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Review

An Overview On Tuberculosis And Advanced Diagnostic Methodlgy

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Abstract

Tuberculosis (TB), is caused by Mycobacterium tuberculosis complex, is one of the ancient diseases which affect more likely in humans. Tuberculosis is a major cause of morbidity and mortality worldwide. It is determined that 25% of world's population are infected with Mycobacterium tuberculosis, with a 5-10% lifetime risk of progression into Tuberculosis disease. Tuberculosis is highly widespread among the low socioeconomic section of the population and marginalized sections of the society. Methods based on the detection of Mycobacterium tuberculosis (Mtb) are inadequate sensitive, methods based on the identifying of Mtb-specific immune responses cannot always differ from active disease from latent infection, and few of the serological markers of infection with Mtb are insufficiently specific to differentiate tuberculosis from further inflammatory diseases. New tools based on technologies such as mass spectrometry, high-throughput sequencing, and artificial intelligence have the potential to solve this tight corner. The aim of this review was to provide an updated overview of optimize classical diagnostic methods, as well as new molecular and other techniques, for appropriate diagnosis of patients with tuberculosis infection. Treatment regimen for sensitive active TB and latent TB was provided.

Keywords: Tuberculosis, acive TB, latent TB, Respiratory route**,** immunocompromised, mycobacterium, Rifampin.

INTRODUCTION

Tuberculosis (TB) is one of the most earliest diseases of mankind and has co-existed with humans for many thousands of years. The oldest known molecular evidence of TB was detected in a fossil of an vanished bison, it is radiocarbon dated at 17,870±230 years [and in 9000, year old human remains which were redeemed from a neolithic settlement in the Eastern Mediterranean. Although as early as 1689, it was invented by Dr. Richard Morton that the pulmonary form was associated with "tubercles," due to the variety of manifestations, TB was identified as a group of diseases until the 1820s and was eventually named as "tuberculosis" in 1839 by J. L.

Schönlein. In 1882, the bacillus causing tuberculosis, *Mycobacterium tuberculosis*, was discovered by Robert Koch; and for this discovery, he was awarded Nobel prize in medicine in 1905. Tuberculosis is caused by a group of closely related bacterial species termed *Mycobacterium tuberculosis* complex. The cause of human tuberculosis is *Mycobacterium tuberculosis*. Other members of the *M. tuberculosis* complex that can cause tuberculosis include *M. bovis*, *M. microti* and *M. africanum. M. microti* is not the cause of TB in humans; infection with *M. africanum* is infrequent, while *M. bovis* has a broad host range and is the main cause of tuberculosis in other animal species. Humans become infected by *M. bovis*, usually via milk, milk products or meat from an effected animal. It is evaluate that in the pre-antibiotic era, *M. bovis* was incharge for about 6% of tuberculosis deaths in humans.In spite of newer procedure for diagnosis and treatment of TB, unluckily, millions of people are still suffering and dying due to this disease. TB is one of the most top three infectious killing diseases in the world: Although it can affect people of any age, individuals with weakened immune systems. This bacterium lives and multiplies in the macrophages, thus avoiding the natural defense system in the patients. TB based on stage there are two stages: asymptomatic latent tuberculosis infection (LTBI) or active tuberculosis disease. TB is caused by Mycobacterium tuberculosis, which can produce either a silent, latent infection, active disease. Untreated TB causes progressive tissue destruction and eventually death. TB rates in the United States continue to demenish. In contrast, TB remains out of control in many developing countries and $1/3^{rd}$ of the world's population currently is infected .

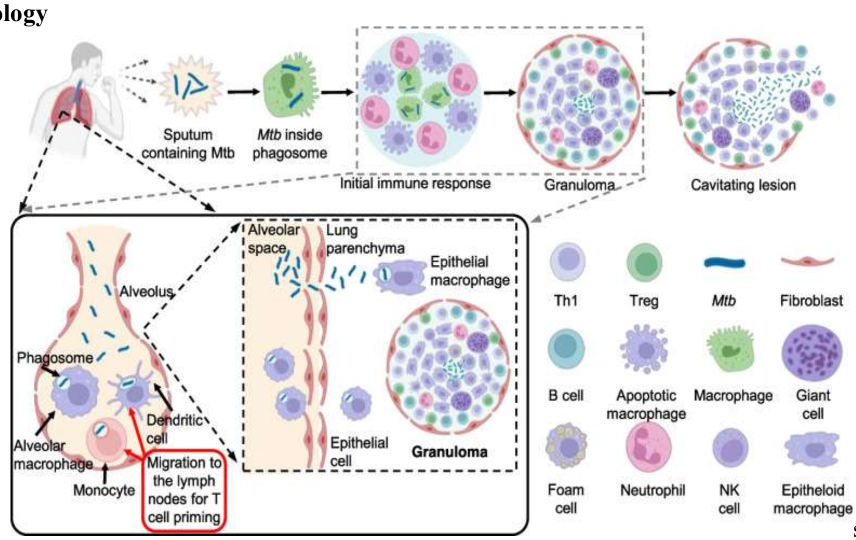
Epidimology

- Over $1/4^{th}$ of the world's population is infected by M. tuberculosis, and roughly 1.3 million people died due to active TB in 2017. In the United States, an estimated 9 million people are infected with latent TB, meaning that they are not currently sick but that they could fall unwell with TB at any time.
- In 2017, 9,105 new TB cases were reported in the US, this is 1.6% lower than the cases reported in 2016. annual incidence of TB in the United States declined by approximately 5% per year from 1953 to 1983.In 1984, this decresed , and then the incidence of TB rose from 1988 reaching its peak in 1992. Despite this good news, the eradication of TB from the United States remains difficult: HIV/AIDS kills 3 million people each year, TB kills 2 million and malaria kills 1 million. Even though tubercle bacilli was identified nearly 130 years ago, a definitive understanding of pathogenesis of this disease is still unidentified.
- One of the reason is that TB among immigrants to the United States from more incidence countries remains a source of extra cases.*M. tuberculosis* bacilli have infected nearly 1/3rd of the world's population with 10% risk of developing TB disease. Worldwidely 10.4 million cases of TB reported in 2017, accounting to 133 cases/1,00,000 population, of which 90% of cases were adults (aged ≥15 years), 64% in males, 9% were people living with HIV (72% of them in Africa). Estimated 558 000 of new cases (range- 483 000–639 000) of Rifampicin resistant TB (RR-TB), of which was most in three countries.

Etilogy

M. tuberculosis is a small bacillus with a waxy outer layer. It is 1 to 4 µm in length. It may be straight or slightly curved in shape. It does not stain well with fluorochrome stain must be used instead of that. Aefter Ziehl-Neelsen staining with carbol-fuchsin mycobacteria maintain the red colour inspite of acid–alcohol washes .They are called acid-fast bacilli (AFB). On culture, M. tuberculosis grows very slowly, doubling about for every 20 hours. This is slow as compared with gram-positive and gram-negative bacteria, which doubling about for every 30 minutes.Members of the *Mtb* family, also as *Mtb* complex, competent of causing human disease include *M tuberculosis*, *M bovis, M africanum, and M canetii* . These are closely related species, *M tuberculosis* being the main human pathogen in world. *Mtb* is aerobic, non spore forming, and nonmotile. The cell wall contains a unique high concentration of lipids that confer a characteristic acid-fast staining property and likely contribute to immunomodulation and virulence. *Mtb* are slow growing microorganism that produce a generation time of roughly 20 hours. Visible growth on solid media usually takes from 3 to 8 weeks, and this characteristic contributes to the challenge of establishing a diagnosis. Humans are the only known reservoir of *M tuberculosis,* although other animals can become sinfected. Genetic variability exists among isolates from around the world and may confer differences in virulence. *Mtb* are intracellular pathogens capable of causing subacute and progressive disease, also referred to as active TB. In addition, the bacteria can remain dormant within infected cells where they may or may not cause disease. The molecular and immunologic mechanisms responsible for dormancy and reactivation remain unknown and represent an important area of *Mtb* research.

Pathophysiology



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TB is spread from person to person by airborne droplet nuclei that can remain suspended in the air for several hours. The fate of the suspended droplet nuclei depends on environmental conditions: the bacilli can be destroyed by exposure to ultraviolet light, the droplet nuclei may innocuously land on inanimate surfaces, or they can be inhaled into a person's airway, where they may or may not establish infection. The longer one is exposed to an enclosed space where TB droplet nuclei are present, the greater the likelihood of transmission. In modern times, TB is acquired most often via the respiratory route. It can also be acquired via ingestion of contaminated milk, and while this route of infection is of historical importance, it is a rare occurrence today. Also, it is exceedingly rare for TB to be contracted through contact with nonintact skin.

Once inhaled, droplet nuclei can land on upper airway mucosa where infection is unlikely to be established or reach the alveoli where the infectious processes may begin **.** Tuberculosis Pathophysiolgy Flow Chart). Depending on complex and poorly understood pathogen virulence factors in concert with host immunomodulatory mechanisms, the bacillus can either be killed, persist in a latent state or progress to active tuberculosis disease. These discreet categorical stages likely represent an oversimplification of a complex and dynamic host-pathogen relationship. Moreover, aspects of the long-held conceptual pathophysiological model, described in some detail below, have been questioned as knowledge about immune mechanisms has evolved.

Alveolar macrophages play a central role in the immunomodulatory process. The bacilli are internalized by the macrophages where they are either killed or establish the primary infection. In the latter case, the bacilli gain access to lung parenchyma and can migrate to pulmonary lymph nodes, where they prime T cells. The primed T cells orchestrate the recruitment of T cells, B cells, monocytes, multinucleated giant cells, dendritic cells, and fibroblasts, forming a granuloma that surrounds infected macrophages within the lung parenchyma. The immunologic mechanisms that govern granuloma formation and the life cycle of the tubercle bacillus within the granuloma are poorly understood and represent areas of intensive research. Occasionally, this primary granuloma, referred to as a Ghon focus, and the associated draining hilar or mediastinal lymph nodes can calcify and reach a size visible on a chest x-ray; this finding is termed the Ranke complex. In children, the intrathoracic lymph nodes can enlarge, obstruct, and erode into bronchi. In immunocompromised adults and immunocompetent children, the Ghon focus may evolve into pneumonia, a form of progressive primary TB disease, primarily in the lower lung zones where cavitation is uncommon. In young children, progressive primary disease can rapidly disseminate within the lung itself and to other organs, most notably the central nervous system, where it can cause life-threatening *Mtb* meningitis.

In most immunocompetent adults, the bacilli will be contained by the granuloma and establish a latent infection. It may escape immunologic controls and disseminate lymphohematogenously within the lung and to virtually any other organ. Dissemination within the lung favors location in apical posterior segments. The reason for preferential dissemination to apical lung segments is speculative and has been attributed to regional differences in oxygen tension, differences in lymphatic flow, and differences in regional pulmonary immune function. The organs most commonly associated with extrapulmonary dissemination include pleura, lymph nodes, kidneys, long bones, vertebrae, and meninges. *Mtb* bacilli will grow within the organs to which they have disseminated until cellular immunity or tuberculin reactivity is established, at which time the bacilli become dormant; this is latent TB infection. This occurs 3 to 8 weeks after infection in immunocompetent people. CD4 and CD8 T cells appear to play a central role in latency.

Latent TB is not necessarily synonymous with *Mtb* dormancy. People labeled as having latent TB may cycle between periods of dormancy and subclinical TB disease. This concept is supported by surveillance studies conducted in regions of high TB endemicity.

If innate and acquired immunity fails to contain *Mtb*, people will develop active TB disease. Granulomas can undergo caseation necrosis, erode into an airway, and form a cavity within which *Mtb* bacilli proliferate. The cavity communicates with the airway and is the source of TB transmission. Because of its high concentration of proliferating bacilli and poorly vascularized inner contents, the cavity represents an environment promoting drug resistance development. Areas of cavitation no longer carry out respiratory functions and are a nidus for opportunistic bacteria and fungi. Lung parenchyma adjacent to cavities becomes fibrotic. Pulmonary blood vessels may erode into cavities and cause massive hemoptysis; this clinical finding is termed a Rasmussen aneurysm. Not all granuloma cavitate; they can involute and heal due to poorly understood immunological mechanisms.
Approximately 5% of recently infected people with TB will develop active disease within the first 2 years after infection. An additional 5% will develop active TB at a later time in their lives. Expressed differently, 90% of people infected with TB will not develop active disease. The risk factors associated with the development of active disease are noted above in the epidemiology section.

People with active TB disease can be asymptomatic at 1 end of the clinical spectrum or severely ill at the other end of the spectrum. Specific disease manifestations are a function of the organs involved; the apical posterior segments of the lung are the most commonly involved structures in adults and adolescents. In young children and older individuals, pneumonia involving the lower lobes is common. Constitutional symptoms are nonspecific and often include cough, fever, weight loss, night sweats, and malaise. Asymptomatic TB disease is well described, and prevalence has been reported to be quite high when active case finding is performed among high-risk populations.

Endobronchial TB represents a unique complication resulting from the spread of organisms from a pulmonary cavity, a pneumonic focus, or an adjacent lymph node into the airway. The endobronchial inflammatory process can produce mucosal ulcerations, granulation tissue, edema, and airway narrowing.

Reactivation of a latent focus of infection represents the most common mechanism leading to active disease, and previously infected people are generally immune to exogenous reinfection. However, it is possible to become reinfected when exposed to a large *Mtb* inocula or if there is significant underlying immunocompromise. This has important therapeutic and epidemiologic implications since it can be a challenge to determine whether a person with a prior history of TB has relapsed due endogenous reinfection, which is failure to eradicate their infection, or due to exogenous reinfection via a newly acquired infection.

Clinical presentation

The classical presentation of TB is weight loss, fatigue, a productive cough, fever, and night sweats. The onset of TB may be gradual, and the diagnosis may not be considered until a chest radiograph is performed. Unfortunately, many patients do not seek medical attention until more dramatic symptoms, such as hemoptysis, occur. At this point, patients typically have large cavitary lesions in the lungs. These cavities are loaded with M. tuberculosis. Expectoration or swallowing of infected sputum may spread the disease to other areas of the body.Physical examination is nonspecific but suggestive of progressive pulmonary disease. Human Immunodeficiency Virus 5 Patients coinfected with HIV may have atypical presentations.As their CD4+ counts decline, HIV-positive patients are less likely to have positive skin tests, cavitary lesions, or fever. Pulmonary radiographic findings may be minimal or absent. HIV-positive patients have a higher incidence of extrapulmonary TB and are more likely to present with progressive primary disease. Because their symptoms are not specific to TB, a thorough workup for TB is essential.Extrapulmonary Extrapulmonary TB typically presents as a slowly progressive decline in organ function. Patients may have low-grade fever and other constitutional symptoms. Patients with genitourinary TB may present with sterile pyuria and hematuria. Lymphadenitis often involves the cervical and supraclavicular nodes and may appear as a neck mass with spontaneous drainage. Tuberculous arthritis and osteomyelitis occur most commonly in the elderly and usually affect the lower spine and weight-bearing joints. TB of the spine is known as Pott's disease. 3 Abnormal behavior, headaches, or convulsions suggest tuberculous meningitis. Involvement of the peritoneum, pericardium, larynx, and adrenal glands also occurs. The Elderly 5 TB in the elderly is easily confused with other respiratory diseases. Many clinical findings are muted or absent altogether. Compared with younger patients, TB in the elderly is far less likely to present with positive skin tests, fevers, night sweats, sputum production, or hemoptysis. Weight loss may occur but is nonspecific. In contrast, mental status changes are twice as common in the elderly, and mortality is six times higher. TB is a preventable cause of death in the elderly that should not be overlooked. Children 5 TB in children, especially those younger than 12 years, may present as a typical bacterial pneumonia and is called progressive primary TB. Clinical disease often begins 1 to 2 months after exposure and precedes skin-test positivity. Unlike adults, pulmonary TB in children often involves the lower and middle lobes. Dissemination to the lymph nodes, GI and genitourinary tracts, bone marrow, and meninges is common. Because of delays in recruitment of cellular immunity, cavitary disease is infrequent, and the number of organisms present typically is smaller than in an adult.

Because cavitary lesions are uncommon, children do not spread TB readily. However, TB can be rapidly fatal in a child, and it requires prompt chemotherapy.

Diagnosis

Microscopy

Sputum smear microscopy still remains one of the basic methods for identifying Mtb in developing countries. The most regular practice is acid-fast staining using carbol fuschin solution. The lipid-rich cell wall of Mtb resists decolorization with acid-containing reagents, which means that acid-fast organisms can be visualized on microscopic examination of smears prepared from sputum, alveolar lavage fluid, or other specimens. The major limitation of smear microscopy is a lack of sensitivity, which varies widely (20 to 80%) in different studies and is particularly poor in paucibacillary TB, including child TB, extrapulmonary TB, or HIV coinfected TB . Specificity is likely to vary considerably depending on the local prevalence of infections with nontuberculous mycobacteria (NTM). In regions with a low incidence of NTM, the specificity of smear microscopy can reach up to 98%.

Fluorescence microscopy can save manpower and improve work efficiency, as well as increasing the sensitivity of smear microscopy. The possibility of false-positive results is a potential shortcoming of fluorescence microscopy, because of non-specific fluorochrome dye incorporation. The instability of fluorescent staining has been reported. Unlike conventional microscopy using conventional artificial light, fluorescence microscopy uses an intense light source, such as a halogen or high-pressure mercury vapor lamp, which is expensive and vulnerable. However, in comparison to intense light sources, light-emitting diodes (LEDs) are more robust, sustainable, and have a longer lasting battery life expectancy, and these qualities make LED microscopy feasible for use in resource-limited settings. Accordingly, the WHO recommends that conventional fluorescence microscopy can be replaced by LED microscopy .

Fluorescein diacetate (FDA) is a new stain solution where only living cells actively convert the nonfluorescent FDA into the green fluorescent compound following enzymatic activity . FDA staining can be used to detect the viable Mtb and simply predict the quantitative culture results within 1 h, indicating whether patients are responding to TB therapy . "TBDx" is an innovative smear microscopy system that automatically loads slides onto a microscope, focuses and digitally captures images, and then classifies smears as positive or negative using computerized algorithms. Despite showing potential for detection of Mtb, these new microscopy methods still need more validation of their performance in clinical practice.

Sputum smear microscopy is relatively fast, inexpensive, and specific for Mtb diagnosis in high-TB burden areas. Thus, it is still a worthwhile method for Mtb diagnosis, especially in resource-limited countries. The most important limitation of microscopy is a low sensitivity for diagnosis of TB, especially in paucibacillary samples. In addition, routine microscopy cannot differentiate between live and dead bacilli, which leads to the difficulty of the method for early detection of treatment failure or drug resistance. Generally, the performance of smear microscopy is far from the current needs for diagnosis of TB in clinical practice.

Culture

Solid and liquid culture

Culture is still the WHO-recommended gold standard for the diagnosis of TB, as Mtb isolation is not only important for disease diagnosis but also permits the detection of drug resistances. Traditional Mtb culture can be performed on either a solid (e.g., Lowenstein–Jensen) or liquid (e.g., Middlebrook 7H9) medium. Notably, solid culture is less expensive than liquid culture and less prone to contamination by other bacteria or fungi, but liquid culture is faster, more sensitive, and convenient (growth is detected automatically).

A technical detail that should be mentioned is sample decontamination. Samples (such as sputum) that are contaminated with normal flora must undergo decontamination before culture. The laboratory can use a routine decontamination reagent such as NaOH together with N-acetyl-L-cysteine (NALC), which kills rapidly growing bacteria and fungi but has a limited effect on Mtb growth. The laboratory should determine the optimal concentration of decontamination reagent, to avoid over-decontamination (which reduces the yield of Mtb) and under-decontamination (which leads to failed cultures, because of high rates of bacterial or fungal growth).

Rapid identification from positive cultures

Rapid identification assays capable of distinguishing between Mtb complex and NTM after positive cultures are the basis for initiating early anti-TB therapy. Traditional biochemical assays are slow and have a long turnaround time (2–3 weeks). Mtb protein 64 (MPT-64) is one of the Mtb-specific antigens secreted during bacterial growth. Immunochromatographic (ICT) assays are based on the principle of a double-sandwich enzyme-linked immunosorbent assay, which detects MPT-64 antigen. A recent review reported a high sensitivity (range, 98.1 to 98.6%) and high specificity (range, 99.2 to 100%) of ICT assays for rapid identification of Mtb complex . In addition, ICT assays are rapid, simple, and without the need of additional special equipment. Therefore, the WHO recommends using ICT assays for rapid identification of Mtb complex from positive cultures.

Phenotypic test for dst

Testing on solid agar using the proportion method is still regarded as the reference standard method for DST of Mtb, which is performed by counting the number of Mtb colonies that grow on agar with or without antibiotics. The absolute concentration method is based on the comparison of growth intensity in the presence of cutoff concentrations and on drugfree controls. Commercial automated liquid culture systems (e.g., the mycobacteria growth indicator tube system) use a modification of the proportion method and offer reliable results for two important first-line drugs (isoniazid (INH) and rifampin (RIF)), while the testing for resistance to second-line drugs is less reliable and reproducible.

Other methods have also been reported, such as microscopic-observation drug susceptibility assays (using inverted microscope to observe the characteristic spiral or comma shaped microcolonies of growing mycobacteria in liquid culture), thin-layer agar (identification of isolates based on the characteristic morphology of mycobacteria in solid culture), and colorimetric redox indicator (observation by a change in the color of the culture medium containing anti-TB drugs) . The current data suggest that these assays could be used for a rapid and accurate DST, in settings where WHO-endorsed assays are not available.

Generally, the sensitivity of Mtb culture is higher than smear, and Mtb culture is still one of the most important methods for diagnosis of TB. However, false-negative results of Mtb culture are inevitable in real clinical practice, and using the method for diagnosing TB in paucibacillary samples is still challenging, leading to its inability to rule out TB. Another limitation is that the growth of Mtb in conventional mediums takes from 4 to 8 weeks, with an additional 4 weeks for DST using the conventional proportion method. In addition, Mtb culture requires biosafety facilities and specially trained laboratory technicians to perform the experiment. Hence, Mtb culture is recommended to be performed at national reference or central laboratories, in some areas.

Molecular tests

Xpert mtb/rif

Xpert MTB/RIF (Cepheid), an automated molecular test for detection of Mtb and RIF resistance directly from clinical specimens, is one of the most commonly used molecular tests for diagnosis of TB worldwide. It is based on a hemi-nested real-time polymerase-chain-reaction (PCR) assay to amplify an Mtb-specific sequence of the rpoB gene. The turnaround time of this assay is short (2–3 h), and the problem of cross-contamination is eliminated because of self-contained cartridges. It uses three specific primers and five unique molecular probes to ensure a high degree of specificity, and NTM does not confound testing..

The sensitivity of Xpert MTB/RIF is 99.8% for smear- and culture-positive cases and 90.2% for smear-negative but culture-positive cases, and the estimated specificity is 99.2% for a single direct MTB/RIF test . As compared with phenotypic DST, the MTB/RIF test correctly identifies RIF resistance with 97.6% sensitivity and 98.1% specificity. Notably, there is some proportion of Xpert MTB/RIF positive results in culture-negative cases, which still should be diagnosed as TB because of the high specificity of this assay . It is worth noting that INH is not detected by this test, while INH resistance accounts for part of the first-line treatment failures . Similar to smear microscopy, both live and dead bacilli are detected by Xpert MTB/RIF, making this test incapable of assessing post-therapy efficacy in the current format.

Ultra, the next generation of Xpert MTB/RIF, has a larger chamber for DNA amplification than Xpert MTB/RIF. This new assay has two multicopy amplification targets for TB, namely, IS6110 and IS1081, and therefore allows a lower detection limit than Xpert MTB/RIF. These modifications have increased Ultra's overall sensitivity from 83% to 88%, with a slight decrease in specificity from 98% to 96%. The WHO recommended Xpert Ultra as the initial TB diagnostic test for adults and children, regardless of HIV status, in 2017 . Another PCR-based cartridge, Xpert MTB XDR-TB, has been designed for detection of mutations associated with resistance to multiple first- and second-line TB drugs or XDR-TB. In 2020, the Xpert MTB XDR-TB cartridge was launched, but further clinical validation is needed.

Loop mediated thermal amplification (lamp)

LAMP is an isothermal PCR amplification technique, and the reaction process proceeds at a constant temperature using an auto-cycling strand displacement reaction targeted at the six regions of the gyrB and 16S rRNA genes . The sensitivity of TB-LAMP is slightly lower than that of Xpert MTB/RIF, while the specificity of the two methods is comparable. Nevertheless, TB-LAMP does not require sophisticated laboratory equipment and can be performed in peripheral settings, contributing to its use as a simple, rapid, specific, and cost-effective nucleic acid amplification method. Currently, the TB-LAMP assay is recommended by the WHO as a potential replacement for smear microscopy, due to its superior diagnostic performance.

Line probe assay (lpa)

LPA detects TB DNA and genetic mutations associated with drug resistance, after DNA extraction and PCR amplification. The basis of the LPA is that the pre-labeled amplification product is captured by the DNA probe solidified on the membrane strip and detected by colorimetry, and the results of LPA appear as a linear band

. LPA can detect drug resistance to first-line TB drugs (INH and RIF), and there are different versions of commercial products, including GenoType MTBDRplus 1.0 (Hain Lifescience) and INNO-LiPA Rif TB kit (Innogenetics) . The newer generation of LPA, GenoType MTBDRplus 2.0, is more sensitive for the detection of Mtb strains from smear-positive and smear-negative specimens . Genotype MTBDRsl (Hain Lifescience) can detect mutations associated with fluoroquinolones and second-line drugs such as kanamycin, amikacin, and capreomycin .

Micro real time pcr

Truenat MTB, Truenat MTB Plus, and Truenat MTB-Rif Dx (Molbio Diagnostics) are micro real-time PCR-based assays for Mtb detection that produce results in 1 h . Truenat MTB and Truenat MTB Plus detect Mtb bacilli in sputum after DNA extraction, and Truenat MTB-Rif Dx has an optional add-on chip for sequential RIF resistance detection . In 2019, the WHO reported that the Truenat MTB series displayed comparable sensitivities and specificities with Xpert MTB/RIF and Xpert MTB/RIF Ultra for the detection of TB and RIF resistance.

Over the last decade, the development of molecular tests such as PCR has played a key role in the control of TB. These assays not only detect TB based on amplification of a targeted genetic region of the Mtb complex, but also detect the drug resistance of key drugs, such as RIF and INH. Importantly, molecular tests work more quickly than conventional Mtb culture and are also available at different levels of laboratories. Thus, they are becoming more and more important for TB diagnosis and are helping to improve the quality of TB care.

Immunological diagnosis

Antibody detection

Serologic tests rely on antibody recognition of Mtb antigens by the humoral immune response. Owing to the poor diagnostic sensitivity and specificity, the WHO does not recommend any commercial serologic assays for the diagnosis of TB, in case of misdiagnosis and resource waste .

Antigen detection

The presence of circulating Mtb antigens can be detected from clinical specimens such as sputum, serum, and urine, based on the principle of sandwich enzyme-linked immunosorbent assay. Lipoarabinomannan (LAM) is a specific component of the cell envelope of Mtb and can be a potential biomarker for TB diagnosis . FujiLAM is a urine lateral flow LAM test. The sensitivity and specificity of FujiLAM are 70% and 93%, respectively, in adult TB, while the sensitivity and specificity in children with TB are 51% and 87%. It performs better, with a higher diagnostic sensitivity, in patients with HIV infection or a $CD4^{+}$ T cell count <200 cells/μL, both in adults and children.

Tuberculin skin testing (TST)

TST is a classical method based on detection of type IV hypersensitivity using purified protein derivative (PPD) of tuberculin. Mtb-infected patients can produce sensitized T lymphocytes with the ability to recognize Mtb antigens. When the sensitized T lymphocytes are stimulated by Mtb antigens again, a variety of soluble lymphokines are released to increase the vascular permeability, local redness, swelling, and induration. The average diameter of induration is measured after 72 h of PPD injection as the results of TST. An average diameter of induration <5 mm or no response is considered as negative; ≥5 mm is considered as positive.

The following factors can influence TST results

(I) Bacillus Calmette-Guerin (BCG) vaccination: Since BCG and PPD share antigenic components, the specificity of TST can be affected by BCG vaccination. The effect of BCG vaccination on TST in infancy is minimal, especially ≥10 years after vaccination. BCG vaccination strategy (whether or not to multiply) also affects TST results, and the effect of BCG booster immunization on TST is more pronounced compared with the current BCG one-time vaccination.

(II) NTM infection: NTM is not a clinically important cause of false-positive TST, except in populations with a high prevalence of NTM infection and a very low prevalence of TB infection.

(III) The immune status of the host: in view of the fact that TST detection is based on Mtb-specific immune response, the immune status of the host will affect the accuracy of TST. Therefore, the sensitivity of TST for diagnosis of TB is reduced in patients with immunocompromised conditions. A systematic review of the investigation of Mtb infection in immunocompromised populations showed that TST sensitivity decreased to 31% in hemodialysis patients. Another study showed that immunosuppressed organ transplant recipients will likely develop anergy to the tuberculin antigen, which leads to false-negative TST results.

INTERFERON-GAMMA (IFN-γ) RELEASE ASSAYS(IGRAS)

IGRAs are based on secretion of IFN-γ by lymphocytes exposed to Mtb-specific antigens (TBAg), such as early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) . IGRA results are not affected by previous BCG vaccination and most infections caused by NTM, leading to a higher specificity than TST in detection of Mtb infection . The two most common commercially available IGRAs are T-SPOT.TB (T-SPOT; Oxford Immunotec) and Quanti FERON-TB (QFT; Qiagen) .

T-SPOT

T-SPOT assay, based on the enzyme-linked immunospot (ELISPOT) method, detects the number of IFN-γ-producing cells after Mtb-specific antigen stimulation. Currently, T-SPOT assay has been widely used for the diagnosis of Mtb infection . T-SPOT has proven useful, not only in detecting Mtb infection in children and HIV patients , but also in the assessment of risk for TB development in chronic inflammatory diseases, prior to anti-TNF treatment and screening for latent tuberculosis infection (LTBI) in immigrant groups, health care workers, and college students . T-SPOT has also been reported to be a useful adjunct test for diagnosing extrapulmonary TB . In spite of the significance of the T-SPOT assay in diagnosing Mtb infection, the most critical limitation of this assay is its inability to distinguish active TB from LTBI . Thus, this limitation led a WHO expert group to discourage the use of T-SPOT for the diagnosis of active pulmonary TB in low- and middle-income countries, because of an unsatisfactory specificity.

Our previous studies have expanded the application of the T-SPOT assay in the following three aspects:
(I) The ratio of TB-specific antigen (TBAg) to phytohaemagglutinin (PHA) (TBAg/PHA): the larger of the ESAT-6/PHA and CFP-10/PHA of T-SPOT assay is defined as the TBAg/PHA ratio. We have found that calculation of the TBAg/PHA ratio of the T-SPOT assay can increase the specificity of this assay for diagnosis of active TB . The theoretical basis is that TBAg/PHA ratio can eliminate the impact of individual immune variation on a T-SPOT assay. Specifically, active TB patients with immunocompromised conditions show decreased TBAg results, leading to increased difficulty in distinguishing active TB from LTBI, because low TBAg results are mostly attributed to LTBI. However, PHA results, the positive control of T-SPOT assay, are correspondingly decreased in these situations, because they can reflect the immune status of the host. Thus, the TBAg/PHA ratio is still at a high level and better than directly using TBAg results in this condition. Conversely, LTBI individuals with high TBAg results may have much higher PHA results, leading to there still being a low TBAg/PHA ratio in this condition. Furthermore, very recently, we reported the potential value of TBAg/PHA ratio in the treatment monitoring of TB.
(II) Mean spot sizes (MSS): the MSS of ESAT-6 spot-forming cells in the T-SPOT assay is calculated with an automated ELISPOT reader (CTL Analyzers). Our study showed that the MSS of ESAT-6, but not CFP-10, of T-SPOT assay in active TB patients was significantly higher than that in LTBI individuals, supporting the evidence that the MSS of ESAT-6 can be used as an adjunct tool for diagnosis of active TB . Expectedly, our findings demonstrated that a combination of the MSS of ESAT-6 and TBAg/PHA ratio of the T-SPOT assay showed potential in discriminating active TB from LTBI .
(III) Non-blood T-SPOT assay: non-blood samples, including pleural and peritoneal fluids, can also be used to perform T-SPOT assay. Our data showed that the performance of a peripheral blood T-SPOT in diagnosing TB pleurisy was limited, especially with a decreased sensitivity. However, using 1×10^5 pleural fluid mononuclear cells for performing T-SPOT can improve the diagnostic accuracy of TB pleurisy, with a sensitivity and specificity of 89.76 and 96.70%, respectively . In addition, our findings showed that, except for pleural and peritoneal fluids, other non-blood samples such as cerebrospinal fluid are not suitable for performing T-SPOT, due to it being difficult to harvest a sufficient number of lymphocytes for performing the experiment.

QFT

A QFT assay is based on the enzyme linked immunosorbent assay to detect IFN-γ secreted into the supernatant of culture medium after Mtb-specific antigen stimulation. The advantages and limitations of QFT are similar to those of T-SPOT. The operational procedures of QFT are simpler than T-SPOT, as it does not require the isolation of peripheral blood mononuclear cells, but instead uses whole blood cells. However, the sensitivity of QFT for detecting Mtb infection is slightly lower than that of T-SPOT, and this is more pronounced in patients with immunocompromised conditions.

The fourth-generation QuantiFERON-TB Plus (QFT-Plus) assay, including another TB antigen tube that contains additional shorter peptides from ESAT-6 and CFP-10, is designed to detect both the $CD4^+$ and $CD8^+$ T cell responses . It was developed with the hope of improving the detection of LTBI among immunocompromised hosts. However, studies comparing QFT-Plus to QFT currently do not support the superior performance of QFT-Plus in individuals with either active TB or LTBI.

In general, given that the immunological diagnosis of TB is based on detection of host antigen-specific responses, and not depending on Mtb load, immunological methods, especially the quantitative and high sensitivity methods like T-SPOT, have the potential for diagnosis of bacterial-negative TB. In addition, there is no doubt that the IGRAs are currently the best methods for screening LTBI, no matter whether in immunocompetent or immunocompromised individuals. Considering that Xpert MTB/RIF and TBAg/PHA ratio can make up for each other's shortcomings based on detecting different aspects, we anticipate that a combination of these two methods may represent a good algorithm for prompt diagnosis of TB in highly endemic areas.

Next-generation sequencing (NGS)

Next-generation sequencing is considered a promising method for performing DST of TB and it produces results much faster than phenotypic culture-based testing . Unlike probe-based assays that are limited to probe-specific targets, NGS can provide detailed and accurate sequence information for whole genomes by using whole-genome sequencing or multiple gene region sequencing. The WHO has published guidance on the role of NGS technologies for detecting mutations associated with drug resistance in Mtb complex . Currently, some developed countries, such as the US and UK, have transitioned from phenotypic culture to whole-genome sequencing for DST for first-line drugs , and the US Centers for Disease Control sequences isolates from all culture-confirmed TB cases nationwide . With the reduction of sequencing cost, NGS will be of importance for the surveillance of drug resistance of TB.

Mass spectrometry

Routine matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has proven to be useful for the identification of mycobacteria by direct analysis of deposits of a colony on MALDI-TOF-MS target . Previous studies have shown that MALDI-TOF-MS is a fast and economical way to identify both Mtb complex and NTM species . When an identification score of 1.3 is used, the positive predictive value of the identification of mycobacteria can reach up to 100% . Given that MALDI-TOF-MS can provide results within a few hours and is faster than sequencing and hybridization-based techniques, it has potential as a rapid and reproducible method for the identification and typing of mycobacterium species.

Recently, a novel MALDI-TOF MS methodology, based on characterized species-specific lipid profiling of intact bacteria, was able to avoid a special treatment to bacteria for releasing molecules of interest. This method is fast (<10 min), highly sensitive (<1000 bacteria required), and specific for identification of Mtb complex strains . Our preliminary study showed that serum the CFP-10 signal detected by nanotechnology and MALDI-TOF MS exhibited high diagnostic sensitivity and specificity for TB in infants and potential utility for monitoring anti-TB treatment. In addition, some researchers used MALDI-TOF MS and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify a TB-specific serum peptide profile and establish diagnostic models for rapid and accurate diagnosis of TB. Nevertheless, these new methods still need further exploration and validation.

Artificial intelligence (AI)

One of the main uses of AI in TB is using machine learning to automate the diagnosis of disease. The common strategy is to establish expert systems using a machine learning method based on the clinical, radiological, and laboratory data of TB patients. Interestingly, machine learning has been reported to aid clinicians in diagnosing pulmonary TB or predicting drug-resistant TB. For instance, Lopes et al. presented three proposals for the application of pre-trained convolutional neural networks as image feature extractors to detect TB disease . Jaeger et al. reported the possibility of discriminating automatically between drug-resistant and drug-sensitive TB in chest X-rays by means of image analysis and machine learning methods, using an artificial neural network in combination with a set of shape and texture features . We also successfully developed a GBM model based on machine learning method, by using laboratory data independently, and this model may be of great benefit for serving as a tool in the identification of active TB . Moreover, another area of AI driven interventions in a health context is morbidity and mortality risk assessment. Hussain et al. proposed a methodology using three machine learning algorithms, to facilitate TB programs by quantifying the risk of TB treatment failure, contributing to running TB programs more effectively . Despite the potential role of AI in TB control, more studies should be carried out to validate the real performance of AI in clinical practice.

Treatment

Latent infection

TSTs and IGRAs are unable to predict the progression from latent infection to active disease. Several observational studies have demonstrated that the risk of developing active TB is highest during the first 2 years after acquiring infection and that reactivation is rare beyond 10 years after infection. It has been estimated that the number needed to treat (NNT) to prevent 1 case of active TB ranges from 36 in recently infected contacts to 179 in those remotely infected. Thus, while evidence clearly demonstrates that treatment of latent TB reduces the likelihood of developing TB disease in populations at high risk, the evidence is less clear that treatment of those at low or intermediate risk reduces the incidence. The rationale to treat those with latent TB is best defined by the WHO; it is *"premised upon the probability that the condition will progress to active TB disease in specific risk groups, on the underlying epidemiology and burden of TB, the feasibility of the intervention, and the likelihood of a broader public health impact."*

The preferred 3-month regimen of once-weekly INH plus rifapentine (3HP) is attractive due to its short duration and decreased incidence of liver toxicity relative to the 6- and 9-month INH regimens .Reports of a flu-like syndrome occurring in recipients of the 3HP regimen have raised concerns. However, a large, multinational trial identified that 11% of participants experienced symptoms of a systemic drug reaction (SDR), most frequently within the first month of therapy. Of those experiencing SDR, 48% were able to complete treatment, and serious adverse events were rare.

The WHO guidelines on TB preventive therapy align with those of the CDC. In addition, the WHO guidelines include an alternative regimen consisting of 1 month of daily 3HP plus INH. Moreover, the WHO recommends that *"in settings with high TB transmission, adults and adolescents living with HIV who have an unknown or a positive TST or IGRA and are unlikely to have active TB disease should receive at least 36 months of daily INH preventive therapy."* The latter recommendation is made regardless of immune status and whether or not ART is being administered.

Currently, there are no studies providing robust data to help guide TB preventive therapy for individuals having close contact with people infected with MDR-TB. The WHO guidelines suggest a targeted approach based on individual risk assessment. Trials to ascertain the efficacy and safety of fluoroquinolones and delamanid are currently in progress. However, trials will require long follow-ups to assess the absence of disease as the clinical endpoint. It will be challenging to devise TB preventive therapy protocols that can be applied to various drug resistance patterns and assess safety and efficacy in different populations (eg, children, adults, PLHIV, the immunosuppressed, and those with existing comorbidities).

Active tuberculosis infection

The goal of anti-TB therapy is to eradicate disease and eliminate transmission in all cases. While the goal seems unambiguous, it has proven exceedingly difficult to achieve in practice. Historically, anti-TB regimens have required many months of treatment and patient adherence has been a challenge. While these challenges exist everywhere, they are particularly problematic in parts of the world with the highest TB prevalence and where public health infrastructure and literacy about TB are lacking. From a global public health perspective, TB treatment guidelines are designed to be straightforward and somewhat standardized. This "one-size-fits-all" approach has been questioned since a standard 6-month regimen may be too long for some and not long enough for others. It is conceivable that in the future, the application of a pretreatment risk stratification algorithm could inform optimized treatment duration schedules on an individualized basis.

Detailed recommendations on dosing, first- and second-line anti-TB drugs, drug-drug interactions, management of treatment interruption, adverse effects of treatment, culture-negative TB, extrapulmonary TB, HIV coinfection, children, advanced age, pregnancy, breastfeeding, and comorbidities are beyond the scope of this activity and may be found in the referenced guidelines**.** Drug Regimens for Microbiologically Confirmed PULMONARY TUBERULOSIS Caused by Drug-Susceptible Organisms). In addition, the management of TB is understood to be very complex, and for that reason, the CDC has established an exceptionally useful online resource, TB Centers of Excellence for Training, Education, and Medical Consultation.

The treatment for drug-susceptible pulmonary TB disease had not, until 2022, changed in 50 years. The long-held standard regimen involves the use of 4 drugs: isoniazid (INH), rifampin (RIF), ethambutol (ETH), and pyrazinamide (PZA). Before the development of that therapeutic combination, the first anti-TB drug, streptomycin, was used as monotherapy in the 1940s. Streptomycin initially provided significant benefit when administered for 6 months but ultimately failed due to the emergence of resistance. The resistance to monotherapy, along with a better understanding of both the pathophysiology of Mtb infection and drug toxicities, informed decisions that led to the current standard of treatment.

There are 2 phases of treatment – an initial intensive phase that provides bactericidal activity directed at rapidly replicating organisms and the continuation phase that is meant to sterilize slowly replicating and dormant tubercle bacilli. The intensive phase of therapy requires the use of INH, RIF, ETH, and PZA, and the continuation phase uses INH and RIF. In treating pulmonary TB, the intensive phase usually extends for 2 months, during which it is hoped that there will be significant reductions in mortality, lung inflammation, rapidly replicating mycobacteria, and transmission. Should drug susceptibility testing indicate that the isolate is sensitive to both INH and RIF, the EMB can be discontinued. In that case, the intensive phase would consist of INH, RIF, and PZA. The continuation phase extends for an additional 4 months. The rationale for the 4-drug combination is that bactericidal killing of rapidly replicating organisms will reduce the chances of emerging resistance.

Patients receiving anti-TB therapy require monitoring to assess the efficacy and safety of the regimens. Sputum smears and cultures should be evaluated monthly until 2 consecutive cultures are negative. In patients with chest x-ray evidence of cavitation and who remain culture positive at 2 months, the recommendation is to extend the continuation phase for an additional 3 months (ie, a total of 9 months of therapy). Extending the continuation phase should also be considered for the following individuals: PLHIV, the malnourished, active smokers, the immunosuppressed, and those with extensive pulmonary disease.

The "maximum" dose for a given patient is the dose that produces the desired response with an acceptable level of toxicity. This can only be determined on a case-by-case basis. Artificially capping doses may deprive patients of needed drug. Primary Antituberculosis Drugs Isoniazid Isoniazid is one of the two most important TB drugs. It is highly specific for mycobacteria, with a MIC against M. tuberculosis of 0.01 to 0.25 mcg/mL (mg/L; 0.07 to 1.82 μmol/L).

It is bactericidal and is thought to inhibit mycolic acid synthesis and disruption of the cell wall in susceptible organisms. Most nontuberculous mycobacteria such as M. avium are resistant to isoniazid, although Mycobacterium kansasii and Mycobacterium xenopi are susceptible. The most common mechanisms of resistance result from mutations in the katG or inhA genes. Isoniazid is readily absorbed from the GI tract and from intramuscular injection sites. It also can be given as a short IV infusion over 5 minutes if diluted in about 20 mL of normal saline. Isoniazid should be given on an empty stomach whenever possible.N-Acetyltransferase 2 forms the principal metabolite acetylisoniazid, which lacks antimycobacterial activity. The rate at which humans acetylate isoniazid is determined genetically; slow acetylation is an autosomal recessive trait and reflects a relative lack of N-acetyltransferase.

Fast acetylators have isoniazid half-lives of less than 2 hours. Approximately 50% of whites and blacks and 80% to 90% of Asians and Native Alaskans are rapid acetylators. Slow acetylators have isoniazid half-lives of 3 to 4 hours and may be at an increased risk of neurotoxicity. The association of acetylator status and risk of hepatotoxicity, however, appears to be weak. Poor absorption and rapid clearance of isoniazid for patients receiving highly intermittent therapy are associated with poor clinical outcomes. Transient elevations of the serum transaminases occur in 12% to 15% of patients receiving isoniazid and usually occur within the first 8 to 12 weeks of therapy.

Overt hepatotoxicity, however, occurs in only 1% of cases. Risk factors for hepatotoxicity include patient age, preexisting liver disease, excessive alcohol intake, pregnancy, coadministration of other medications that are potentially hepatotoxic, and the postpartum state. Isoniazid also may result in neurotoxicity, most frequently presenting as peripheral neuropathy or, in overdose, as seizures and coma. Patients with pyridoxine deficiency, such as pregnant women, alcoholics, children, and the malnourished, are at increased risk. Isoniazid may inhibit the metabolism of phenytoin, carbamazepine, primidone, and warfarin.40 Patients who are being treated with these agents should be monitored closely, and appropriate dose adjustments should be made when necessary. Rifampin The introduction of rifampin into routine use during the 1970s allowed for true short-course treatment of TB (6-9 months).

Without rifampin, treatment is generally 18 months or longer. Drug resistance to rifampin is an ominous prognostic factor because it is frequently associated with isoniazid resistance and leaves the patient with few good therapeutic options. Clinicians must take care to protect susceptibility to rifampin by carefully treating their patients. Rifampin shows bactericidal activity against M. tuberculosis and several other mycobacterial species, including Mycobacterium bovis and M. kansasii. It also is active against a broad array of other bacteria. Alteration of the target site on RNA polymerase, primarily through changes in the rpoB gene, leads to most forms of rifampin resistance Rifampin usually is given orally, but it also can be given as a 30-minute IV infusion. Oral doses are best given on an empty stomach. Patients with AIDS, diabetes, and other GI problems appear to have difficulty absorbing rifampin after oral doses, and this has been associated with therapeutic failures in some cases.

Rifampin is metabolized to desacetyl rifampin, which retains some of rifampin's activity; most of rifampin and its metabolite are cleared in the bile. Rifampin generally is given at 600 mg daily or intermittently, although this dose does not take full advantage of rifampin's concentration-dependent killing.Higher doses should be tested in humans within the context of clinical trials. Elevations in hepatic enzymes have been attributed to rifampin in 10% to 15% of patients, with overt hepatotoxicity occurring in less than 1%.More frequent adverse effects of rifampin include rash, fever, and GI distress. Allergic reactions to rifampin have been reported and occur more frequently with intermittent rifampin doses 900 mg or more twice weekly. These reactions may take the form of a flu-like syndrome with development of fever, chills, headache, arthralgias, and, rarely, hypotension and shock. Alternatively, hemolytic anemia or acute renal failure may occur, requiring permanent discontinuation.

Rifampin's potent induction of hepatic enzymes, especially cytochrome P450 3A4, may enhance the elimination of many other drugs, most notably the protease inhibitors used to treat HIV . HIV-positive patients may benefit from the use of rifabutin instead of rifampin. Furthermore, women who use oral contraceptives must use another form of contraception during therapy because increased clearance of the hormones may lead to unexpected pregnancies. Patient records should be reviewed for potential drug interactions before dispensing rifampin. Rifampin may turn urine and other secretions orange-red and may permanently stain some types of contact lenses. Other Rifamycins Rifabutin is used for disseminated M. avium infection in AIDS patients and is quite active against M. tuberculosis.

Most rifampin-resistant organisms are resistant to rifabutin. Because rifabutin is a less potent enzyme inducer than rifampin, it may be used for patients who are receiving protease inhibitors. For HIV-positive patients, the ATS/CDC recommends regimens with three or more doses of the TB drugs per week . Rifapentine is a long-acting rifamycin that can be used once weekly in the continuation phase of treatment (after the first 2 months) in carefully selected HIV-negative patients. It is approximately as potent an enzyme inducer as rifampin, so similar drug interactions . Pyrazinamide Adding pyrazinamide to the first 2 months of treatment with isoniazid and rifampin shortens the duration to 6 months for most patients.Pyrazinamide may be bacteriostatic or bactericidal depending on the concentration and the susceptibility of the organism.

It is usually well absorbed and displays a fairly long halflife.66,67 The most common toxicities of pyrazinamide are GI distress, arthralgias, and elevations in the serum uric acid concentrations.37 Most patients do not experience true gout. Hepatotoxicity is the major limiting adverse effect and is dose-related when pyrazinamide is given daily.

A fixed-combination product (Rifater, Aventis) of rifampin 120 mg, isoniazid 50 mg, and pyrazinamide 300 mg is designed to prevent drug resistance by keeping the self-medicating patient from using only one drug at a time. If the patient is receiving DOT, there is no particular advantage to this product. The typical dose of Rifater will be five to six tablets daily. When pyrazinamide is discontinued after 2 months of treatment, the combination product Rifamate (isoniazid 150 mg and rifampin 300 mg).

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