. **Clinical failure:** A child with clinically documented TB or any participant with bacteriologically-confirmed TB may meet the definition for clinical (i.e., not cultureconfirmed) failure if he/she completes 4 full months of anti-TB treatment but remains ill with persistence, progression, or recurrence of signs or symptoms of TB that are determined to be due to TB and not due to another underlying cause.

c. **Clinical relapse*:** A child with clinically documented TB or any participant with bacteriologically-confirmed TB may meet the definition for clinical relapse (i.e., not culture-confirmed) if he/she meets the definition for clinical response or bacteriologic cure or treatment completed/bacteriologic status indeterminate, and is subsequently diagnosed with a recurrent episode of TB that is not confirmed by culture.

*The term "relapse" is used per the WHO definition. It is recognized that without genotype results, a status between relapse and **reinfection** cannot be discriminated.

4. **Other Outcome Status Criteria**

These definitions apply to either DS or DR TB:

a. **Death:** A participant who dies for any reason after consenting to participate and prior to the end of study.

b. **Treatment incomplete:** A participant who drops out of treatment (defined as treatment interruption for 2 or more consecutive months), but continues in study follow-up.

c. **Lost to follow-up/unknown:** A participant who no longer participates in study visit follow-up or an outcome status cannot be determined.

5. **Completion of Therapy Status**

These definitions apply to either DS or DR TB:

a. **Completion of adequate therapy:** Considered accomplished when the participant is not a treatment failure, does not meet criteria for incomplete treatment, and has received at least 90% of the recommended number of doses of RNTCP-specified multidrug anti-TB therapy within 1 year of treatment initiation for DS participants and 2 years for MDR/XDR participants.

b. **Incomplete:** Treatment is considered incomplete if the participant has defaulted from treatment, defined as treatment interruption for 2 or more consecutive months.

Cohort B - Household Contacts of Pulmonary TB

Inclusion Criteria

To be considered eligible for enrollment, an individual must meet the following criteria:

1. Is an adult or child with significant recent exposure (within the past 6 months) to an adult with untreated or inadequately treated pulmonary TB (see the MOP for detailed definitions).
2. Has no clinical signs or symptoms of active TB that include, but are not limited to: persistent cough, hemoptysis, fever, unintended weight loss or failure to thrive (children),

fatigue or lethargy, night sweats, pleuritic chest pain, draining lymph node, or other evidence of extra-pulmonary TB. If clinical signs or symptoms of TB are present, CXR and/or sputum culture results must be included in the overall evaluation to rule out active TB.

Exclusion Criteria

To be considered eligible for enrollment, an individual must not meet any of the following criteria:

1. Plans to move from his/her current residence, which would interfere with the participant's ability to complete all study visits (through the Month 24 Visit).
2. Has an active psychiatric condition, or alcohol or drug dependence that, in the opinion of the site investigator or designee, might interfere with the ability to give true informed consent and to adhere to the study requirements.

4 Outcome Measures for Cohort B

Case Definitions for Outcome Measures

Active TB is the outcome measure of interest in this cohort. All participants must be assigned only one outcome, as defined below:

1. **No TB:** Participant had no indication of active TB (pulmonary or extra-pulmonary) over the 24-month follow-up period.
2. **Definite case:** Culture-confirmed or GeneXpert-confirmed Mtb from any anatomical site over the 24-month follow-up period.
3. **Probable case (adult or child of any age):**
   a. Signs or symptoms consistent with active TB that include persistent cough, hemoptysis, fever, unintended weight loss or failure to thrive (children), fatigue or lethargy, night sweats, pleuritic chest pain, draining lymph node, or other evidence of extra-pulmonary TB (see the MOP for definitions); and
   b. Acid-fast bacilli (AFB) seen on microscopic examination of sputum or biopsy specimen, but without culture confirmation.
4. **Probable case (child ≤14 years of age[2]):**
   a. Signs or symptoms consistent with active TB that include persistent cough, hemoptysis, fever, unintended weight loss or failure to thrive (children), fatigue or lethargy, night sweats, pleuritic chest pain, draining lymph node, or other evidence of extra-pulmonary TB (see the MOP for definitions); and
   b. AFB smear and culture-negative, not done, or results unknown; and
   c. A CXR that is consistent with intrathoracic disease due to TB or radiographic or other evidence of extra-pulmonary TB; and
   d. There is at least one of the following:
   i. A positive clinical response to standard multidrug anti-TB treatment; ii. Documented exposure to a case of active TB; or iii. Immunological evidence of Mtb infection (e.g., reactive TST or positive IGRA).
5. **Possible case (adult or child of any age[2]):**
   a. Signs or symptoms consistent with active TB that include persistent cough, hemoptysis, fever, unintended weight loss or failure to thrive (children), fatigue or lethargy, night sweats, pleuritic chest pain, draining lymph node, or other evidence of extra-pulmonary TB (see the MOP for definitions); and

b. AFB smear and culture-negative, not done, or results unknown; and

c. There is at least one of the following:

i. A CXR that is consistent with intrathoracic disease due to TB or radiographic or other evidence of extra-pulmonary TB; ii. A positive clinical response to standard multidrug anti-TB treatment; iii. Documented exposure to a case of active TB; or iv. Immunological evidence of Mtb infection (e.g., reactive TST or positive IGRA).

6. **Other Outcome Status Criteria**

a. **Death:** A participant who dies for any reason after consenting to participate and prior to the end of study.

Lost to follow-up/unknown: A participant who no longer participates in study visit follow-up or an outcome status cannot be determined

Table 2.Schedule of Events for Cohort B – Household Contacts

Activities / Visit	SCREENING	BASELINE	MONTH 4-6[e]	MONTH 12[e] (+/-2 months)	MONTH 24[e] (+/-2 months) and PREM DC	TB ACTIVATION EVALUATION
Informed consent	X					
Eligibility assessment	X	X				
Demographic, medical history, and clinical data		X				
IGRA or TST		**X**	**X**[j]	**X**[k]		
Participant status[f]			X[e]	X[e]	X[e]	X
Smear and culture from TB activation site[a]						X
Mtb isolate subculture for storage						X
Sputum DST[a]						X
Sputum for storage						X
Whole blood (PAXgene) for storage		**X**[i]	**X**[l]	**X**[l]		X
Whole blood (PBMC, **plasma**) for storage		**X**[i]	**X**[l]	**X**[l]		X
Whole blood (IGRA) for storage		X[i]	X[l]	X[l]		X
Whole blood (genetic analyses) for storage		**X**[i]	**X**[l]	**X**[l]		X
Saliva (genetic analyses)[g] for storage		X[i]	**X**[l]	**X**[l]		X
Urine for storage		X[i]	**X**[l]	**X**[l]		X
CXR[b]						X
HIV test if status is unknown[c]						X
CD4 count if HIV-infected[d]						X
CBC and lymphocyte count[h]						X
HbA1c[h]						X

[a] Smear and culture to determine participant's bacteriologic status. Speciation and drug sensitivity to be performed if TB is suspected. All those determined to have active TB will have all specimens saved in the biorepository and will be requested to roll over to Cohort A.

[b] CXR to rule out active TB unless one was taken as a part of standard of care or the Parent Protocol; pregnant women are not required to have a CXR.

[c] HIV testing to be performed per national guidelines. HIV testing not required if there is documentation of a confirmed positive test at any time in the past or the last negative HIV test

was obtained ≤90 days prior to the study visit; or the participant is a child <18 years of age who was not born to an HIV-positive mother; abstract the data from the participant's medical chart or research record.

[d] CD4 count will only be performed on participants who are HIV-positive and who have not had a CD4 count performed in the preceding 6 months as a part of standard of care or the Parent Protocol.

[e] Visits may take place via phone contact, unless a repeat TST or IGRA is needed or TB disease is suspected.

[f] Clinical assessment, as needed.

[g] CRUs may choose not to collect saliva specimens for participants <5 years of age.

[h] CBC, lymphocyte count, and HbA1c are not required if collected within 4 weeks prior to the TB Activation Evaluation Visit as part of standard of care or as part of the Parent Protocol.

[i] If blood volume in combination with other clinical or protocol blood collection requirements exceeds the allowable volume by the ICMR or IRB/IEC guidelines, request the participant to return at the earliest possible time point to collect the baseline specimen. j Month 4-6 Visit: Repeat TST or IGRA to be performed if baseline test was negative and a repeat test was not performed as part of standard of care or the Parent Protocol between 6 weeks after the Baseline Visit and before the Month 4-6 Visit. If the Parent Protocol conducts the repeat TST or IGRA test at Month 12 and not at the Month 4-6 Follow-Up Visit, the Parent Protocol schedule may be followed. k Month 12: Repeat TST or IGRA to be performed if baseline test was negative and a repeat test was not performed before the Month 12 Visit. l Months 4-6 and 12: Only collect biorepository specimens if a repeat TST or IGRA is being conducted for participants who had a negative baseline result or if the repeat test was conducted prior to the visit and Central Biorepository specimens were not collected for storage.

Table 3. Central Biorepository Study Specimen Collection and Storage Chart (Adults and Children)

Specimen Type[b]	**Adults and Children (≥5 years of age)**	**Children (<5 years of age)[a]**
Whole blood (PAXgene RNA)	2.5 mL	2.5 mL
Whole blood (IGRA)-Cohort B only	4 mL (1 mL/tube)	4 mL (1 mL/tube)
Whole blood (genetic analyses)	**3-6** mL (BD EDTA)	**3-6** mL (BD EDTA)
PBMC	**8-**10 mL (BD Heparin)	**4-**6 mL (BD Heparin)
Plasma	Harvested from BD Heparin (PBMC) tubes above	Harvested from BD Heparin (PBMC) tubes above

Saliva (genetic analyses)		6 mL	6 mL CRUs may choose not to collect saliva
Urine		Spot urine (10 mL)	Spot urine (10 mL)
Sputum		Whatever volume is possible to collect	Whatever volume is possible to collect
Extracted host RNA		Prepared from PAXgene tube	Prepared from PAXgene tube
Mtb isolate	**Cohort A**	Subculture of original Mtb isolate, and relapse or failure isolate	Subculture of original Mtb isolate, and relapse or failure isolate
	Cohort B	Subculture of confirmatory Mtb isolate from each participant who develops active TB	Subculture of confirmatory Mtb isolate from each participant who develops active TB

Common Protocol Phase II

Dx Cohort

Inclusion Criteria

1. Presents with one of the following:
   a) Presumed Pulmonary TB (PTB) case: Signs or symptoms consistent with active pulmonary TB that include, but are not limited to: persistent cough, hemoptysis, fever, unintended weight loss or failure to thrive (children), fatigue or lethargy, night sweats, or chest pain. CXR findings consistent with TB.
   b) Presumed Extrapulmonary (EPTB) case (Lymph node, pleural, and TBM only): The symptoms are depending on the organ affected. Most common symptoms are swelling and/or discharging sinus in neck, axilla or groin; headache, vomiting, fever, weight loss or loss of appetite; pleuritic chest pain, breathlessness, or non-productive cough.
2. Has documentation of, or willingness to be tested for HIV infection. Adults (≥18 years of age) and children born to an HIV-infected mother must provide documentation of HIV status (confirmed positive test any time in the past or last negative test within 90 days prior to the Screening Visit) or be willing to be tested as part of the study.
3. Has provided written consent or witnessed oral consent in the case of illiteracy, prior to his/her first sample or other study-specific data being collected, or parents/legal guardians have provided consent for all minors and children have provided assent, as dictated by the CRU's IRB/IEC and country-specific regulations.
4. Agrees to the collection and storage of blood, urine, saliva, sputum, and stool specimens for use for future research. The participant may decline collection of specimens for human genetic research and still be eligible for the study.

Exclusion Criteria

To be considered eligible for enrollment, an individual must not meet any of the following criteria:

1. Confirmed PTB or EPTB case at the time of enrollment.
2. Plans to move from his/her current residence, which would interfere with the participant's ability to complete all study visits.
3. Has an active psychiatric condition, or alcohol or drug dependence that, in the opinion of the site investigator or designee, might interfere with the ability to give true informed consent and to adhere to the study requirements.
4. Is currently imprisoned.
5. Received >3 days of any drugs with anti-TB activity within 30 days prior to enrollment, including:
   i. First line anti-TB drugs in any combination: isoniazid (INH), rifampicin, pyrazinamide, ethambutol, and streptomycin;
   ii. Fluoroquinolones: e.g., ofloxacin, ciprofloxin, levofloxacin, moxifloxacin, nalidixic acid, sparfloxacin, and gatifloxacin;
   iii. Injectable second line anti-TB drugs: e.g., kanamycin, amikacin, and capreomycin;
   iv. Oral second line anti-TB drugs: e.g., p-aminosalicylic acid, cycloserine, terizidone, ethionamide, prothionamide, thiocetazone; or
   v. Other bacteriostatic second-line anti-TB drugs: e.g., clofazamine, linezolid amoxicillin/clavulanate, imipenem/cilastin, meropenam, clarithromycin, bedaquiline, and delamanid.

6. Any prior episode of TB disease in the past two years, regardless of disease site or receipt of treatment.
7. Unable to provide any specimen (Sputum/ induced sputum/ nasopharyngeal aspirate/ gastric lavage/ EPTB specimen) for TB diagnosis

Selection of participants for follow-up

Based on results of mycobacteriology testing performed at enrolment, certain participants will have a Month 2 follow-up visit. The following operational definitions will be used to classify participants **based on enrolment test results**:

1. Discordant participant: any enrollment with Ultra test result of "MTB detected" AND no MGIT culture with Mtb growth at week 6 after inoculation AND no LJ culture positive for growth consistent with Mtb at week 6 after inoculation (up to week 10 in negative cultures). Smear and Xpert MTB/RIF results are not components of the definition.
2. Eligible for Negative Control Group: any enrollment with Xpert Ultra tests with result of "MTB not detected" AND two LJ and two MGIT cultures negative for growth (not contaminated) at week 6 (up to week 10) after inoculation. Target total number of negative controls will be two times the number of discordants that are included in the follow-up study.
3. Eligible for Positive Control Group: individuals with an enrollment Ultra test result of "MTB detected" AND any MGIT or LJ culture positive for Mtb within 6 weeks after inoculation. Target total number of positive controls will be the same as number of discordants included in the study.

Outcome Measures

Data for the Dx Cohort will be collected to support several key outcome measures as defined below:

Discordant TB: A study participant with a positive Xpert Ultra test (MTB detected) and all negative cultures (no MTB growth) at the end of month 2 visit/ last study visit irrespective of their other micro results including smear status or TB histopathology.

Confirmed TB: A study participant with a confirmed culture (positive for M TB) irrespective of any other smear, histopathology or molecular test results anytime in the study.

Clinical TB: A study participant with all negative AFB smears or negative TB histopathology, negative cultures (no MTB growth) and negative molecular tests (Xpert Ultra or Xpert MTB/RIF) but started on anti-TB treatment (ATT) based on clinical decision.

TB status unknown: A study participant with a, negative or inconclusive Xpert Ultra test (test indeterminate) or test not done and at least 50% of available cultures contaminated with other cultures negative/ not tested at the end of the last study visit and not started on ATT or status of ATT unknown.

Not Tuberculosis: All smears, cultures and molecular tests tested negative in the study including any other results available from standard of care tests done outside the study and not started on ATT.

Table 03a: Schedule of Events for Dx Cohort

Activities / Visit	Baseline – Day 1	Baseline– Day 2	Month 2 (discordants, negative controls or status unknown)
Informed consent	X		
Eligibility assessment	X		
Demographics	X		
Medical history, clinical questionnaires (symptoms, medications, MMRC, Karnofsky)	X		X
COVID-19 Questionnaire (screening, impact on TB and testing history)	X		X
Psychosocial questionnaire[a] (tobacco, AUDIT, PHQ-9, BAI)	X		
Impact of TB symptoms on income	X		X
Anthropometrics	X		X
Pregnancy Test[b]	X		
CXR[c]	X		
HIV Test (Rapid)[d]	X		
CD4 count if HIV-infected[d]		X	
CBC and lymphocyte count	X		
HbA1c[a]	X		
Sputum smear[e]	X (S1)[j]		
Sputum smear, Xpert Ultra and culture (MGIT and LJ)[f]	X (S2)[j]	X (S3)[j]	X
Sputum DST	X		X
EPTB sample for smear, Xpert Ultra, culture, pathology and storage[g]	X		X[k]
Mtb isolate subculture for storage	X		X
Whole blood (PAXgene) for storage (x 2)	X		X
Whole blood (plasma) for testing and storage[h]	X		X
Whole blood for storage (genetic analyses)	X		
Saliva for storage[e] (diagnosis or genetic analyses)	X		
Urine for storage	X		X
Sputum for storage (half in TRIzol, half untreated)		X (S4,5)[j]	X[l]
Stool for storage[i] (diagnosis, microbiome)	X		

AUDIT=alcohol use disorders identification test; CXR=chest radiograph; CBC=complete blood count; COVID-19=Coronavirus disease of 2019; LFT=liver function test; MGIT=mycobacteria growth indicator tube; LJ= Lowenstein Jensen; culture; DST=drug-susceptibility testing; EPTB=extrapulmonary TB (lymph node pleural, and TBM only); PHQ-9=Patient Health Questionnare-9, BAI=Beck's Anxiety Inventory

[a] Applicable for adults (≥18years) only

[b] Urine pregnancy test to be done before a performing a CXR in women of reproductive age (usually 10 to 50years)

[c] CXR at baseline, unless a good quality digital CXR was done within 4 weeks prior to the Baseline as part of standard of care evaluations or through the Parent Protocol and is available for the study team. Pregnant women can have a CXR done if adequate shield can be provided (at discretion of study clinician). Do not repeat if enrolled for cohort A.

[d] HIV testing to be performed per national guidelines. HIV testing not required if there is documentation of a confirmed positive test at any time in the past or the last negative HIV test was obtained ≤90 days prior to the study visit; or the participant is a child <18 years of age who was not born to an HIV positive mother; abstract the data from the participant's medical chart or research record; CD4 count will only be performed on participants who are HIV-infected and who have not had a CD4 count performed in the preceding 6 months.

[e] For children (<15years) enrolled in the Dx cohort refer to Table 04b for micro and specimen collections.

[f] MGIT extension for discordant and negative controls

[g] EPTB specimens from lymph node pleural, and TBM cases will undergo smear/Xpert MTB/RIF, histopathology, Xpert Ultra and cultures by MGIT and LJ. Anything leftover will go for storage.

[h] Plasma samples will be used for Aim 1 (cytokine) and cross-cutting Aims (SARS-CoV-2 antibody) testing.

[i] Stool collection only for Paediatric (<15years) study participants.

[j] For sputum samples:

- Day 1- Sputum 1 (S1): Sputum 1 (S1): Spot sputum (preferably early morning specimen).
- Day 1- Sputum 2 (S2): Spot sputum collection.
- Direct florescence smear microscopy will be performed on both S1 and S2 samples. Both S1 and S2 will be mixed and homogenized using 10% sputasol or 0.1% DTT. The sample will be split, one half used for Direct Xpert ULTRA (version 2) and the other half subjected to NALC-NaOH decontamination followed by sedimentation, and the following studies will be performed on the sediment: concentrated fluorescence smear microscopy; Lowenstein Jensen cultures and MGIT liquid culture (with 4 weeks extended incubation for smear/ Xpert positive negative cultures)
- Day 2- Sputum 3 (S3), Spot sputum collection (preferably early morning).
- Day 2-Sputum 4 (S4). Spot sputum collection.
- Direct florescence smear microscopy will be performed on both S3 and S4 samples. Both S3 and S4 will be mixed and homogenized using 10% sputasol or 0.1% DTT. The sample will be split, one half subjected to NALC-NaOH decontamination followed by sedimentation, and the following studies will be performed on the sediment: concentrated fluorescence smear microscopy; Lowenstein Jensen culture and MGIT liquid culture (with 4 weeks extended incubation for smear negative cultures). The other half will be equally split and one half treated with Trizol and frozen for storage, and the other half directly frozen for storage. S5 spot sputum sample may be collected in a TRIzol prefilled sputum collection tube for sputum RNA stablity.
- For induced sputum or nasopharyngeal aspirates or gastric lavage, 2 specimens will be collected on day 1, four hours apart. Direct fluorescence smear microscopy or Xpert MTB RIF; Lowenstein Jensen culture and MGIT liquid culture (with 4 weeks extended incubation for smear negative cultures) will be performed on both samples. Detailed procedures for included sputum or nasopharyngeal aspirate or gastric lavage specimen processing and storage are mentioned in Lab MOP.

[k] Only if specimen was collected as standard of care for TB diagnosis

[l] If symptomatic

All TB cases including EPTB will be offered sputum/ respiratory specimen collection.

Table 03b: Microbiologic SOE for Pediatric Dx Cohort

Activities / Visit	Baseline – Day 1	Baseline–Day 2	Month 2 (discordants, negative controls or status unknown)
Informed consent	X		
Eligibility assessment	X		
Demographics	X		
Medical history, clinical questionnaires (symptoms, medications, MMRC, Karnofsky)	X		X
COVID-19 Questionnaire (screening, impact on TB and testing history)	X		X
Psychosocial questionnaire[a] (tobacco, AUDIT, PHQ-9, BAI)	X		
Impact of TB symptoms on income	X		X
Anthropometrics	X		X
Pregnancy Test[b]	X		
CXR[c]	X		
HIV Test (Rapid)[d]	X		
CD4 count if HIV-infected[d]		X	
CBC and lymphocyte count	X		
HbA1c[a]	X		
Sputum smear[e]	X (S1)[j]		
Sputum smear, Xpert Ultra and culture (MGIT and LJ)[f]	X (S2)[j]	X (S3)[j]	X
Sputum DST	X		X
EPTB sample for smear, Xpert Ultra, culture, pathology and storage[g]	X		X[k]
Mtb isolate subculture for storage	X		X
Whole blood (PAXgene) for storage (x 2)	X		X
Whole blood (plasma) for testing and storage[h]	X		X
Whole blood for storage (genetic analyses)	X		
Saliva for storage[e] (diagnosis or genetic analyses)	X		
Urine for storage	X		X
Sputum for storage (half in TRIzol, half untreated)		X (S4,5)[j]	X[l]
Stool for storage[i] (diagnosis, microbiome)	X		

AUDIT=alcohol use disorders identification test; CXR=chest radiograph; CBC=complete blood count; COVID-19=Coronavirus disease of 2019; LFT=liver function test; MGIT=mycobacteria growth indicator tube; LJ= Lowenstein Jensen; culture; DST=drug-susceptibility testing; EPTB=extrapulmonary TB (lymph node pleural, and TBM only); PHQ-9=Patient Health Questionnare-9, BAI=Beck's Anxiety Inventory

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[d] HIV testing to be performed per national guidelines. HIV testing not required if there is documentation of a confirmed positive test at any time in the past or the last negative HIV test was obtained ≤90 days prior to the study visit; or the participant is a child <18 years of age who was not born to an HIV positive mother; abstract the data from the participant's medical chart or research record; CD4 count will only be performed on participants who are HIV-infected and who have not had a CD4 count performed in the preceding 6 months.

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- Day 2- Sputum 3 (S3), Spot sputum collection (preferably early morning).
- Day 2-Sputum 4 (S4). Spot sputum collection.
- Direct florescence smear microscopy will be performed on both S3 and S4 samples. Both S3 and S4 will be mixed and homogenized using 10% sputasol or 0.1% DTT. The sample will be split, one half subjected to NALC-NaOH decontamination followed by sedimentation, and the following studies will be performed on the sediment: concentrated fluorescence smear microscopy; Lowenstein Jensen culture and MGIT liquid culture (with 4 weeks extended incubation for smear negative cultures). The other half will be equally split and one half treated with Trizol and frozen for storage, and the other half directly frozen for storage. S5 spot sputum sample may be collected in a TRIzol prefilled sputum collection tube for sputum RNA stablity.
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All TB cases including EPTB will be offered sputum/ respiratory specimen collection.