Estimation of the Prevalence of Latent TB Infection in a wholesale perishable market Complex in Chennai and assessment of risk factors for the progression of LTBI to Active TB Disease- Research Protocol

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Background and Justification:

PTB is an airborne communicable disease caused by Mtb. TB disease can be contacted by anyone in an endemic nation like India. Vulnerable and key population in particular is at a higher risk of contacting TB when compared to the general population and it is said that LTBI progresses to Active TB within a mean duration of 1-2 years. Many studies have been carried out to detect LTBI and TB disease prevalence among vulnerable population like prisoners, hostelers, healthcare workers and is also being implemented as a part of the NTEP. It is important to consider the staff of wholesale perishable market complex like Koyambedu market complex in Chennai which is a closed, damp, dark, dusty and overcrowded environment also as key population and they possess a threat of faster TB spread among themselves and also to the community. Not many studies are reported worldwide to detect the prevalence of LTBI in wholesale perishable market complex. Hence, we propose to estimate the prevalence of LTBI and also assess the environmental and occupational risk factors for the progression of LTBI to active TB disease which will serve as a platform for the better understanding about the spectrum of TB disease among population in such a setting and lead to future studies with regards to whether perishable goods can act as a source or carrier of Mtb leading to aerosol generation and thus serving as a hotspot for TB transmission. Those participants who have been identified to be IGRA positive will be referred to NTEP for Active TB disease screening. Participants will be declared as LTBI positive only after ruling out active TB disease which will also help to identify potential individuals for TPT under NTEP. We propose to conduct this study in Koyambedu Market complex in Chennai which is one of Asia's largest and busiest closed perishable market complex open round the clock with the wholesale market operating between 10 p.m. and 10 a.m. and the retail market operating between 10 a.m. and 10 p.m, receiving about 100,000 visitors and 500 to 600 vehicles every day.

Literature Review:

As per the Global TB Report 2021, the estimated incidence of all forms of TB in India for the year 2020 was 188 per 100,000 population (129-257 per 100,000 population). A total of 19,33,381 incident TB patients (new & relapse) were notified during 2021 which was 19% higher than that of 2020 (16,28,161). The National Tuberculosis Elimination Program (NTEP) has been resiliently progressing towards the goal of Ending Tuberculosis by 2025 through various efforts like Active Case Finding, uninterrupted supply of TB drugs, bidirectional screening of patients with comorbidities for TB, TB screening in frontline workers, newer diagnostic tools, treatment regimens, policies, enhanced engagement with private sector etc.,

In most individuals, Mtb infection is eliminated by host defences, and infection remains latent (hidden). Although latency and active (i.e., symptomatic, infectious) TB disease are a part of dynamic spectrum, persons with latent TB infection (LTBI) are classically considered to be asymptomatic and not infectious. However, latent TB bacilli may remain viable and "reactivate" later to cause active TB disease. Identification and treatment of LTBI can substantially reduce the risk of development of disease and are important TB control strategies

Vulnerable and key population in particular is at a higher risk of contacting TB when compared to the general population and it is said that Latent TB Infection progresses to Active TB within a mean duration of 1-2 years. Screening of the vulnerable population like PL-HIV, HHC of active TB patients, hostelers, prisoners, frontline workers for active TB and LTBI has been routinely implemented in the program.   Prevention, which is one of the 4 pillars of 'Prevent' is one of the four critical pillars (Detect – Treat – Prevent – Build) of India's National Strategic Plan for Elimination of Tuberculosis (NSP 2017-25) that focuses on preventing the emergence of TB in a vulnerable population by offering tuberculosis preventive therapy (TPT) consisting of 3 months of weekly rifapentine and isoniazid (3HP) or 6 months of daily isoniazid regimen (6H).

The laboratory investigations for the detection of LTBI include Tuberculin Skin Test (TST) also known as Mantoux Test and Immunological Assay namely the Interferon Gamma Release Assay (IGRA). The TST, performed using the Mantoux technique, consists of the intradermal injection of 5 tuberculin units (TU) of PPD-S purified protein derivative (PPD) or 2 TU PPD RT23 (these are considered equivalent. In a person who has cell-mediated immunity to these tuberculin antigens, a delayed-type hypersensitivity reaction will occur within 48 to 72 h. The reaction will cause localized induration of the skin at the injection site, and the transverse diameter should be measured (as millimeters of induration) by a trained individual and interpreted using risk-stratified cutoffs. It is important to note that cell-mediated immunity to tuberculin antigens can sometimes reflect exposure to similar antigens from environmental mycobacteria or Mycobacterium bovis bacillus Calmette-Guérin (BCG) vaccination or a previous infection that has been cleared (through immunological mechanisms or treatment). The TST has several known limitations. False-positive and false-negative results can occur. There are two important causes of false-positive results: nontuberculous mycobacterium (NTM) infection and prior BCG vaccination. NTMs are not a clinically important cause of false-positive TST results, except in populations with a high prevalence of NTM sensitization and a very low prevalence of TB

infection. The impact of BCG on TST specificity depends on when BCG is given and on how many doses are administered. If BCG is administered at birth (or during infancy) and not repeated, then its impact on TST specificity is minimal and can be ignored while interpreting the results. In contrast, if BCG is given after infancy (e.g., school entry) and/or given multiple times (i.e., booster shots), then TST specificity is compromised. False-negative TST results may occur because of limited sensitivity in particular patient subgroups (e.g., immunosuppressed individuals [due to medical conditions such as HIV infection or malnutrition] or those taking immunosuppressive medications) or because of preanalytical or analytical sources of test variability (e.g., improper tuberculin handling or placement or incorrect interpretation of test results). Unfortunately, individuals for whom the TST has limited sensitivity are often the very individuals that are at increased risk of progression to active disease if infected. Anergy induced by active TB itself can cause false-negative TST results. The TST is also known to have problems with reproducibility, with inter- and intrareader variability in measurements of induration. Nonspecific variability is expected, and interpretation of repeat testing is complicated by immunologic recall of pre-existing hypersensitivity to TB (i.e., boosting), conversions (i.e., new infection), and reversions (of positive results to negative). Cut-offs used for TST conversions are different from the cut-offs used for diagnosis of LTBI.

IGRAs are tests of cell-mediated immune response; they measure T-cell release of IFN-γ following stimulation by antigens specific to the M. tuberculosis complex (with the exception of BCG substrains), i.e., early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These antigens are encoded by genes located within the region of difference 1 (RD1) locus of the M. tuberculosis genome. They are more specific than PPD for M. tuberculosis because they are not encoded in the genomes of any BCG vaccine strains or most species of NTM, other than M. marinum, M. kansasii, M. szulgai, and M. flavescens. However, not all NTMs have been studied for cross-reactivity. The following are the commercial IGRAs that are available in many countries: the QuantiFERON-TB Gold In-Tube (QFT) or the QuantiFERON-TB Gold In-Tube (QFT) Plus assay (Cellestis/Qiagen, Carnegie, Australia), T-SPOT.TB assay (Oxford Immunotec, Abingdon, United Kingdom). C-Tb (SSI, Copenhagen, Denmark) is a skin test based on recombinant ESAT-6 and CFP10, and is designed to combine the operational advantages of the TST with the specificity of commercially available IGRAs for the diagnosis of *M tuberculosis* infection in individuals at risk of developing active tuberculosis and it is still under trial and yet to be launched in the program. The QFT assay is an enzyme-linked immunosorbent assay (ELISA)-based, whole-blood test that uses peptides from the RD1 antigens ESAT-6 and CFP-10 as well as peptides from one additional antigen (TB7.7 [Rv2654c], which is not an RD1 antigen) in an in-tube format. The result is reported as quantification of IFN-γ in international units (IU) per milliliter. An individual is considered positive for M. tuberculosis infection if the IFN-γ response to TB antigens is above the test cutoff (after subtracting the background IFN-γ response of the negative control). To improve the efficacy of IGRA, QuantiFERON-TB Gold Plus (QFT-Plus) has been recently developed as a next-generation QuantiFERON-TB Gold In-Tube (QFT-GIT) test. QFT-Plus contains two TB-specific antigen coated tubes, TB1 and TB2.The antigen tubes contain 6-kDa early secretory antigenic target (ESAT-6) and 10-kDa culture filtrate protein (CFP-10), but not TB7.7. The long synthetic peptides in TB1 tubes are designed to stimulate CD4-T cells, as does QFT-GIT. However, the QFT-Plus includes an additional TB2 antigen tube which contains six short peptides that may also induce CD8 T-cell specific immune responses. Test performance of the QFT-Plus has mostly been reported in low incidence settings or in immuncompromised patients. The T-SPOT.TB assay is an enzyme-linked immunosorbent spot (ELISPOT) assay performed on separated and counted peripheral blood mononuclear cells (PBMCs) that are incubated with ESAT-6 and CFP-10 peptides. The result is reported as the number of IFN-γ-producing T cells (spot-forming cells). An individual is considered positive for M. tuberculosis infection if the spot counts in the TB antigen wells exceed a specific threshold relative to the negative-control wells. Indeterminate IGRA results can occur due to a low IFN-γ response to the positive (mitogen) control or a high background response to the negative control. Based on published meta-analyses, IGRAs have a specificity for LTBI diagnosis of >95% in settings with a low TB incidence, and specificity is not affected by BCG vaccination. Among populations where BCG is administered, the specificity of TST is much lower (approximately 60%) and variable, depending on when and how often BCG is given. Because QFT plus is not affected by BCG vaccination status or HIV infection and is commercially available,

Test Name	Sensitivity	Specificity
TST at 13mm threshold	54%	90%
QFT-GIT	73%	93%
QFT Plus	98.9%	98.1%
T-Spot-TB (Elispot)	83%	80.9%
C-Tb	Yet to be launched commercially	

it can be useful for evaluation of LTBI. Koyambedu Market complex in Chennai which is one of Asia's largest and busiest closed perishable market complex open round the clock with the wholesale market operating between 10 p.m. and 10 a.m. and the retail market operating between 10 a.m. and 10 p.m, receiving about 100,000 visitors and 500 to 600 vehicles every is a closed, damp, dark, dusty and overcrowded environment. Their workers should also be considered as vulnerable population as they possess a greater threat of developing active TB disease thus leading to the faster spread of the disease among themselves and also to the community. Early detection of LTBI and its treatment can prevent the development of Active TB disease. Hence, the prevalence estimation of LTBI and also assessment of the environmental and occupational risk factors for the progression of LTBI to active TB disease will serve as a platform for the better understanding about the spectrum of TB disease among population in such a setting and lead to future studies with regards to whether perishable goods can act as a source or carrier of Mtb leading to aerosol generation and thus serving as a hotspot for TB transmission

Study Objectives:
1. Estimate the Prevalence of LTBI among the static adult population of Koyambedu Market Complex
2. Screening for active PTB disease among IGRA positive individuals
3. Assessment of environmental and occupational risk factors for the progression of LTBI to Active TB disease

Materials and Methods:
Sample Size and Sampling Method with Inclusion and Exclusion Criteria:
*The Koyambedu market complex will be divided into 4 clusters of 50 participants/cluster (200 in total) and the distribution of the type of staff/cluster is mentioned below (confirmation needed from MMC regarding the exact number of workers to calculate the exact sample size)
1. Vegetable Market Cluster-50- Shopkeepers/Owners-5, Vendors-15, Labourers-25, Parking lot staff- 5
2. Flower Market Cluster -50- Shopkeepers/Owners- 5, Vendors-15, Labourers-25, Parking lot staff- 5
3. Fruit Market Cluster- 50- Shopkeepers/Owners-5, Vendors-15, Labourers-25, Parking lot staff-5
4. Grocery Market Cluster-50- Shopkeepers/Owners-5, Vendors-15, Labourers-25, Parking Lot staff-5

*The participants in each cluster will be selected by simple random sampling after obtaining the approval from the Market Management Committee
*Floating Population like Vehicle drivers and small-time retailers and buyers will be excluded from the study
Study Design: Cross-Sectional
Study Period and Timelines: 2 years
- 4 months for preparatory activities
- 16 months for data and sample collection and testing
- 4 months for data entry, cleaning, analysis and publication

Methods:
1. Informed Consent obtained from the selected participants and enrolled in the study (Annexure I)
2. Risk factor assessment for the progression of LTBI to active PTB disease by detailed interview questionnaire (Annexure II)
3. Screening of all 200 study participants for IGRA by QFT Plus ELISA

Introduction:
QFT-Plus is a test for cell-mediated immune (CMI) responses to peptide antigens that simulate Mycobacterial proteins. These proteins, ESAT-6 and CFP-10, are absent from all BCG strains and from most Nontuberculous Mycobacteria with the exception of M. kansasii, M. szulgai, and M. marinum . Individuals infected with MTB-complex organisms usually have lymphocytes in their blood that recognize these and other Mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine IFN-γ. The detection and subsequent quantification of IFN-γ by standard ELISA forms the basis of this test.
Principle:
QFT-Plus has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain peptide antigens from the MTB–complex–associated antigens, ESAT-6 and CFP-10. Whereas the TB1 tube contains peptides from ESAT-6 and CFP-10 that are designed to elicit CMI responses from CD4+ T-helper lymphocytes, the TB2 tube contains an additional set of peptides targeted to the induction of CMI responses from CD8+ cytotoxic T lymphocytes. In the natural history of MTB infection, CD4+ T cells play a critical role in immunological control through their secretion of the cytokine IFN-γ. Evidence now supports a role for CD8+ T cells participating in the host defence to MTB by producing IFN-γ and other soluble factors, which activate macrophages to suppress growth of MTB, kill infected cells, or directly lyse intracellular MTB. MTB-specific CD8+ cells have been detected in subjects with LTBI and with active TB disease where IFN-γ producing CD8+ cells may be frequently found. Moreover, ESAT-6 and CFP-10 specific CD8+ T lymphocytes are described as being more frequently detected in subjects with active TB disease versus LTBI, and may be associated with a recent MTB exposure. In addition, MTB-specific CD8+ T cells producing IFN-γ have also been detected in active TB subjects with HIV co-infection and in young children with TB disease. The mitogen and nil tubes are identical between the two tests, and, as a reminder, the mitogen tube contains phytohemaglutanin, which serves as a positive control for T-cell activity, while the nil tube essentially measures the level of background or circulating InterFERON-gamma in the patient.
Procedure Overview:
The QFT-Plus test is performed in two stages. First, 4mL of whole venous blood is drawn from the participant with a syringe and 1ml is transferred into each of the QFT-Plus Blood Collection Tubes, which include a Nil tube, TB1 tube, TB2 tube, and a Mitogen tube in the order of draw mentioned above. The tubes should be shaken 10 times just firmly enough to make sure the

entire inner surface of the tube is coated with blood. This will dissolve antigens on tube walls. Blood should be maintained and transported at room temperature (22°C ± 5°C) before transfer to QFT-Plus tubes for incubation, which must be initiated within 16 hours of blood collection.

The Mitogen tube is used as a positive control. This may be important where there is doubt as to the individual's immune status. The Mitogen tube also serves as a control for correct blood handling and incubation. The QFT-Plus tubes should be incubated at 37°C as soon as possible, and within 16 hours of collection. Following a 16 to 24 hour incubation period, the tubes are centrifuged, the plasma is removed and the amount of IFN-γ (IU/ml) is measured by ELISA.

A QFT-Plus assay is considered positive for an IFN-γ response to either TB Antigen tube that is significantly above the Nil IFN-γ IU/ml value. The plasma sample from the Mitogen tube serves as an IFN-γ positive control for each specimen tested. A low response to Mitogen (<0.5IU/ml) indicates an indeterminate result when a blood sample also has a negative response to the TB antigens. This pattern may occur with insufficient lymphocytes, reduced lymphocyte activity due to improper specimen handling, incorrect filling/mixing of the Mitogen tube, or inability of the patient's lymphocytes to generate IFN-γ. The Nil tube adjusts for background (e.g., excessive levels of circulating IFN-γ or presence of heterophile antibodies). The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the TB Antigen tubes and Mitogen tube.

Calculations and Test Interpretation

QFT-Plus Analysis Software may be used to analyze raw data and calculate results. It is available from www.QuantiFERON.com. Please make sure that the most current version of the QFT-Plus Analysis Software is used.

The software performs a quality control assessment of the assay, generates a standard curve, and provides a test result for each subject, as detailed in the Interpretation of Results section.

As an alternative to using the QFT-Plus Analysis Software, results can be determined according to the following method.

Quality control of test

The accuracy of test results is dependent on the generation of an accurate standard curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.

For the ELISA to be valid:
- The mean OD value for Standard 1 must be ≥0.600.
- The %CV for Standard 1 and Standard 2 replicate OD values must be ≤15%.
- Replicate OD values for Standard 3 and Standard 4 must not vary by more than 0.040 optical density units from their mean.
- The correlation coefficient (r) calculated from the mean absorbance values of the standards must be ≥0.98.

The QFT-Plus Analysis Software calculates and reports these quality control parameters.

If the above criteria are not met, the run is invalid and must be repeated.

The mean OD value for the Zero Standard (Green Diluent) should be ≤0.150. If the mean OD value is >0.150 the plate washing procedure should be investigated.
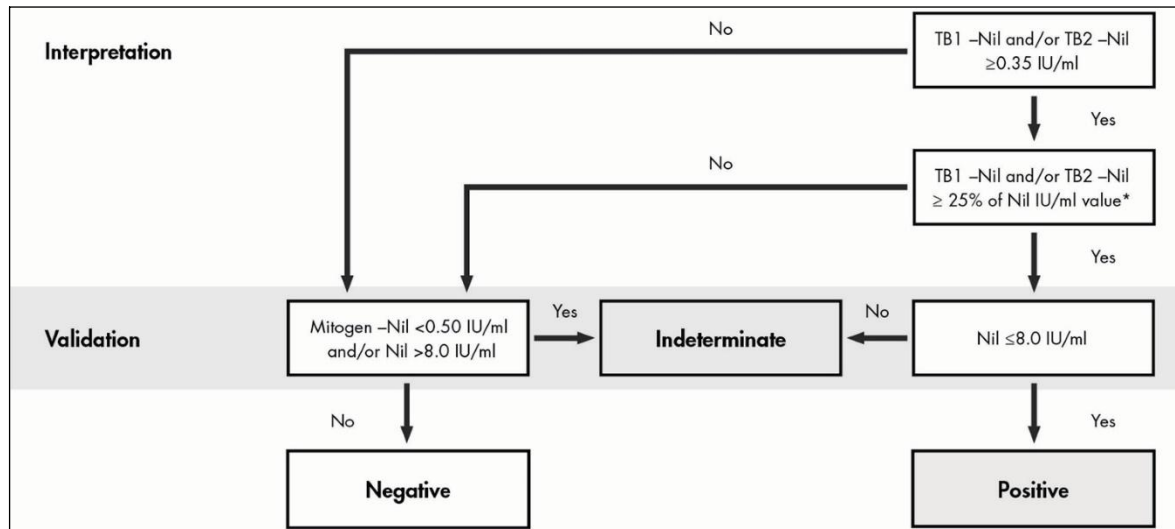
Interpretation of QFT Plus Results:

Nil (IU/ml)	TB1 minusNil (IU/ml)	TB2 minusNil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT-PlusResult	Report/Interpretation
≤8.0	≥0.35 and ≥25% of Nil value	Any	Any	Positive	*M. tuberculosis* infectionlikely
	Any	≥0.35 and ≥ 25% of Nil value			
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	≥0.5	Negative	*M. tuberculosis* infectionNOT likely
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	<0.5	Indeterminate	Likelihood of *M. tuberculosis* infection cannot be determined

>8.0	Any		

*Responses to the Mitogen positive control (and occasionally TB Antigens) can be outside the range of the microplate reader. This has no impact on test results. Values >10 ml are reported by the QFT-Plus software as >10 lU/ml.

QFT-Plus interpretation flowchart.

- For TB1 minus Nil or TB2 minus Nil to be valid, amount ≥25% of Nil IU/ml value must be from the same tube as the original ≥0.35 IU/ml result.

4. Referral of IGRA Positive individuals to the nearest TU for Active TB disease screening and treatment initiation for TB disease by NTEP.
5. Identification of LTBI positive individuals for TPT initiation and referral to NTEP.

Operational Definitions:
1. IGRA Positive: ≥0.35IU/ml by QFT Plus
2. LTBI Positive: IGRA Positive without Active PTB
3. Active TB (Clinical/Microbiological)- IGRA positive participants confirmed to be TB patients by NTEP and registered with NIKSHAY for treatment initiation

Statistical Analysis Plan:
1. Prevalence of LTBI- IGRA Positive after ruling out Active PTB disease/ Total Population * 1000
2. Percentage of participants positive for PTB after screening IGRA positive for Active TB
3. Risk factor assessment for progression of LTBI to active TB disease by Multivariate Regression Analysis

Potential Benefits to the Community:
1. Assessment of Prevalence of LTBI among vulnerable population like a Market complex
2. Screening for Active PTB among IGRA positive individuals and linking the PTB patients to NTEP NIKSHAY system for treatment initiation
3. Identification of potential group of individuals for TPT under NTEP
4. Assessment of risk factors for the progression of LTBI to Active TB disease in a closed and overcrowded setting like a market complex
5. Future studies with regards to perishable goods acting as a source or carrier of Mtb and thus spread of TB in the market and also to the community

Human Subject Protection and Confidentiality:
Informed consent will be obtained from the participants prior to enrolment. Information will be provided regarding the blood draw for IGRA, and risk assessment interview prior to getting informed consent from the participants. Confidentiality of the participants and study data will be maintained. (Annexure I).

Clinical Information Form/ Risk Assessment Questionnaire

Participant ID:_____   Date of interview:_____
Date of Birth: _____   Age: _____yrs
Gender: Male / Female / Transgender   Education: Literate/Illiterate

Marital Status: Married/Unmarried/Divorced/Separated/Widow
Weight: _____Kg   Height: _____cm   BCG Vaccination Status: Yes/No
Smoking Status: Past Smoker/Current Smoker/Non Smoker
If Current Smoker, No. of units per day: _____
Alcohol Status: Past Alcoholic/Current Alcoholic/Non Alcoholic
If Current Alcoholic, What quantity (pegs) each day: _____

Comorbidities:

Diabetes mellitus   Yes / No / Not Known   If yes, duration (weeks) _____
Taking insulin?   Yes / No / Not Known   If yes, duration (weeks) _____
Taking diabetes tablets?   Yes / No / Not Known   If yes, duration (weeks) _____
Hypertension   Yes / No / Not Known   If yes, duration (weeks) _____
Other (specify) _____   If yes, duration (weeks) _____
Taking other medicines: _____Yes / No (specify)   If yes, duration (weeks) _____
Ever tested for HIV   Yes / No / Don't know
If yes, where?   Govt/Private/NGO/Other: _____(specify)
If yes, result?   Positive / Negative/ Don't know
If positive, on ART?   Yes / No
If yes, where?   Govt/Private/NGO/Other: _____ (specify)
If yes, duration (months): _____

Risk Assessment:

Last Known contact with a TB Patient: Yes/No/Don't Know
Place of Work: Vegetable/Fruit/Flower/Grocery
Nature of Work: Shop owner/Vendor/Labourer/Parking Lot
Duration of employment: _____years
No: of duty hours: _____ hours
Shift: Morning/Night
No: of duty hours: _____hrs
Average No: of retailers/consumers met in a day: _____
Mode of Travel to work: Public Transport/ Own vehicle
Time Taken for Travel: _____hrs

Symptom screening:

1. Do you have symptoms? If yes, since how long?   Duration

Symptom	No	Yes	Days	Weeks	Months
Cough		☐	☐	____	____	____
Sputum		☐	☐	____	____	____
Blood stained sputum		☐	☐	____	____	____
Chest pain	☐	☐		____	____	____
Weight loss		☐	☐	____	____	____
Fatigue, malaise	☐		☐	____	____	____
Fever		☐	☐	____	____	____
Other ( )	☐	☐		____	____	____
TB suspect by symptom	☐	☐		____	____	____

2. Current TB treatment: ☐ No   ☐Yes
3. Past history of TB treatment: ☐ No   ☐Yes

IGRA Test:

Venous Blood Drawn: _____ml   Result Date: _____
Nil_____ IU/ml Mitogen_____IU/ml TB1_____ IU/ml   TB2_____IU/ml
IGRA Result: Positive/Negative   Eligible for Active TB Screening ☐ No ☐Yes
_____X_____

SECTION II

Data Extraction from NIKSHAY:
Chest X-Ray data: **Any significant signs suggestive of EPTB:**
Any Lesion in lung fields: ☐ Yes   ☐ No   _____
Cytopathology/Histopathology Results: _____
Sputum examination Results:

CBNAAT Results	Smear Results	Diagnosis of TB:
☐ NA ☐ Invalid ☐ TB ☐ Neg ☐ Rif Resistant ☐ Rif Sensitive	☐ **Neg** ☐ **Scanty** ☐ **1+** ☐ **2+** ☐ **3+**	PTB/EPTB/No TB
1st Line LPA Results		_____
☐ NA ☐ Invalid ☐ TB ☐ Non-TB ☐ INH Sensitive ☐ INH Resistant ☐ Rif Resistant ☐ Rif Sensitive		_____ ____X_____

References:

1. India TB Report 2022
2. World Health Organization. Global Tuberculosis Report. Geneva: World Health Organization; 2020
3. Lakshmi, K. (23 October 2018). "Partial ban on lorries at wholesale market planned". The Hindu. Chennai: Kasturi & Sons. p. 2. Retrieved 23 October 2018.
4. Chennai Metropolitan Development Authority (CMDA), Development of Wholesale Food-grains Market-Government of India
5. Kit insert QFT Plus
6. Landry J, Menzies D. 2008. Preventive chemotherapy. Where has it got us? Where to go next? Int. J. Tuberc. Lung Dis. 12:1352–1364.
7. Menzies D, Gardiner G, Farhat M, Greenaway C, Pai M. 2008. Thinking in three dimensions: a web-based algorithm to aid the interpretation of tuberculin skin test results. Int. J. Tuberc. Lung Dis. 12:498 –505.
8. Farhat M, Greenaway C, Pai M, Menzies D. 2006. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? Int. J. Tuberc. Lung Dis. 10:1192–1204.
9. Zwerling A, Behr M, Varma A, Brewer TF, Menzies D, Pai M. 2011. The BCG World Atlas: a database of global BCG vaccination policies and practices. PLoS Med. 8:e1001012. http://dx.doi.org/10.1371/journalpmed.1001012.
10. Sester M, Sotgiu G, Lange C, Giehl C, Girardi E, Migliori GB, Bossink A, Dheda K, Diel R, Dominguez J, Lipman M, Nemeth J, Ravn P, Winkler S, Huitric E, Sandgren A, Manissero D. 2011. Interferon-gamma release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. Eur. Respir. J. 37:100 –111. http://dx.doi.org/10.1183/09031936.00114810
11. Geluk A, van Meijgaarden KE, Franken KL, Subronto YW, Wieles B, Arend SM, Sampaio EP, de Boer T, Faber WR, Naafs B, Ottenhoff TH. 2002. Identification and characterization of the ESAT-6 homologue of Mycobacterium leprae and T-cell cross-reactivity with Mycobacterium tuberculosis. Infect. Immun. 70:2544 –2548. http://dx.doi.org/10.1128/IAI.70.5.2544-2548.2002.
12. Dorman S, Belknap R, Graviss EA, Reeves R, Schulger N, Weinfurter P, Wang Y, Cronin W, Hirsch-Moverman Y, Teeter L, Parker M, Garrett DO, Daley CL. 3 December 2013. Interferon-gamma release assays and tuberculin skin testing for diagnosis of latent tuberculosis infection in healthcare workers in the United States. Am. J. Respir. Crit. Care Med.[Epub ahead of print.] http://dx.doi.org/10.1164/rccm.201302-0365OC.
13. Yoshiyama T, Harada N, Higuchi K, Sekiya Y, Uchimura K. 2010. Use of the QuantiFERON-TB Gold test for screening tuberculosis contacts and predicting active disease. Int. J. Tuberc. Lung Dis. 14:819 –827.
14. Andrews JR, Noubary F, Walensky RP, Cerda R, Losina E, Horsburgh CR. 2012. Risk of progression to active tuberculosis following reinfection with Mycobacterium tuberculosis. Clin. Infect. Dis. 54:784 –791. http://dx.doi.org/10.1093/cid/cir951.
15. Kim YJ, Kim SI, Kim YR, Wie SH, Park YJ, Kang MW. 2012. Predictivevalue of interferon-gamma ELISPOT assay in HIV 1-infected patients in an intermediate tuberculosis-endemic area. AIDS Res. Hum. Retroviruses 28:1038 –1043. http://dx.doi.org/10.1089/AID.2011.0360
16. Morten Ruhwald et al Safety and efficacy of the C-Tb skin test to diagnose Mycobacterium tuberculosis infection, compared with an interferon γ release assay and the tuberculin skin test: a phase 3, double-blind, randomised, controlled trial, Lancet Respiratory Medicine, The, 2017-04-01, Volume 5, Issue 4, Pages 259-268