BIOMÉRIEUX

TUBERCULOSIS

A practical guide to diagnosis and management of latent and active TB

PIONEERING DIAGNOSTICS

INTRODUCTION

Tuberculosis (TB) is one of the biggest killers of mankind and approximately a billion people have succumbed to the disease over the last 2 centuries.[1,2] TB is far from eradicated and remains the foremost infectious disease killer over the last decade, with over 1.4 million deaths in 2019.[3] The number of deaths in 2020 is estimated to have been somewhere between 1.5 million and 2 million given the deleterious impact of COVID-19 on TB.[4] For several reasons including reduced access to healthcare, healthcare worker shortages, and repurposing of healthcare facilities, 2020 saw a 20 to 30% decline in TB case detection globally, and this has negatively impacted TB outcomes. Drug-resistant TB threatens to derail control in several parts of the world including Eastern Europe and Russia.[5,6]

This booklet provides an overview and practical guide to the classification, pathogenesis, clinical presentation, diagnosis, management, and prevention of latent and active TB. It is aimed at recently qualified healthcare professionals, junior medical officers, primary care physicians, and general physicians.

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CONTENTS

1 CLASSIFICATION AND EPIDEMIOLOGY

1.1 What is tuberculosis?

Tuberculosis (TB) is a chronic multi-system infection caused by an aerobic bacterium of the *Mycobacterium tuberculosis* complex.[7,8]

The Mycobacterium genus consists of over 150 members that subsist in animals, plants, soil, mammals and water.[9] The *M. tuberculosis* complex can cause disease in mammalian hosts and comprises *M. tuberculosis, M. microti, M. africanum, M. bovis, M. bovis BCG, M. orygis, M. canetti, M. caprae, M. pinnipedii, M. suricattae* and *M. mungi*.[10]

About 1.5% of the human TB burden is thought to be zoonotically derived (mainly from *M. bovis* in infected milk). Rarely, hosts with immune compromise or structural lung disease can be infected by mycobacteria other than TB (MOTT; e.g. *M. kansasii, M. abscesses* etc.)

The bacterium is ~0.4 x 4 microns in size and has a doubling time of ~18 to 20 hours. Almost any organ can be involved but the active phase of the disease most commonly affects the lung, and is characterised by a bronchopneumonia with a tendency to cavitate.

1.2 Spectrum of disease and lifecycle of *M. tuberculosis*

It is now appreciated that tuberculosis is a **spectrum of infection**, rather than a single distinct disease entity.[2] Although the earliest written reference to TB goes back to 1 500 BC[11], there is still no consensus about the nomenclature and definition of the different components of the TB spectrum (**Figure 1**).

At the one end of the spectrum are those with active TB disease where the organism can be cultured from tissues. When such patients are asymptomatic, this is frequently referred to as subclinical TB.

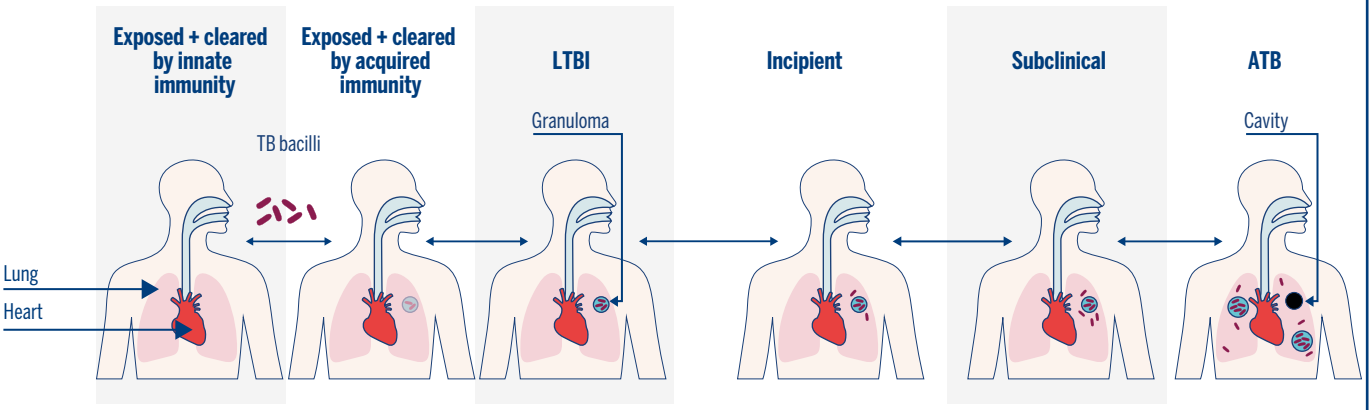
In the middle of the spectrum, latent TB infection (LTBI) is a pathobiological rather than a clinical entity.[7,8] Individuals with LTBI are asymptomatic and TB cannot be detected in the tissues using conventional microbiological tests, including culture or nucleic acid amplification tests. Microscopic visualisation of this pathobiological entity is variable and controversial and probably ranges from being immunologically silent and containing cell wall free forms of the organism to containment within a granuloma (discussed in section 2.3).

On exposure to the organism, the infection may be cleared immediately or transiently leading to an 'infection eliminated' status (**Figure 1**). Failure to clear the organism results in long term asymptomatic infection in a variable proportion of those exposed (approximately 10% to 20% though the exact proportion is unclear and maybe higher).[12]

Thus it has now become apparent that only a minority of those with immunoreactive tests are likely to have LTBI with the potential to relapse or transform into active disease later on.[13] ~95% of those with LTBI contain the infection lifelong, but in ~5% of persons the latent organisms may reactivate resulting in active TB (ATB) later in life or when immunity is compromised (**Figure 2**).

Incipient TB refers to an intermediate pathobiological state characterised by a higher burden of organisms than in LTBI, but less than that seen with sub-clinical or active TB disease. There are no microbiological correlates for latent or incipient TB.

Figure 1. The spectrum of TB.[8]



	Infection eliminated (with innate immunity)	Infection eliminated (with acquired immunity)	Latent TB Infection	Incipient TB	Subclinical TB	Active TB disease
Symptoms	None	None	None	None *	None to mild	Mild to severe
Imaging features (CXR and/or CT) **	Normal	Normal	Normal	Normal	May be normal	Abnormal
TST	Negative	Positive ***	Positive ***	Positive ***	Positive ***	Positive ***
IGRA	Negative	Positive ***	Positive ***	Positive ***	Positive ***	Positive ***
Mycobacterial burden	None	None	+	++	+++	+++++/++++++
Smear microscopy	Negative	Negative	Negative	Negative	Usually negative	Positive or negative
Sputum molecular testing	Negative	Negative	Negative	Negative	Usually negative	Positive
Culture	Negative	Negative	Negative	Negative	May be intermittently positive	Positive
Infectious	No	No	No	Likely No	Sporadically	Yes
Treatment	None	None	Preventative therapy	Preventative therapy****	Multidrug therapy	Multidrug therapy

* There may be mild symptoms that are not considered significant by the patient.

** Normality will depend on the type of imaging (abnormalities may be visible on CT but not chest radiograph), burden of disease, and resolution of the imaging technique.

*** Positive = usually positive but may be negative in a proportion (10 to 20%) either due to false negativity or test reversion to negativity over time.

**** Optimal therapy not known.

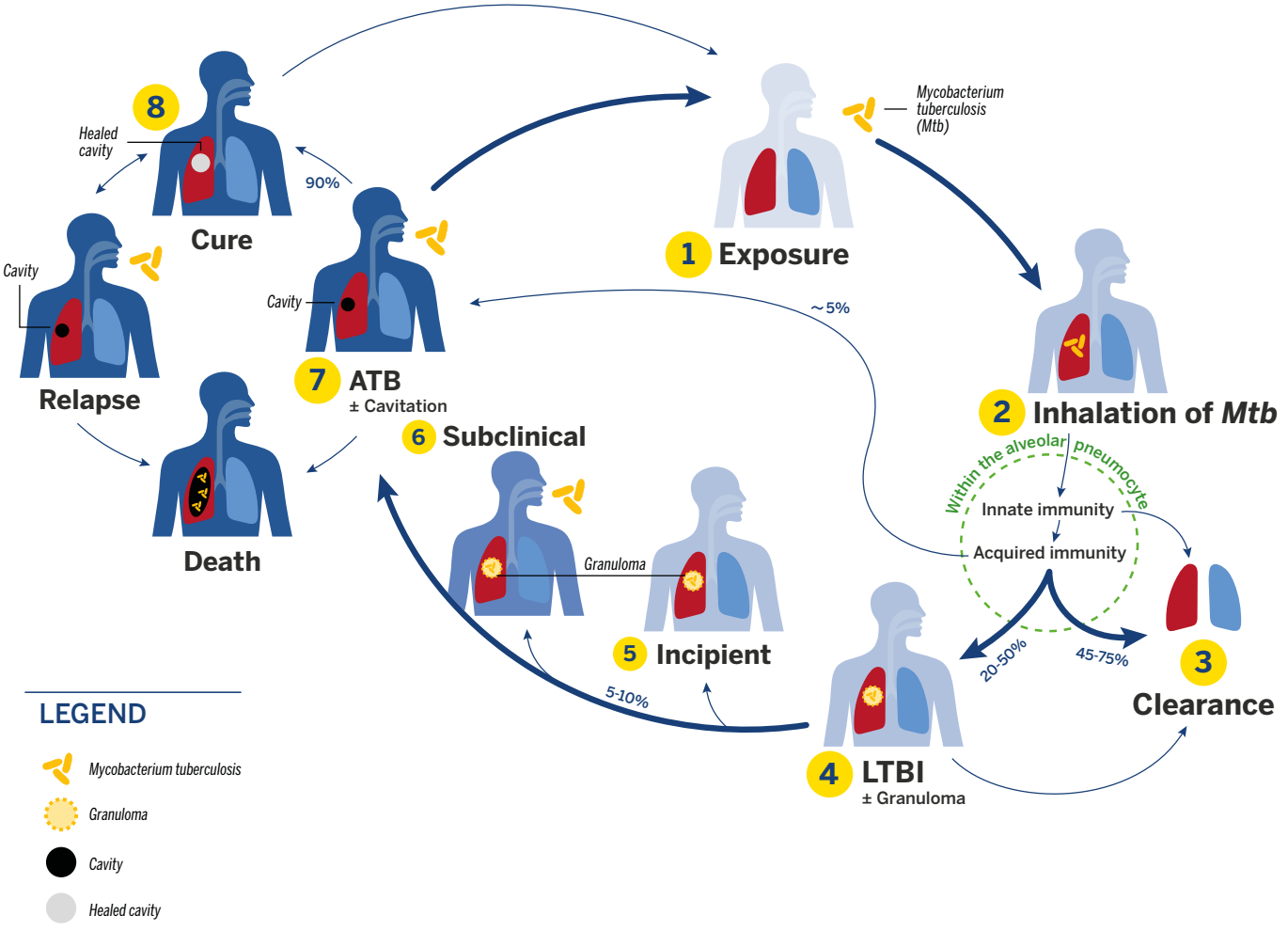
CT: Computerized tomography; CXR: Chest X-ray; IGRA: Interferon gamma release assay; TST: Tuberculin skin test.

The left hand side of the figure and the accompanying table outlines individuals who have eliminated infection, and on the right hand side those with microbiologically proven TB, i.e. subclinical TB and active TB. Endotypes with intermediate burden of disease, i.e. LTBI or incipient TB, are shown in the middle of the figure and the table.

The characteristics of each part of the spectrum are outlined in the table based on clinical characteristics, imaging features, test characteristics, transmission potential, and treatment strategies.

Figure 2. The lifecycle of *M. tuberculosis*.

Original illustration provided by Prof. Keertan Dheda. Used with permission.



Individuals who are exposed to TB aerosol (1) under optimal circumstances (poor ventilation, high mycobacterial load etc.) inhale the organisms into the alveoli (2). In roughly 50% or more cases the infection is likely cleared through innate or adaptive immune mechanisms (3). In a proportion of individuals (varies depending on the force of infection and host factors), however, infection persists in the form of LTBI (4). Individuals with LTBI may progress to incipient (5) and/or subclinical TB (6). There may be stepwise progression or individuals may stagnate at individual steps for months to years.

Alternatively, progression may lead to active TB (7) with onward transmission and repeated exposure and infection of a human host (1,2). 5% to 10% of patients with active TB either due to lack of treatment or due to complications may succumb to the disease or alternatively may be cured (8). With time, some patients may relapse.

1.3 Global epidemiology

In 2019, ~10 million people became newly ill with active TB. Almost two thirds of the burden was concentrated in the 22 high burden countries (**Figure 3**). However, in 2020, due to COVID-19, although the estimated number of people that became newly ill with active TB remained at ~10 million, the number of undetected or undiagnosed cases increased from ~2.9 million in 2019 to almost ~4 million cases, and the number of TB deaths increased from ~1.4 million in 2019 to ~1.5 million, though the number may be as high as ~2 million deaths (**Figure 4** outlines the impact of COVID-19 on TB burden and mortality). This has been due to several factors including reduced willingness to test for TB (related to stigmatisation of both COVID-19 and TB), reduced access to healthcare services, impact of lockdowns, repurposing of clinic and laboratory resources to COVID-19, etc.[14] Nevertheless, it is noteworthy that **more than 2 in every 5 TB cases remain undiagnosed or undetected**. Such cases continue to fuel the TB epidemic through ongoing community-based transmission.

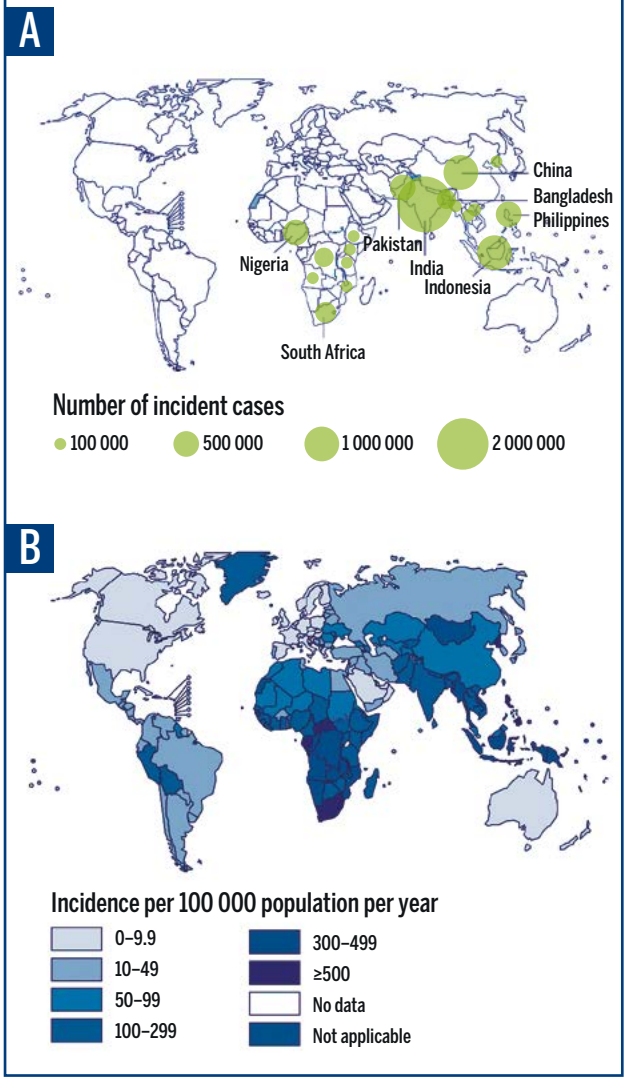
The incidence of TB has been declining at a slow rate over the last decade (~1.5% per year). More encouragingly, mortality has declined substantially over the last 20 years by over 50%.[3,4] It is disappointing, however, that in 2020 mortality is thought to have increased by ~6%, with an increase in mortality that is anticipated to be maintained over the next 3 to 5 years. Thus, the net effect of COVID-19 has set back TB control by ~5 years but potentially up to 10 years!

As outlined in the **WHO 2021 Global TB Report**:[4]

- About 15% of the newly ill patients with active TB in 2020 were HIV-infected with 80% of such cases occurring in Africa.
- In 2020, there were an estimated 500 000 newly ill patients with rifampicin-resistant or multi-drug-resistant active TB (RR/MDR TB). This is defined as resistance to the 2 frontline TB antibiotics, i.e. rifampicin and isoniazid. About 5 to 10% of such cases are thought to be extensively drug-resistant (resistance to fluoroquinolones and at least 1 other group A second line drug).
- ~5% of the global TB burden in 2020 occurred in children.
- Whilst the dominant form of the disease is that of pulmonary TB, ~15% of the total case burden is extrapulmonary TB (EPTB).
- The most common form of EPTB in many countries is pleural TB but other forms include TB lymphadenitis, pericardial TB, TB peritonitis, TB meningitis, etc.

Figure 3. Global epidemiology.[4]

Source: World Health Organization.* Global Tuberculosis Report 2021. Accessed March 1, 2022.

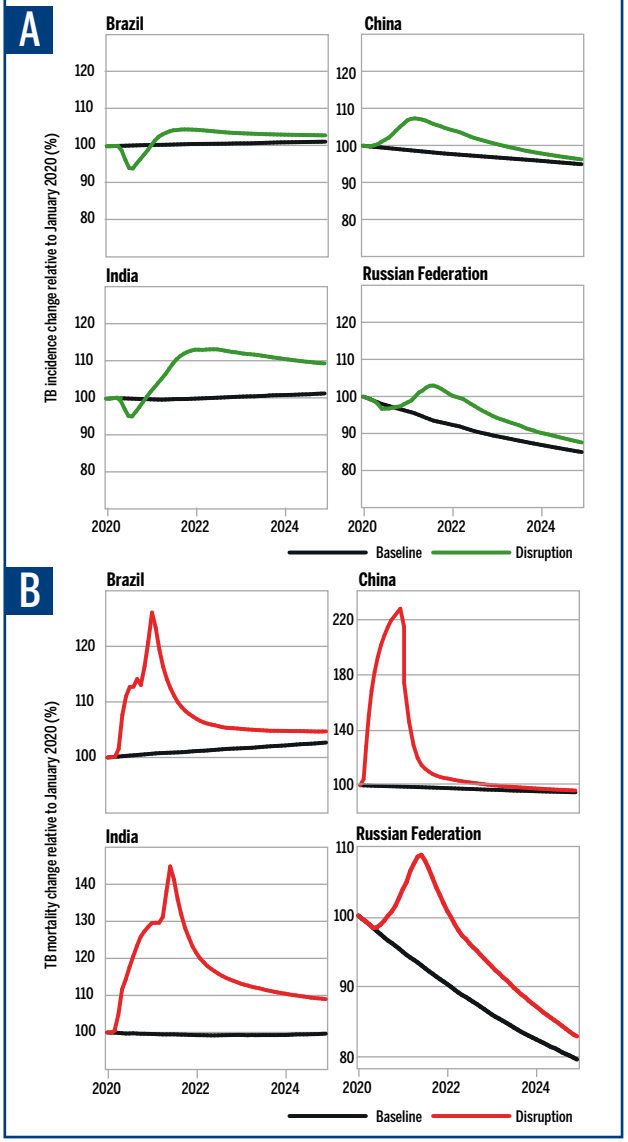


A. Countries with highest burden of TB (total numer of estimated cases in 2020).
B. Countries by per capita indicence (cases per 100 000 inhabitants).
Over two-thirds of TB cases in the world are concentrated in the 22 high-burden TB countries.

** WHO does not endorse any company or branded products over others.*

Figure 4. Impact of COVID-19 on TB burden and mortality.[4]

Source: World Health Organization.* Global Tuberculosis Report 2021. Accessed March 1, 2022.



A. Due to the reduction in the detection of newly ill patients, there was an acute decline in TB incidence in 2020 (newly diagnosed cases) that is anticipated to be followed by an increase in TB burden over the next few years.

B. COVID-19 also caused an acute increase in the TB mortality in 2020 which is anticipated to slowly decline to baseline levels over the next few years.

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1.4 Molecular epidemiology

Molecular epidemiological tools, including next generation whole genome sequencing, have provided insights into **tuberculosis strain variability and its transmission**. There are thousands of different strains which can be grouped into 8 different lineages, which display different phenotypic and genotypic characteristics.[15] Besides providing insights into transmission dynamics, whole genome sequencing can also provide comprehensive information about susceptibility profiles, and targeted deep sequencing approaches are able to confirm drug resistance directly from clinical specimens.[16,17] This opens the doorway to a **precision medicine 'approach' to treat TB (see Chapter 5).**

Key learning points:

- Tuberculosis is a chronic multi-system infection caused by *Mycobacterium tuberculosis* complex.
- TB is not a single entity but a spectrum of disease, which includes exposed individuals where the infection has been eliminated, presumed LTBI, incipient TB, subclinical TB and active TB.
- In 2020, there were an estimated ~10 million newly ill patients with tuberculosis.
- COVID-19 has had a dramatic impact on reducing TB case detection. The number of undetected and undiagnosed cases in 2020 was estimated to be 4 million and mortality in 2020 increased by ~6% (compared to 2019).
- COVID-19 has set back TB control by at least 5 years with the increased TB mortality expected to be sustained over the next 3 to 5 years.

2 PATHOGENESIS

2.1 Transmission

Studies using molecular tools have shown that transmission may occur as a result of casual contact or in congregate settings (e.g. bars, churches, schools etc.) but that, surprisingly, transmission was more likely in a community setting than within the household.[18,19] In such settings, public transport and schools are potential sites for high transmission. Thus, **most transmission occurs in the community**.

However, the current global public health strategy is one of passive case finding, i.e. self-reporting of symptoms by patients leading on to diagnostic testing. Unfortunately, by that time, almost all the onward transmission and hence infection of other individuals has already occurred. Thus, reduction of transmission would greatly **benefit from an active case finding (ACF) strategy**, where the diagnosis and diagnostics are moved out of healthcare facilities (hospitals and clinics) and into the community **(see Chapter 4)**.

Several components impact TB transmission, including:

- **Determinants in the person with active disease** (presence of a cough, cavitary disease, being non-immunocompromised, and having a high concentration of bacilli in the sputum which is associated with greater transmission);
- **Determinants in the exposed host** (background genetics impacting innate and adaptive immunity, duration of exposure, infecting dose);
- **Environmental factors** (good ventilation);
- **The organism itself** (certain clades like the Beijing strains are thought to be more infectious).[2] However, a recent study has indicated that mycobacterial genetic variants are less important than previously thought, and host pathogen interactions are likely more important.[20]

2.2 Latent TB infection (LTBI)

LTBI in the context of the lifecycle of TB and the spectrum of TB infection has already been outlined (**Figures 1 and 2**). Studies from macaques have provided insights into understanding the immunopathogenesis of LTBI.[21] However, in humans, the nature of the organism and the pathological appearance in tissues is not well defined and is controversial.

Individuals with presumed LTBI are able to contain the infection either within granulomas (discussed in section 2.3) or elsewhere. With sub-optimal host immunity there may be progression to active disease. Evidence that T-cell immunity has previously been induced (a positive tuberculin skin test (TST) or interferon gamma release assay (IGRA)) does not equate to LTBI. There is some controversy over what proportion of those with measurable T-cell responses have "definite" LTBI.[12] Other biomarkers that can accurately predict the stage of TB infection have been challenging to find. A recent study showed that blood transcriptional signatures may indicate the presence of incipient TB (**Figure 2**).[22]

2.3 Active TB

In those who develop active TB disease, particles <5μm in diameter harbouring mycobacteria enter the alveolar space where they infect macrophages and dendritic cells.[23] These cells, together with other innate immune mechanisms (including soluble factors), may result in eradication of the organism (sterilising immunity with or without the induction of adaptive immunity). Alternatively, mycobacteria are transported into the lung parenchyma (interstitium) and to the mediastinal lymph nodes where T-cell activation and clonal expansion occurs. The infection may be eradicated even at this stage or may progress to active disease in some individuals over a period of a few weeks to a few months.

Why there is a delay in T-cell priming (for up to several months) in some individuals with TB remains unclear. The archetypal pathobiological lesion of TB is the granuloma, which is thought to restrict the spread of infection and multiplication of the organism. Alternatively, the granuloma acts as an environment permissive to infection. Thus, the central part of the granuloma may undergo liquefactive necrosis resulting in multiplication of the organisms. Multiplication and expansion of this process causing an expanding bronchopneumonia, which may cavitate, causing the expression of large numbers of organisms into the airways, and leading to ongoing transmission.

The **critical virulence factors** that contribute to immunopathology are not well understood, but important factors include the ESX-1 system and lipoarabinomannan amongst others.[24,25] Although some immunodominant antigens have been characterised, and Th1 and other pathways/ molecules (including IFN-γ, IL-12, CD4 T-cells, CD8 T-cells, autophagy, granulysin-granzyme and cathelicidin) are thought to be critical for disease control, in themselves alone they cannot completely explain susceptibility to infection. Thus, clear-cut correlates of protective immunity remain undefined and are poorly understood. Lack of human lung challenge models remains a major unmet research need in the field. More recently, the feasibility and safety of challenging humans with live Bacillus Calmette-Guerin (BCG), and studying immune responses in the human lung, has been described.[26]

Key learning points:

- The transmission of TB occurs by inhaling particles <5 to 10 µm into the air sacs (alveoli) of the lung.
- Transmission of TB depends on factors specific to the person transmitting the disease, genetics and immunity of the exposed host, environmental factors such as ventilation, and virulence characteristics of the organism itself.
- Those who are smear microscopy positive are more likely to transmit disease.
- The archetypal TB lesion is the granuloma, which may encapsulate the organisms or be a site of active replication (this depends on the nature of host immunity).
- Liquefactive necrosis in the tissues leads to discharge of organisms into the airways leading to aerosolisation and onward transmission.

3 CLINICAL PRESENTATION

3.1 Latent TB infection (LTBI)

Individuals with presumed LTBI are asymptomatic **(see Figure 1).**

3.1 Active TB

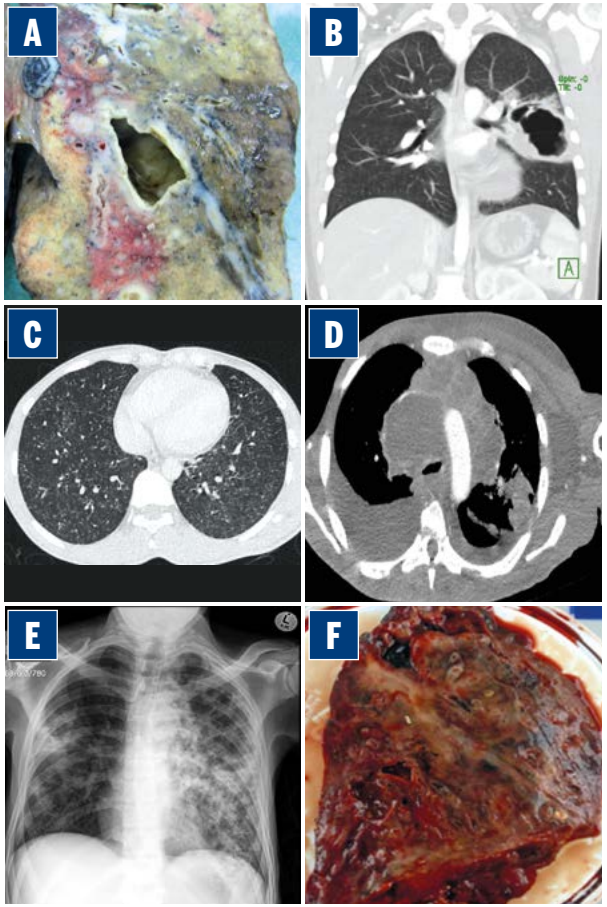
In the 75 to 85% of patients who present with pulmonary tuberculosis, typical symptoms are those of **cough, fever, night sweats, loss of appetite, chest pain, and hemoptysis**.[2] Clinical examination is consistent with an underlying pulmonary bronchopneumonia with or without cavitation (crackles and bronchial breathing). Depending on the genetic background, underlying immunological status, and other factors, ~15 to 25% of individuals present with extrapulmonary TB (EPTB). Almost any organ can be involved, but the most common presentations are those of **pleural effusion** (TB outside the lung but involving the parietal and visceral pleural membranes), and **lymphadenitis** (lymph node enlargement), followed by less common forms of EPTB including TB pericarditis, TB peritonitis, TB meningitis, etc.

In general, those with underlying immunosuppressive conditions and those at the extremes of age (young children and the elderly) present with unusual clinical features which may include atypical, minimal, or no symptoms, presentation with EPTB, atypical features on chest radiography (lack of cavitation and minimal or no infiltrates), and disseminated forms of TB. These features are therefore more common in HIV-infected persons and become more pronounced as the CD4 count decreases (<200 cells per mL and particularly at a CD4 count <50 to 75 cells per mL). Thus, in such individuals, diagnosis is also more challenging. Typical radiological and pathological features of TB are shown in **Figure 5**.

Patients with drug-resistant TB have the same clinical presentation as those with drug-sensitive TB, though at presentation they often have more advanced disease (because of diagnostic delay).

Figure 5. Gross pathology and radiological images of active pulmonary TB.

A-D: courtesy of Prof. Keertan Dheda. E: courtesy of Dr Phindile Gina at Groote Schuur Hospital. F: courtesy of Dr Anil Pooran and Prof. Keertan Dheda.



A: Pathological section of lung showing caseous necrosis and cavitation.

B: CT chest slice showing a large cavity in the left middle zone.

C: Coronal CT chest slice showing bilateral nodularity, typical of miliary TB.

D: TB-related rim-enhancing mediastinal lymphadenopathy with tracheal compression (superior mediastinal syndrome) and right pleural effusion.

E: CXR demonstrating bilateral patchy infiltration, worse on left than right in a 34-year-old female living with HIV who was NAAT positive for *M. tuberculosis*.

F: Pathological section of explanted lung showing caseous necrosis and numerous smaller cavities.

Key learning points:

- Active TB may be asymptomatic (also called subclinical TB) or have typical symptoms.
- About 15 to 20% of those with active TB develop the disease outside of the lungs (also called extrapulmonary TB; EPTB).
- Pleural effusion is often the most common manifestation of EPTB.
- Those with immunosuppressive conditions or who are at the extremes of age (young children and the elderly) may present with atypical clinical features, higher rates of EPTB, and atypical radiological features.

4 DIAGNOSIS

4.1 Latent TB infection (LTBI)

There is no microbiological standard or reference for LTBI.[13] Therefore, by measuring the *in vivo* or *in vitro* immune responses, it can be inferred that someone probably has LTBI. As there is no gold standard, **sensitivity estimates are based on test performance in active TB** (with the assumption that in LTBI performance would be similar or better). Specificity approximations are based on test performance in very low TB incidence settings and along a gradient of exposure (comparing performance in those with high versus low exposure).

In general, test sensitivity is lower in immunocompromised populations (e.g. HIV, renal disease, immune mediated inflammatory diseases, etc.). In such populations, strategies to improve sensitivity include using different readout formats (e.g. ELISPOT format) and combining TST and IGRA. In such cases, it is important to remember that as tuberculin has ESAT-6 and CFP-10 proteins, it will artifactually 'boost' the IGRA signal for at least several months.[27]

There is some evidence that the magnitude of the interferon gamma response might be predictive of a higher mycobacterial burden or progression along the spectrum of TB disease.[28] However, recent studies have indicated that using the magnitude of the interferon gamma response is not clinically useful to distinguish the stage of TB infection or predict disease progression. Thus, better biomarkers that can predict the stage of infection within the spectrum are needed.

Diagnostic testing should only be performed if there is serious intention to treat presumed LTBI. Test interpretation (sensitivity and predictive value) will depend on pre-test probability of disease. Thus, a test is more likely to detect underlying LTBI in those with recent contact with someone with active TB, of more advanced age, with an underlying immunosuppressive condition or taking immunosuppressive medication, with chest X-ray abnormalities, or smokers. The timing of BCG vaccination can impact the specificity of tests like the TST.

A risk stratification calculator, which can take into account all these variables and estimates the likely future cumulative risk of active TB (hence identifying those most likely to benefit from testing and preventative therapy (PT)) is available at www.tstin3d.com.

Potential adverse event rates should also be considered when using PT, such as hepatotoxicity (~1% overall with isoniazid but with risk increasing in those over 35 years of age), as well as the cumulative risk reduction in future development of TB from onset of PT to end of life (risk decreases by ~0.3 to 0.5% a year after PT).

Thus, there are many factors to consider besides test performance alone. It is also critical to consider the entire cascade of LTBI management (triaging, diagnosis, and treatment including sub-optimal adherence to therapy). In many studies, treatment adherence is only ~50 to 60% and thus appropriate follow-up and attention needs to be paid to this.

In low burden settings, testing for LTBI is usually performed in contacts of patients with active TB, immigrants from TB endemic settings (including those who have recently visited such settings), healthcare workers, and others at high risk of exposure and hence LTBI.

Finally, **in many high burden settings**, given the high force of infection (high risk of repeated exposure) in specific sub-populations (e.g. HIV-infected persons and children under 5 years of age), testing for LTBI is not performed despite treatment with PT on the assumption that there is a very high risk of LTBI. Thus, in these subgroups, PT is given empirically. However, this approach will need re-appraisal given recent recommendations by the WHO that HIV-negative close contacts of those with active TB should be considered for PT.[29]

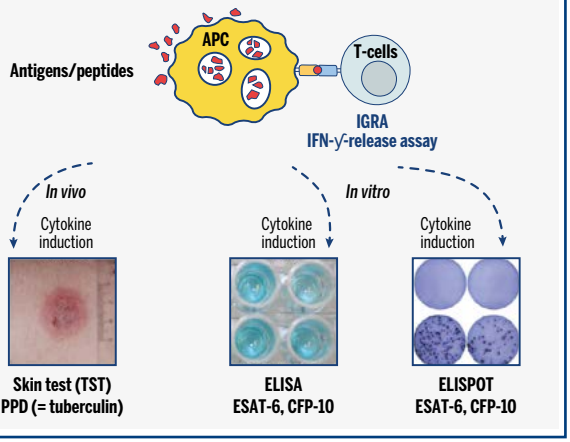
Thus, many factors need to be taken into account in the LTBI cascade of care, and test selection will depend on several factors, including different test formats available (**Figure 6**) and the pros and cons of each test format **(Table 1)**.

It is important to appreciate that the tests outlined in Figure 6 and Table 1 cannot distinguish between latent and active TB. The positive predictive value of these tests for the development of active TB in the short term (over ~2 to 3 year period) is only ~3%.[30]

Thus, both the skin and blood tests for LTBI are imperfect tests but are currently used because there are no better alternatives. Development of tests that can distinguish between latent and active TB, and between recent and remote infection, are a research priority.

Figure 6. Tests for latent TB infection (LTBI).

Adapted from Bumbacea D. *et al.*, *Eur Respir J.* 2012:40(4):990-1013. DOI: 10.1183/09031936.00000712. Copyright 2022.



Immune-based T-cell assays for the diagnosis of a latent infection with *Mycobacterium tuberculosis*. All tests rely on stimulation with either purified protein derivative (PPD) or *M. tuberculosis* specific antigens that elicit a cytokine induction in specific T-cells. Cytokines may be detected *in vivo* by skin testing or *in vitro* by interferon gamma release assays: ELISA or ELISPOT.

4.1.1 Skin tests (TST and RD1-specific skin tests)

The **tuberculin skin test (TST)** relies on a delayed hypersensitivity reaction developing 48 to 72 hours after the administration of tuberculin intradermally.[13] A positive test is indicated by the size of the induration. Advantages and disadvantages of the TST are outlined in **Table 1**. The major disadvantage of the TST is lack of specificity in those who have been BCG vaccinated[27] and/or have been significantly exposed to environmental mycobacteria (common in TB endemic settings). To overcome these hurdles, an **RD-1-specific skin test** (only containing ESAT-6 and CFP-10 proteins and not all the other proteins contained in tuberculin) has been developed. Preliminary clinical trials have been very encouraging.[31] The RD-1 gene encodes for two immunogenic proteins, ESAT-6 and CFP-10, but this gene is absent in BCG. Hence, tests containing only RD-1-associated proteins (described in the next sub-section and unlike BCG) have improved specificity. However, RD-1-associated proteins are found in some environmental mycobacteria.

4.1.2 Blood tests (IGRAs)

The pros and cons of the IGRAs are shown in **Table 1**. The tests are currently accessible in 2 different formats. One uses an **ELISA format** after overnight incubation with RD-1 antigens, and includes readouts to peptides preferentially processed by CD8 T-cells.[32] The second format consists of **spots representing the cytokine footprint of a T-cell producing interferon gamma** and enumeration and requires an ELISPOT reader.[33] Both these formats include overnight incubation with RD-1 antigens, processing of the samples, and using instruments to obtain a readout. There is a need for improved IGRA test formats that are more rapid, robust and automated.

Table 1. Pros and cons of commercially available tests for LTBI.

	Tuberculin skin test (TST)	Interferon gamma release assay (IGRA)
Cost	+	+++
Length of time to diagnosis	++	+
Return visit required	+++	+*
Specificity	+	++++
Physician skill	+	NA
Lab requirements	NA	+++
Phlebotomy needed	No	Yes
PPV for active TB	Poor	Poor
Can discriminate between latent and active TB	No	No
Limited by BCG vaccination	Yes**	No
Comments	Antigens in tuberculin found in many environmental mycobacteria	Does not require a return visit to readout the result

*Return visit may still be required to inform and advise the patient. **Only an issue if BCG is given.
NA: not applicable; PPV: positive predictive value

4.2 Active TB

The diagnosis of active TB generally depends on microbiological proof of disease.[2] However, a combination of factors, including test performance, may result in difficulty in proving the diagnosis. Therefore, empiric treatment is not uncommon (in most settings microbiological proof is only obtained in ~50 to 60% of patients started on treatment). Furthermore, some microbiological tests have sub-optimal sensitivity and specificity. Thus, pre-test probability must be taken into account when interpreting test results (exposure history, symptoms, signs, and radiological findings, host immune status).

Failure to establish the diagnosis is often due to sampling error.[34] Thus, ~10 to 15% of patients with pulmonary TB (PTB) are sputum scarce (inability to produce sputum). Even if sputum is obtained, the sample quality may be poor or there may be very low concentrations of mycobacteria. The frequency of

these factors, in general, tends to be higher in those with immunocompromising conditions, in children, and those with EPTB. In such cases, alternative sampling strategies may be required such as sputum induction[35,36] and bronchoscopy. However, proper instruction in collecting sputum is important in improving yield.[37] In EPTB, a sample is often obtained through needle aspiration of the affected area or organ (e.g. pleural aspiration, pericardial aspiration, etc.). The biological sample is then subjected to testing. Organ biopsy (e.g. of the liver, parietal pleura, peritoneum, etc.) may also be undertaken. Molecular tests and TB culture often have poor yield in extra-pulmonary compartments (<30% to 40%) and better biomarkers for diagnosis are needed.

In children, collection of samples from the gastrointestinal tract is often undertaken in order to circumvent the hurdle of obtaining a sputum sample (gastric aspirates and the string test*).[35] In some cases, typical radiological findings (e.g. cavitary disease on chest X-ray, or abdominal lymphadenopathy in HIV-infected patients with advanced immunosuppression) can provide enough confidence to initiate therapy.

However, one of the **key drawbacks of empiric treatment is that the presence of rifampicin resistance can be missed**. In TB endemic countries where the rate of rifampicin-resistance is more than 5% in either new or retreatment cases, an attempt should be made to clarify the rifampicin-resistant status in everyone evaluated for TB diagnosis. In TB non-endemic countries, those with appropriate risk factors (e.g. previous TB or with adherence issues in the past) should be evaluated for rifampicin resistance.

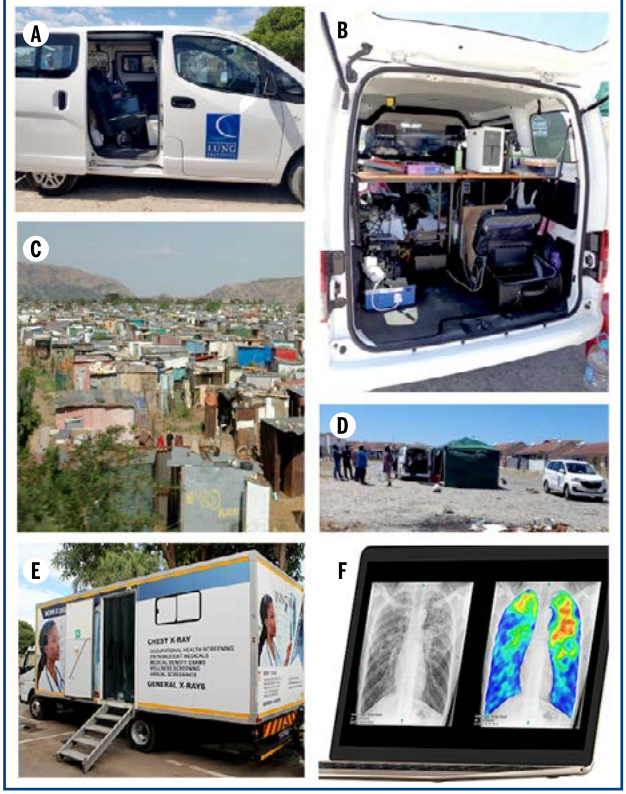
The current public health strategy for diagnosing active TB requires an urgent re-appraisal.[38] Patients self-reporting to healthcare facilities and then undergoing diagnostic testing is known as passive case finding.[2] By contrast, active case finding (ACF) involves taking sample collection and diagnostic strategies out of healthcare facilities and into communities or environments where there are high rates of TB (also called TB hotspots). **An ACF strategy will impact TB transmission and hence burden of disease.**[38]

The need for such strategies has become even more pressing given the reduction in case detection due to COVID-19.[4] ACF strategies may include the use of mobile phone-based screening Apps, targeting testing based on the presence of symptoms, targeted screening of individuals attending healthcare facilities for unrelated reasons, door-to-door finding of cases, and innovative strategies using mobile clinic-based portable X-ray and DNA detection technologies (**Figure 7**).

*String test: involves a string swallowed by patients and exposed to gastrointestinal secretions that is later analyzed for TB diagnosis and drug-resistance detection by nucleic acid amplification tests (NAAT).

Figure 7. Examples of active case finding models in peri-urban settlements using mobile clinics and X-ray units.

A-D: courtesy of Prof. Keertan Dheda. E-F: courtesy of Delft Imaging (www.delft.care)



A low-cost scalable model comprising a panel van (A and B) operated by two non-specialized healthcare workers is used for community-based TB screening and contact tracing (C and D). E and F show an alternative model of active case finding using mobile X-ray units for mass screening together with heatmap-based computer-aided detection (CAD) of TB.

Different diagnostic test options when evaluating patients for active TB are discussed in the following pages and shown in **Figure 8,** and the pros and cons of each diagnostic test are outlined in **Table 2.**

4.2.1 Smear microscopy

With the advent of molecular diagnostics, **smear microscopy is no longer the frontline test** in several TB endemic and TB non-endemic countries. The level of detection varies from 5 000 to 10 000 mycobacteria per mL. Only ~50% of patients are smear positive.[4] Patients who are smear positive are likely to be more infectious.

4.2.2 Mycobacterial culture using sputum or other biological samples

This represents the **semi-gold standard for TB diagnosis**. Automated systems are now commonly used to culture the organisms. Speciation tests are used to confirm the type of mycobacterial strain in positive cases. The level of detection varies from 10 to 100 mycobacteria per mL.

4.2.3 Nucleic acid amplification tests using sputum or other biological samples

There are a range of different **nucleic acid amplification tests (NAAT) formats** that include the line probe assays, isothermal amplification platforms, kit-based formats, some of which are also able to detect rifampicin resistance, semi-automated systems, and fully automated systems where buffered sputum is added to a cartridge.[39] The level of detection of NAAT platforms varies from ~30 to 150 mycobacteria per mL.[40] One important drawback of these tests is that they are unable to distinguish between current TB versus those previously treated for TB and who have now recovered (DNA lingers around for many years and recurrent TB is a major problem in TB endemic countries).

4.2.4 Urine tests

Available tests **detect lipoarabinomannan (LAM) in the urine** using polyclonal antibodies on a simple lateral flow assay, providing a point-of-care (POC) readout within 20 minutes.[41] The current format is only useful in HIV-infected persons, particularly in those with a CD4 count <200 (where average sensistivity is ~30% though sensitivity increases with decreasing CD4 count).[41] This test is useful in HIV endemic settings in hospitalised patients where LAM is used as a screening test, and in HIV-infected outpatient populations (particularly when CD4 count is <200 cells per mL).[42,43] A more sensitive platform is being developed, has shown encouraging results[44] and is in the process of being commercialised. Development of POC tests based on host or mycobacterial markers is an important area of research and development.

4.2.5 Genomic sequencing

Subjecting extracted DNA from a culture isolate to whole genome sequencing has enabled the **documentation of the resistance profiles of many drugs simultaneously**. Although the cost of this technology has reduced dramatically, the main drawback is the need to culture the isolate and hence the readout is obtained 6 to 10 weeks after therapy has started.

Alternative approaches, such as targeted sequencing, use DNA extracted directly from a sputum sample.[45,46] Clinical trials on the utility of such technologies and their impact on clinical practice are underway, but the main drawback is the sub-optimal concentration of DNA in at least half of the sputum samples.

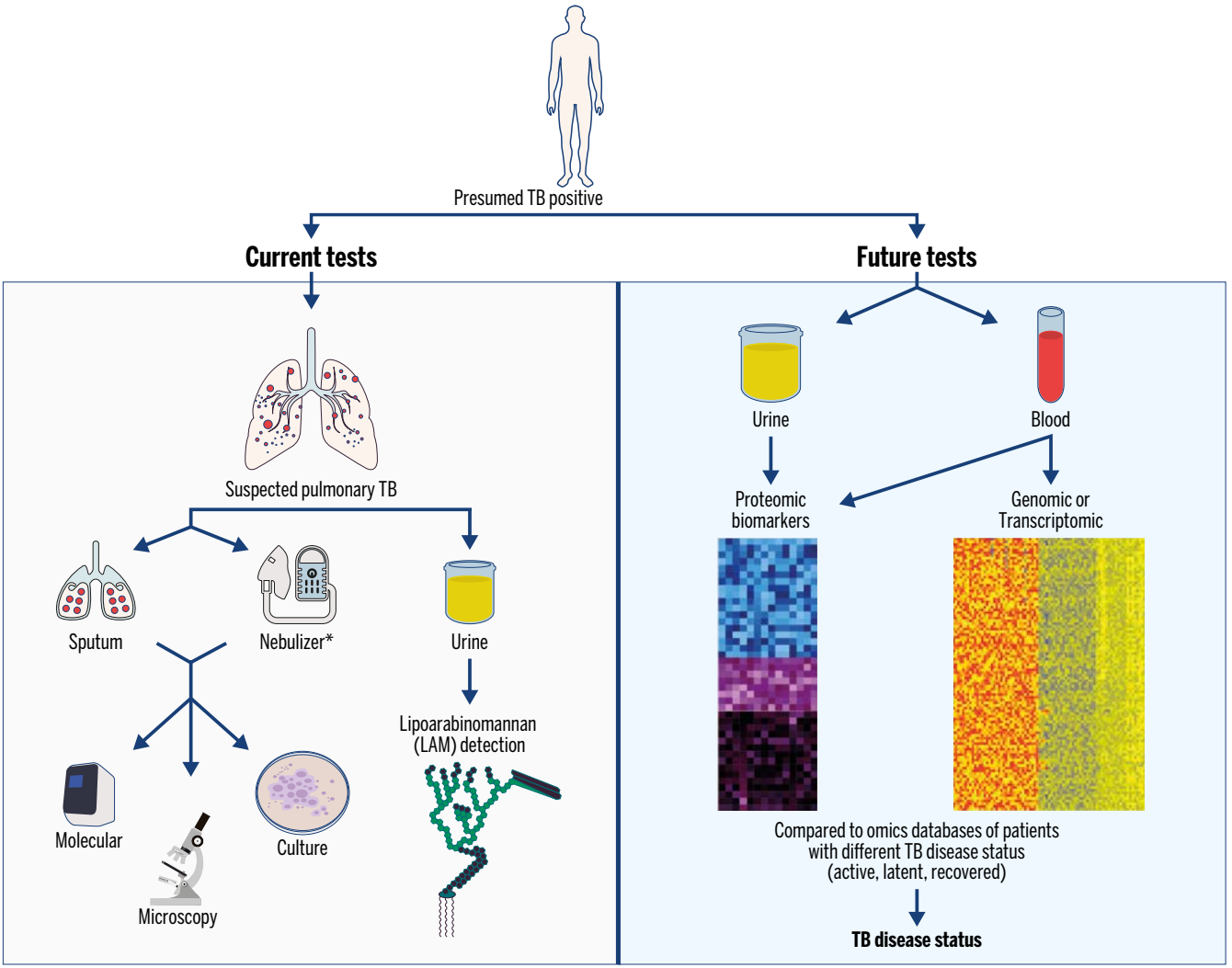
4.2.6 Blood tests

There are currently **no commercially available blood-based tests for active TB**. However, there are encouraging data from recently published trials and transcriptional signatures are currently being trialled for their diagnostic utility in active TB.[47] Other approaches are looking into detecting TB DNA or antigen in human blood.

Key learning points:

- The presence of LTBI can only be inferred from diagnostic tests like the TST and the IGRAs.
- Tests for LTBI should only be performed if there is an intention to treat for LTBI.
- Interpretation of the IGRA and TST will depend on pre-test probability, timing of previous BCG vaccination, and underlying host immunity.
- Diagnosis of active TB depends on microbiological proof of the disease (smear microscopy, culture, NAAT, and antigen detection tests such as urine LAM).
- In ~40 to 50% of treated cases, a microbiological diagnosis cannot be made and therapy is empiric.
- In children, those with EPTB, and those with immunocompromising conditions, a sputum sample may not be obtainable and/or is of poor quality. In such cases, alternative sampling strategies are required.
- In appropriate contexts, tests to determine whether the organism is resistant to rifampicin should be performed.
- Ideally, diagnostic testing for active TB should be moved out of healthcare facilities into the community thus interrupting transmission early on in the lifecycle of TB (this is called active case finding).

Figure 8. Tests used for the microbiological diagnosis of TB.



*Nebulized hypertonic saline or bronchoscopy may be used to obtain samples in sputum scarce persons.

Table 2. Diagnostic assays and tests currently used for the diagnosis of active TB.

	Imaging	Smear microscopy	Culture	Nucleic acid amplification tests (except LPA)	LPA	Urine LAM antigen detection test
Cost	++	++	+++	++++	++++	+
Time to diagnosis	+ (30-60 min)	+++ (same day)	++++++ (4-8 weeks)	+ (same day for automated systems; or up to 2-3 days)	++++ (2-4 days)	+ (~30 min)
Sensitivity	++ (80-90%)	++ (50%)	++++ (80-95%)**	+++ (~80-85%)**	+++ (~80-85%)**	+*** (~30% when CD4 <200 cells/mL)
Specificity	+* (Poor, ~50-70%)	+++ (≥95%)	+++ (≥95%)	+++ (≥95%)	+++ (≥95%)	++ (~90%)
***Mtb* distinguishable from NTM**	No	No	Yes	Yes	Yes	No
High skill and laboratory requirements	+	+	++++	++++	+++++	+
Can detect rifampicin resistance	No	No	+++	++	++	No
Dependence on good quality sputum sample	NA	+++	+++	+++	+++	No
Comments	High sensitivity but poor specificity.	Low cost test commonly used.	Prolonged time to result.	Rapid and sensitive but cannot distinguish between current and previous disease.	As for NAATs but more labour intensive.	Point of care test but poor sensitivity. Useful only in HIV-infected persons.

*Dependent on HIV and smear microscopy status. **Dependent on quality of sample and concentration of mycobacteria and less sensitive in smear-negative samples. *** Dependent on CD4 count.

LPA: line probe assay; NAAT: nucleic acid amplification test; NTM: nontuberculous mycobacteria; Urine LAM: urine lipoarabinomannan assay

For interpretation of diagnostic test results for LTBI and active TB, refer to Figure 1, page 6.

5 TREATMENT AND PROGNOSIS

5.1 Latent TB infection (LTBI)

Treatment options and regimens for LTBI, along with their benefits and limitations, are summarised in **Table 3**.

Controlled trials done several decades ago showed that treatment with 9 months of isoniazid reduced future development of active TB in the short term by ~70%.[48] However, adherence has been a major challenge and has led to the move towards using 2 drugs for a shorter duration (as short as ~1 month; **Table 3**).

Historically, development of isoniazid and/or rifamycin resistance has not been borne out. In environments where there is a high force of infection (high risk of repeated exposure), regimens have been shown to be effective up to about 3 years. In cases where there has been exposure to a patient with rifampicin-resistant TB, after appropriate risk stratification, PT based on susceptibility testing of the isolate should guide treatment (usually a fluoroquinolone with or without additional drugs).[49]

Trials exploring newer drugs (e.g delamanid) for preventative therapy against rifampicin-resistant isolates are currently in progress.[49]

5.2 Active TB (including drug-resistant TB)

Regimens for drug-sensitive, isoniazid mono-resistant, and rifampicin-resistant TB are outlined in **Table 4**.

More recently, a multinational trial showed successful use of a **4 month shortened regimen containing rifamycin and moxifloxacin**.[13] Challenges for the introduction of such regimens will include cost, roll-out of rifamycin in TB endemic countries, and high rates of fluoroquinolone resistance in South East Asia.[3,4,8,50] Another concern is rapid development of fluoroquinolone resistance, which could potentially eliminate one of the key drugs from the MDR-TB regimen. There have been recent changes in the definition of MDR and XDR-TB (**Table 4**).

On-going trials are exploring newer drug combinations to construct shorter treatment regimens and there is a push towards personalised medicine, i.e. tailoring the length and type of regimen to the patient. Factors to consider might include the drug susceptibility profile, radiological disease extent, and mycobacterial burden. There is also a **trend to using an all-oral regimen**, and **regimens shorter than 9 months** for the **treatment of rifampicin-resistant/MDR-TB.**

Recent evidence indicates that a 6 month regimen is feasible, and several trials are underway to optimise the constituents of an all-oral 6 month regimen using newer and repurposed drugs (e.g. bedaquiline, linezolid, delamanid, clofazimine, etc.), which should become available in the near future.

Table 3. Regimens used for the treatment of latent TB infection (LTBI).

Regimen	Duration	Benefits	Limitations
Isoniazid	Daily for 6-9 months	Single drug. No interactions with ART.	Prolonged and may impact compliance. Risk of peripheral neuropathy.
Rifampicin	Daily for 4 months	Single drug. Short duration. Safe and non-inferior to INH monotherapy.	Drug-drug interactions with ART.
Isoniazid and rifampicin	Daily for 3 months	Short regimen Safer + non-inferior to 9m INH monotherapy. Available in child-friendly water dispersible format.	Drug-drug interactions with ART.
Isoniazid and rifapentine	Once weekly rifapentine and daily INH for 3 months	Shortest regimen. Non-inferior to 9m INH monotherapy + safer.	Optimal rifapentine dose not established in children <2 years of age. Expensive. Possible drug-drug interaction with ART.

ART: antiretroviral therapy; INH: isoniazid

Table 4. Regimens used for the treatment of active TB.

	Resistance profile	Drugs	Interval/dose
Newly diagnosed TB	Without RIF resistance	RIF + INH + pyrazinamide + ethambutol	RIF + INH + pyrazinamide + ethambutol for 2 months followed by RIF and INH for 4 months.
INH mono resistant TB	INH mono-resistance	RIF, ethambutol, pyrazinamide, and FQ	Daily for 6 months
		RIF, ethambutol, pyrazinamide (fixed dose tablets may contain INH)	Daily for 9 months
MDR/RR-TB	RIF mono-resistance or resistance to RIF and INH.	Regimen containing at least 4 -5 likely susceptible drugs using group A drugs first and adding the remaining drugs from group B and/ or C. Group A drugs include levofloxacin/ moxifloxacin, bedaquiline and linezolid. Group B drugs include clofazimine and cycloserine (terizidone). Group C drugs include ethambutol, delamanid, pyrazinamide, imipenem-cilastatin OR meropenem, amikacin, ethionamide OR prothionamide, and P-aminosalicyclic acid (PAS).	Total 18 – 20 months. 9 - 11 months are being used in the context of operational research.
Pre-XDR-TB	RIF + FQ resistance	Regimen containing at least 4 -5 likely susceptible drugs using group A drugs and the remaining drugs from group B or C.	Total 18 – 20 months
XDR-TB	RIF + FQ resistance + 1 other group A drug	Regimen containing at least 4 -5 likely susceptible drugs using group A drugs and the remaining drugs from group B or C.	Total 18 – 20 months

FQ: fluoroquinolone; INH: isoniazid; RIF: rifampicin; MDR-TB: multidrug-resistant TB; RR-TB: rifampicin-resistant TB; XDR-TB: extensively drug-resistant TB.

Each level of resistance (e.g. isoniazid monoresistance), how it is defined, and the type of regimen used is outlined.

Key learning points:

- There are several potential treatment regimens for LTBI with newer regimens containing 2 drugs and being given for as little as 4 weeks duration.
- Standard short course therapy for TB that is not rifampicin-resistant is 6 months with 4 drugs.
- Isoniazid monoresistant TB is often treated by adding a fluoroquinolone to the short course regimen.
- Rifampicin-resistant or MDR-TB is treated with an all-oral regimen of second line drugs (the key drugs include a fluoroquinolone, bedaquiline, and linezolid).
- Pre-XDR-TB is defined as resistance to rifampicin and a fluoroquinolone, while XDR-TB refers to resistance to rifampicin, fluoroquinolone, and in addition, resistance to either bedaquiline or linezolid.

6 PREVENTION

There are 5 key strategies to preventing TB:

(i) control of underlying risk factors;

(ii) TB exposure reduction in the general population and healthcare workers (primarily through improved ventilation in work, travel and living spaces);

(iii) reduction in transmission through screening and active case finding strategies;

(iv) use of preventative therapy to treat LTBI;

(v) vaccination.

6.1 Control of risk factors

Control of HIV and provision of antiretroviral therapy is critical to TB control in many settings but particularly in Africa. Other inhaled pollutants, such as exposure to biomass fuels (due to cooking over open fires), outdoor air pollution, and smoking, are also major drivers of TB globally.[2] Economic development and reduction in poverty and overcrowding led to a substantial decline in TB incidence in Europe many decades prior to the introduction of anti-TB drugs.

6.2 Exposure reduction

Healthcare workers are at higher risk of developing TB. Preventative measures include appropriate triaging of patients and administrative controls, use of environmental controls, such as ultraviolet germicidal irradiation and air extraction systems (at least 10 to 12 air changes per hour), and the use of personal protective measures (N95 or FFP2 like masks which are able to filter out TB organisms from inhaled air).

In many TB endemic countries, most TB transmission in the general population occurs outside the household in congregate settings like schools, churches, buses, and taxis, and therefore cough etiquette and good ventilation in vehicles, trains and buildings are essential.

6.3 Reduction in transmission

Various strategies, including active case finding, screening strategies using digital health, and screening of individuals in healthcare facilities have been discussed in **Chapter 4**.

6.4 Preventative therapy (see section 5.1)

6.5 Vaccines

The current vaccine administered in most of the world, **Bacillus Calmette-Guerin (BCG)**, protects against disseminated childhood forms of tuberculosis such as TB meningitis. However, protection wains in the teenage years and BCG only provides ~30% protection in adults.[26]

Encouragingly, a phase 2b study evaluating the **M72/AS01 vaccine candidate** was found to provide ~50% protection against active TB compared to individuals who were BCG vaccinated at birth.[51] Phase 3 trials are planned. This was an important development because it showed that **vaccines could provide a greater level of protection than natural infection and BCG vaccination**. There are several vaccine candidates in the pipeline at various stages of development (selected candidates are outlined in **Table 5**).

Exactly why some people get TB and some people don't is poorly understood, and thus better proxy measures of protection are urgently needed. This is a major hurdle in vaccine development. Development of a human lung challenge model for TB is also a major unmet need. More recently, the safety of a live BCG lung challenge model was published.[26] Further trials are ongoing.

The extraordinary speed at which a COVID-19 vaccine could be developed and rolled out globally has been instructive. The TB field could benefit from many of the lessons learnt from COVID-19, including technology transfer.

Table 5. Selected vaccine candidates that are currently in phase II or phase III trials.

Vaccine candidate	Phase	Mode of action	Origin	Application
TB/FLU-04L	II a	Viral vector vaccine (influenza virus)	*M. tuberculosis*	Adult/ adolescents/ therapeutic
ID93/GLA-SE	II a	Protein/adjuvant	4 *Mtb* antigens (Rv2608, Rv3619, Rv 3620, Rv1813)	Adult/ adolescents/ therapeutic
H56:IC31	II b	Protein/adjuvant	3 *Mtb* antigens (Ag85B, ESAT-6, Rv2660c) + adjuvant IC31	Adult/ adolescents/ therapeutic
MTBVAC	II a	Live attenuated vaccine (double deletion of phoP-fadD26 virulence genes)	*M. tuberculosis*	Newborn/ adult / adolescents
BCG revaccination	II a	Live attenuated vaccine (loss of >100 genes with RD* deletions)	*M. bovis*	Adult/ adolescents
VPM1002	III	Live attenuated vaccine (urease C deletion and listeriolysin insertion)	*M. bovis*	Newborn/ adult/ adolescents/ therapeutic
Vaccae™	III	Whole cell inactivated/ fragmented mycobacteria (heat)	*M. vaccae*	Adult/ adolescents
M72/AS01E	III	Protein/adjuvant (Planned to start shortly)	2 *Mtb* antigens (32A, 39A)	Adult/ adolescents

*RD: regions of difference

A full list of candidates can be found at: https://newtbvaccines.org/tb-vaccine-pipeline/

Key learning points:

- There are 5 key strategies to preventing TB which include control of underlying risk factors, exposure reduction, reduction in transmission, vaccination, and use of preventative therapy to treat LTBI.
- The BCG vaccine is protective against disseminated childhood forms of TB but protection wanes during adulthood.
- The M72/AS01 vaccine was found to have ~50% protection against active TB; phase III trials are due to start shortly.

CONCLUSIONS AND FUTURE PERSPECTIVES

TB is far from eradicated and remains one of the foremost infectious disease killers globally. COVID-19 has resulted in reduced case detection and thus TB mortality increased by ~6% in 2020 with excess mortality forecast for the next few years. **Two in five TB cases remain undiagnosed or undetected**. Recent research has led to newer and shorter treatment regimens and options for LTBI, active TB, and MDR-TB.

Major research priorities include:

- Understanding the immunopathogenesis of TB (comprehending why some people get TB and others don't);
- Determining whether those who are asymptomatic can transmit disease;
- Determining why some people fail to develop LTBI despite heavy exposure;
- Developing a sputum independent triage test and rule-in test for active TB;
- Developing effective ACF models for TB;
- Developing better treatment approaches so that one short treatment regimen could be used for all types of TB irrespective of drug resistance pattern;
- Developing an effective preventative vaccine.

However, in addition to an effective vaccine, the eradication of TB will require improved funding for research and development, implementation of interventions, and more importantly political will together with a sustained reduction in global poverty and overcrowding. Indeed, a recent study found that **ending extreme poverty and expanding social protection coverage globally** could reduce the TB burden by ~85% worldwide.[52]

10 KEY QUESTIONS AND ANSWERS

1 Has TB been eradicated?

TB is far from eradicated. Over 10 million people become newly ill with tuberculosis every year. The COVID-19 pandemic has resulted in reduced detection of TB and almost 4 million TB cases remained undetected or undiagnosed in 2020. The declining TB mortality over the last decade has been abruptly reversed by the advent of the COVID-19 pandemic. There has been a resurgence of drug-resistant TB in Eastern Europe and Russia.

2 How to determine if patients with TB are infectious?

Patients with a high concentration of TB bacilli in the sputum (e.g. those who are smear microscopy positive), who have a strong cough, and who do not have underlying immunocompromising conditions are more likely to be infectious. However, only a modest proportion of those who are smear positive are highly infectious and thus better biomarkers are needed to more accurately determine who is infectious. Such biomarkers could target patients for transmission-limiting interventions especially if they have rifampicin-resistant TB. However, such biomarkers could also serve as a gateway to better study the disease and interrupt transmission.

Research methods to determine the infectiousness of patients include subjecting animals to infectious aerosol (e.g. guinea pigs) or enumerating the number of culturable bacilli in particles <10μm (also called cough aerosol sampling). Transmission may be inferred in several ways including TST or IGRA conversion, comparing whole genome sequencing readouts in TB isolates, and documenting downstream development of active TB in those who were significantly exposed. However, such methods are imprecise and often impractical to use in clinical practice.

3 Is there a microbiological test for LTBI?

There is no microbiological reference standard or test for LTBI because the organism cannot be recovered from the tissues in such persons. The likely presence of LTBI can only be inferred from tests that measure immunological responses to TB antigens. Such tests include interferon gamma release assays (IGRAs) and the tuberculin skin test (TST).

4 What are the main pros & cons of current tests for LTBI?

TST is the original test for TB detection and is inexpensive, but has several drawbacks:

- Requires a return visit to complete the test, a reliable supply of tuberculin, and training to accurately read the result (significant inter-reader variability);
- May lack specificity in BCG vaccinated persons;
- May be associated with skin ulceration during testing.

IGRAs do not require a return visit, provide an objective laboratory result and are more specific than the TST, however they also have some drawbacks:

- More expensive;
- Short-term variability;
- Need for more complex laboratory infrastructure, overnight incubation with antigens, and downstream laboratory processing to obtain a read-out.

Neither TST nor IGRA are able to distinguish between active TB and LTBI, nor predict risk of LTBI progression to active TB.

Both assays are negatively impacted by immune depression (e.g. HIV co-infection).

5 What proportion of active TB that is treated is microbiologically proven and what are the unmet diagnostic needs?

Although there are several microbiological tests for active TB, including culture, NAAT, LAM urine antigen detection tests, etc., a microbiological diagnosis can often not be proven. This is mainly related to the inability to obtain an adequate sample or because of the very low concentration of mycobacteria within the sample. Such an occurrence is more common in those with immunocompromising conditions, those at the extremes of age (young children or the elderly), or those with EPTB. Up to a third of TB patients fall into this category. In some cases, the microbiological load is beyond the detection limit of sensitive tests like the NAATs. Thus, in about 40 to 50% of cases, the diagnosis is strongly suspected (but cannot be proven) and empiric treatment is given. Therefore, a major unmet need is to develop a sensitive and reasonably priced sputum-independent test for TB.

6 What are the indications for treatment of presumed latent LTBI in low and high burden settings?

In low burden settings, the main indications for treating LTBI are positive tests in immigrants who are screened for TB and in individuals who have come into contact with someone with active TB.

By contrast, in high burden settings, LTBI is often not searched for or only treated in certain special situations (e.g. HIV-infected persons or children under the age of 5 years as these individuals are at very high risk of progression to active TB).

However, more recently it has been recognised that good TB control will not be possible without treating LTBI because of the large disease reservoir that it represents (later conversion to active TB). The WHO has therefore recommended that HIV-uninfected contacts of those with active TB should be offered preventative therapy if appropriate.

7 What are the recent advances in the treatment of drug-sensitive and drug-resistant TB?

Although the standard short course therapy for TB comprises 6 months of treatment with 4 drugs, a recent large multicentric trial found that a 4-month regimen containing a long-acting rifamycin and a fluoroquinolone was equally effective. Concerns and hurdles around implementing such a regimen globally include cost (the 6-month regimen is a low-cost and highly effective regimen) and high rates of fluoroquinolone-resistance in some parts of the world like South East Asia, which has a very high burden of TB. Rifampicin-resistant or MDR-TB is now treated with an all-oral regimen (injectables are no longer used). The backbone of such regimens includes new and repurposed drugs such as fluoroquinolones, bedaquiline, and linezolid.

This is a rapidly changing landscape and oral regimens of 9 to 12 months are currently being used in some parts of the world. It is likely that very soon a pan-oral 6-month regimen will be in use.

8 What common adverse events or side effects may occur when initiating treatment for latent or active TB?

The key drugs used to treat latent and/or active TB are the rifamycins and isoniazid. Both drugs can result in hepatitis and isoniazid may be associated with peripheral neuropathy (therefore pyridoxine is used concomitantly with treatment). Pyrazinamide is used to treat active TB and may be associated with hepatitis or a skin reaction. Ethambutol may result in ocular toxicity. Linezolid is a repurposed drug that is used in the treatment of rifampicin-resistant TB and may be associated with anaemia and neurotoxicity.

9 What methods can be used to determine whether a patient has been successfully treated for TB?

Most patients with active TB have culture converted by between 4 and 6 weeks after treatment initiation. Symptoms usually improve considerably over the first 6 weeks. Radiological improvement is usually evident by 8 weeks. Culture positivity, failure in resolution of symptoms, and absence of any radiological improvement after 8 weeks of treatment should raise suspicion about poor adherence or drug-resistance.

Better biomarkers to monitor treatment would be useful particularly in patients with drug-resistant TB where treatment regimens are generally longer and resolution of symptoms is often delayed.

10 How should healthcare workers protect themselves against becoming infected with TB?

The most important aspect of prevention is triaging and administrative controls. Patients with suspected TB should be placed in a designated well-ventilated area so that contact with other patients and healthcare workers are minimised. Such patients may include those with an appropriate history, presence of cough, and appropriate radiological findings. In those with suspected pulmonary TB, a negative NAAT test using a good sputum specimen, should provide enough confidence to allow de-isolation of the patient.

In low and middle income countries where TB is more common, environmental controls such as ventilation systems (opening the windows or air extraction systems providing at least 12 air changes per hour), and ultraviolet germicidal eradiation (often ceiling-based) reduces the risk of acquiring TB.

Finally, personal protective equipment such as an N95 or FFP2 like mask should be used. Such masks are capable of filtering almost all particles <5µm in diameter. Healthcare workers should ideally be fit tested to wear such masks. It is important to check that such masks are compatible with existing regulatory standards (commonly called N95 or FFP2 in different countries).

ABBREVIATIONS & ACRONYMS

ACF	Active Case Finding
BCG	Bacillus Calmette-Guerin (vaccine)
COVID-19	Corona-virus disease 2019
CT	Computerized tomography
CXR	Chest X-ray
DNA	Deoxyribonucleic acid
EPTB	Extrapulmonary TB
IGRA	Interferon Gamma Release Assay
LTBI	Latent TB Infection
MDR-TB	Multidrug-resistant TB
MOTT	Mycobacterium other than tuberculosis
NAAT	Nucleic acid amplification tests
NTM	Nontuberculous mycobacteria
POC	Point of care
PT	Preventative therapy
PTB	Pulmonary TB
RR-TB	Rifampicin-resistant TB
TB	Tuberculosis
TST	Tuberculin Skin Test
XDR-TB	Extensively drug-resistant TB
WHO	World Health Organization

REFERENCES

1. Paulson T. Epidemiology: A mortal foe. *Nature* 2013; **502**(7470): S2-3.
2. Dheda K, Barry CE, 3rd, Maartens G. Tuberculosis. *Lancet* 2016; **387**(10024): 1211-26.
3. WHO. Global Tuberculosis Report 2020. Geneva: WHO, 2020.
4. WHO. Global Tuberculosis Report 2021. Geneva: WHO, 2021.
5. Dheda K, Gumbo T, Maartens G, *et al.* The Lancet Respiratory Medicine Commission: 2019 update: epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant and incurable tuberculosis. *Lancet Respir Med* 2019; **7**(9): 820-6.
6. Dheda K, Limberis JD, Pietersen E, *et al.* Outcomes, infectiousness, and transmission dynamics of patients with extensively drug-resistant tuberculosis and home-discharged patients with programmatically incurable tuberculosis: a prospective cohort study. *Lancet Respir Med* 2017; **5**(4): 269-81.
7. American Thoracic Society. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. *American Journal of Respiratory and Critical Care Medicine* 2000; **161**(4): 1376-95.
8. Pai M, Behr MA, Dowdy D, *et al.* Tuberculosis. *Nature Reviews Disease Primers* 2016; **2**(1): 16076
9. Rastogi N, Legrand E, Sola C. The mycobacteria: an introduction to nomenclature and pathogenesis. *Rev Sci Tech* 2001; **20**(1): 21-54.
10. Barbier M, Wirth T. The Evolutionary History, Demography, and Spread of the *Mycobacterium tuberculosis* Complex. *Microbiol Spectr* 2016; **4**(4).
11. Zysk KG. The Science of Respiration and the Doctrine of the Bodily Winds in Ancient India. *Journal of the American Oriental Society* 1993; **113**(2): 198-213.
12. Behr MA, Edelstein PH, Ramakrishnan L. Is *Mycobacterium tuberculosis* infection life long? *BMJ* 2019; **367**: l5770.
13. WHO. Latent tuberculosis infection: updated and consolidated guidelines for programmatic management. Geneva: WHO, 2018.
14. Dheda K, Perumal T, Moultrie H, *et al.* The intersecting pandemics of tuberculosis and COVID-19: population-level and patient-level impact, clinical presentation, and corrective interventions. *The Lancet Respiratory Medicine* Published online March 23, 2022. https://doi.org/10.1016/S2213-2600(22)00092-3.
15. Peters JS, Ismail N, Dippenaar A, *et al.* Genetic Diversity in *Mycobacterium tuberculosis* Clinical Isolates and Resulting Outcomes of Tuberculosis Infection and Disease. Annual Review of Genetics 2020; **54**(1): 511-37.
16. Gygli SM, Keller PM, Ballif M, *et al.* Whole-Genome Sequencing for Drug Resistance Profile Prediction in *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* 2019; **63**(4):e02175-18.
17. Cohen KA, Manson AL, Desjardins CA, Abeel T, Earl AM. Deciphering drug resistance in *Mycobacterium tuberculosis* using whole-genome sequencing: progress, promise, and challenges. *Genome Medicine* 2019; **11**(1):45.
18. Verver S. Transmission of tuberculosis in a high incidence urban community in South Africa. *International Journal of Epidemiology* 2004; **33**(2): 351-7.
19. Verver S, Warren RM, Munch Z, *et al.* Proportion of tuberculosis transmission that takes place in households in a high-incidence area. *The Lancet* 2004; **363**(9404): 212-4.
20. Chai Q, Wang L, Liu CH, Ge B. New insights into the evasion of host innate immunity by *Mycobacterium tuberculosis*. *Cellular & Molecular Immunology* 2020; **17**(9): 901-13.
21. Cadena AM, Fortune SM, Flynn JL. Heterogeneity in tuberculosis. *Nat Rev Immunol* 2017; **17**(11): 691-702.

22. Kwan PKW, Periaswamy B, De Sessions PF, *et al.* A blood RNA transcript signature for TB exposure in household contacts. *BMC Infectious Diseases* 2020; **20**(1):403.
23. Scriba TJ, Netea MG, Ginsberg AM. Key recent advances in TB vaccine development and understanding of protective immune responses against *Mycobacterium tuberculosis*. *Semin Immunol* 2020; **50**: 101431.
24. Chan J, Fan XD, Hunter SW, Brennan PJ, Bloom BR. Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. Infection and Immunity 1991; **59**(5): 1755-61.
25. Roy S, Ghatak D, Das P, Bosedasgupta S. ESX secretion system: The gatekeepers of mycobacterial survivability and pathogenesis. *European Journal of Microbiology and Immunology* 2020; **10**(4): 202-9.
26. Davids M, Pooran A, Hermann C, *et al.* A Human Lung Challenge Model to Evaluate the Safety and Immunogenicity of PPD and Live Bacillus Calmette-Guérin. *American Journal of Respiratory and Critical Care Medicine* 2020; **201**(10): 1277-91.
27. Van Zyl-Smit RN, Zwerling A, Dheda K, Pai M. Within-Subject Variability of Interferon-g Assay Results for Tuberculosis and Boosting Effect of Tuberculin Skin Testing: A Systematic Review. *PLoS ONE* 2009; **4**(12): e8517.
28. Ledesma JR, Ma J, Zheng P, Ross JM, Vos T, Kyu HH. Interferon-gamma release assay levels and risk of progression to active tuberculosis: a systematic review and dose-response meta-regression analysis. *BMC Infectious Diseases* 2021; **21**(1):467.
29. WHO. WHO consolidated guidelines on tuberculosis. Module 1: Prevention - tuberculosis preventive treatment. Geneva: World Health Organization, 2020.
30. Lalvani A, Berrocal-Almanza LC, Halliday A. Predicting progression to active tuberculosis: A rate-limiting step on the path to elimination. *PLOS Medicine* 2019; **16**(5): e1002814.
31. Hoff ST, Peter JG, Theron G, *et al.* Sensitivity of C-Tb: a novel RD-1-specific skin test for the diagnosis of tuberculosis infection. *European Respiratory Journal* 2016; **47**(3): 919-28.
32. Barcellini L, Borroni E, Brown J, *et al.* First evaluation of QuantiFERON-TB Gold Plus performance in contact screening. *European Respiratory Journal* 2016; **48**(5): 1411-9.
33. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008; **149**(3): 177-84.
34. Heinmöller E, Renke B, Beyser K, Dietmaier W, Langner C, Rüschoff J. Pitfalls in diagnostic molecular pathology – significance of sampling error. *Virchows Archiv* 2001; **439**(4): 504-11.
35. Brown M, Varia H, Bassett P, Davidson RN, Wall R, Pasvol G. Prospective Study of Sputum Induction, Gastric Washing, and Bronchoalveolar Lavage for the Diagnosis of Pulmonary Tuberculosis in Patients Who Are Unable to Expectorate. *Clinical Infectious Diseases* 2007; **44**(11): 1415-20.
36. Olsen S-RJ, Long R, Tyrrell GJ, Kunimoto D. Utilization of Induced Sputum and Bronchoscopy for Diagnosis of Pulmonary Tuberculosis. *Chest* 2008; **134**(4): 154P.
37. Alisjahbana B, van Crevel R, Danusantoso H, *et al.* Better patient instruction for sputum sampling can improve microscopic tuberculosis diagnosis. *International Journal of Tuberculosis and Lung Disease* 2005; **9**(7): 814-7.
38. World Health Organization (WHO). Optimizing active case finding for tuberculosis: Implementation lessons from South-East Asia. New Delhi: World Health Organization, Regional Office for South-East Asia, 2021.
39. WHO. WHO operational handbook on tuberculosis. Module 3: diagnostics - rapid diagnostics for tuberculosis detection, 2021 update. Geneva: WHO, 2021.
40. García-Basteiro AL, DiNardo A, Saavedra B, *et al.* Point of care diagnostics for tuberculosis. *Pulmonology* 2018; **24**(2): 73-85.
41. WHO. The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV: policy guidance. Geneva: WHO, 2015.
42. WHO. Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV: policy update 2019. Geneva: WHO, 2019.
43. National Department of Health. Guidance on the use of the lateral flow urine lipoarabinomannan assay for the diagnosis of active tuberculosis in people living with HIV. Pretoria: National Department of Health, 2021.
44. Kerkhoff AD, Sossen B, Schutz C, *et al.* Diagnostic sensitivity of SILVAMP TB-LAM (FujiLAM) point-of-care urine assay for extra-pulmonary tuberculosis in people living with HIV. *European Respiratory Journal* 2020; **55**(2):1901259.
45. Doyle RM, Burgess C, Williams R, *et al.* Direct Whole-Genome Sequencing of Sputum Accurately Identifies Drug-Resistant *Mycobacterium tuberculosis* Faster than MGIT Culture Sequencing. *Journal of Clinical Microbiology* 2018; **56**(8):e00666-18.
46. Kayomo MK, Mbula VN, Aloni M, *et al.* Targeted next-generation sequencing of sputum for diagnosis of drug-resistant TB: results of a national survey in Democratic Republic of the Congo. *Scientific Reports* 2020; **10**(1):10786.
47. Roe JK, Thomas N, Gil E, *et al.* Blood transcriptomic diagnosis of pulmonary and extrapulmonary tuberculosis. *JCI Insight* 2016; **1**(16):e87238.
48. Ferebee SH. Controlled chemoprophylaxis trials in tuberculosis. A general review. *Bibl Tuberc* 1970; **26**: 28-106.
49. Furin J, Cox H, Pai M. Tuberculosis. *Lancet* 2019; **393**(10181): 1642-56.
50. Seung KJ, Keshavjee S, Rich ML. Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis. *Cold Spring Harbor Perspectives in Medicine* 2015; **5**(9): a017863.
51. Van Der Meeren O, Hatherill M, Nduba V, *et al.* Phase 2b Controlled Trial of M72/AS01EVaccine to Prevent Tuberculosis. *New England Journal of Medicine* 2018; **379**(17): 1621-34.
52. Carter DJ, Glaziou P, Lonnroth K, *et al.* The impact of social protection and poverty elimination on global tuberculosis incidence: a statistical modelling analysis of Sustainable Development Goal 1. *Lancet Glob Health* 2018; **6**(5): e514-e22.

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