Review Article

Diagnosis of tuberculosis: Newer Techniques

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ABSTRACT: *Tuberculosis (TB) is still one of the deadly infectious diseases worldwide. It is estimated that around one-third of the world's population is infected with latent TB (LTBI) with 5–10% life time risk of developing active TB [1, 2]. Presence of good diagnosis is important for the control and ultimate elimination of the disease [3]. However, diagnosis of LTBI and active TB using the existing tools is challenged by low sensitivity (in smear microscopy); need of prolonged time for results and need of sophisticated laboratories and expertise (in TB culture) [4]; relatively expensive and heterogeneous diagnostic accuracy (in molecular techniques) [5]; false positive reactions [6], lower sensitivity in immune-suppressed peoples [6, 7], anamnestic recall of immunity [8], potential for inter- and intra- operator variability of results [9], inconvenience for patients in tuberculin skin test, a test, based on the fact that infection with M. tuberculosis bacterium produces a delayed-type hypersensitivity skin reaction to certain components of the bacterium [10]. Putting all the challenges of the techniques together reinforces the need for improved diagnostic tools*

Recently, important advances have been achieved in these fields that have led to substantial improvements in the accuracy and the timing of the diagnosis of tuberculosis. Novel methods allow for a better identification of latently infected individuals who are at risk of developing active tuberculosis, they also offer the possibility for a rapid diagnosis of active tuberculosis in patients with negative sputum smears for acid-fast bacilli and enable prompt identification of drug-resistant strains of Mycobacterium tuberculosis directly from respiratory specimen with a high accuracy. In addition, promising methods that will further optimize the diagnosis of tuberculosis are under development. In the future, therapeutic interventions based on the results of novel diagnostic procedures can be made earlier leading to improvements in patient care.

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INTRODUCTION:

In clinical practice the rapid detection of individuals with tuberculosis can be difficult,**2** as only 44% of all new cases (and only 15–20% of children**3**) are identified by presence of acid-fast bacilli (AFB) on

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sputum smears.**1** The gold standard for the diagnosis of tuberculosis is the detection of *Mycobacterium tuberculosis*, the causative microorganism of tuberculosis. In fact, whenever *tuberculosis M.* is recovered from human specimens by microbiological culture the diagnosis of active tuberculosis is regarded as definite.

However, culture growth of *tuberculosis M.* may take 2 or more weeks on average. The ad hoc decision to initiate anti-tuberculosis treatment can be difficult in cases where AFB are not found on sputum smear microscopy despite the clinical suspicion of tuberculosis. The clinical diagnosis of active tuberculosis then classically relies on the results of different methods, including the tuberculin skin test (TST), chest radiography, amplification of *tuberculosis M.* nucleic acids and/or pathological examinations from biological specimens (**1 Fig.**).

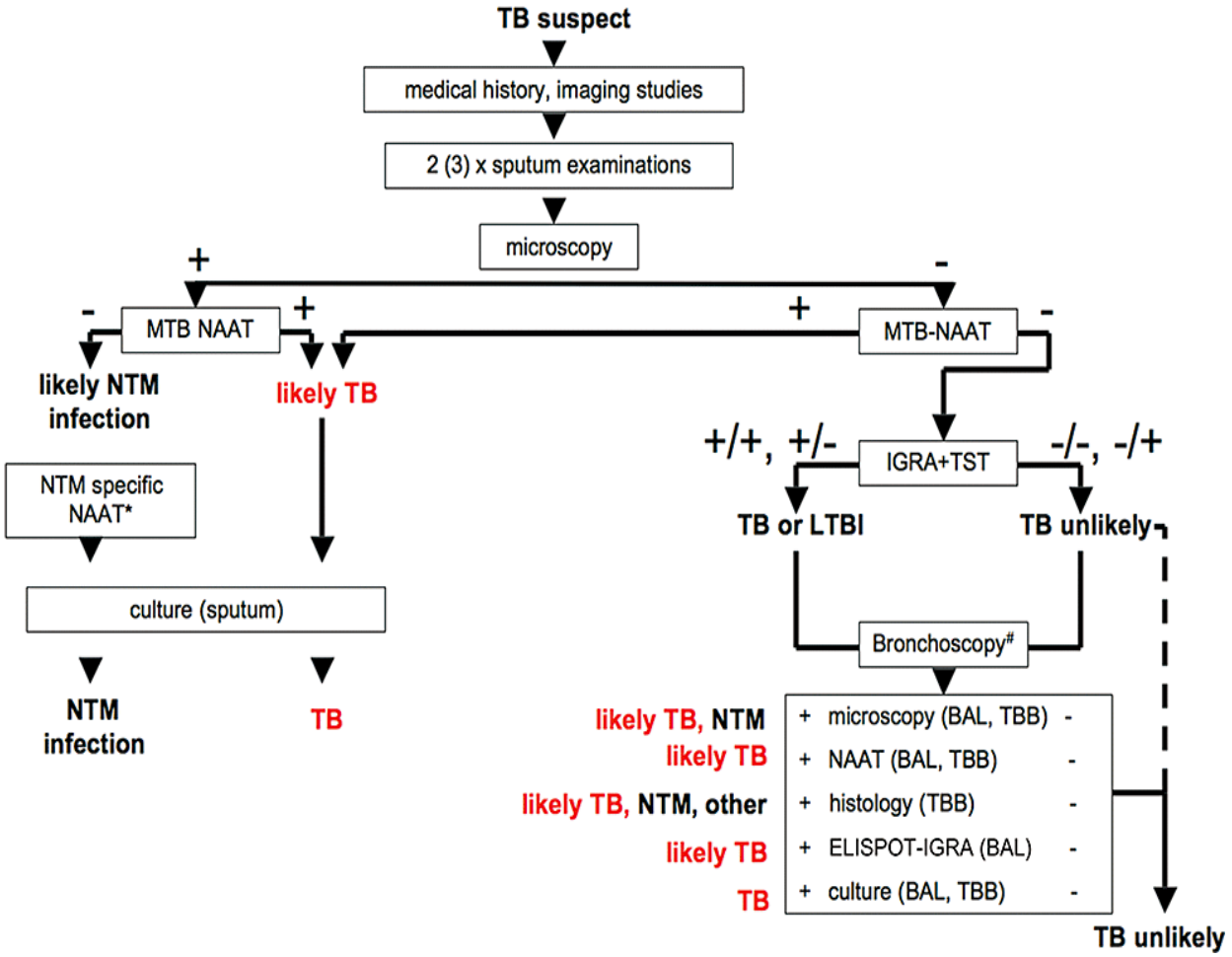

Figure 1: Flow diagram for the diagnosis of tuberculosis in clinical practice.

CURRENT TRENDS IN THE EPIDEMIOLOGY OF TUBERCULOSIS:

In high-prevalence countries, most tuberculosis patients are in their 20s to 40s, resulting in tremendous socioeconomic loss

as this is the most productive generation. In contrast, among low-prevalence countries, tuberculosis is drifting to involve the elderly, socioeconomically marginalized people, medical high-risk groups (e.g. diabetics[5] and those treated with immunosuppressive agents, such as TNF-alpha blockers[6], which presents a challenge to both medical and welfare services.

As a consequence of the global efforts in tuberculosis control under the Directly Observed Treatment Short-course strategy since the 1990s, the incidence of tuberculosis is estimated to have started to decline for the first time around 2003, although very slowly.[1] At the same time, issues that had been given only lower priority in the developing world have emerged as unavoidable challenges. One of these issues is multi-drug resistant (MDR) tuberculosis that strikes a half million people annually and is a malignant burden to the patients and community, as well as to national tuberculosis programmes with its poor treatment outcome.[7] In line with this problem, extensively drug resistant (XDR) strains of *tuberculosis M.* are emerging recently.[8] The use of effective secondary drugs based on the result of high-performance drug sensitivity tests is necessary in order to address these issues, which requires technical innovation.[7]

A second newly emerging issue is co-infection of the HIV and *tuberculosis M.*. Currently, 15% of the new tuberculosis patients are infected with HIV, and in some areas or countries this proportion exceeds 50%. One quarter of the global tuberculosis deaths are due to HIV, and this is equal to one-third of new HIV-positive tuberculosis cases and to 23% of the estimated two million HIV-related deaths in 2007.[1] Diagnosing tuberculosis in these subjects with sputum smear examination alone cannot prevent their infectiousness and save their lives; more aggressive case-finding and treatment of smear-negative cases are required. Another issue is tuberculosis in children for whom *Mycobacterium bovis* Bacille Calmette Guerin (BCG) vaccination has been virtually the only control measure in developing countries. This also requires accurate diagnosis in the early stage of tuberculosis.[9]

Tuberculosis in children

Because of its paucibacillary nature, tuberculosis of children is difficult to diagnose. Bacteriological confirmation seldom exceeds 30–40% among children in developed as well as developing countries.[106], [107] years, of whom about half were HIV-infected, and found the following frequency of symptoms and signs in the HIV-seronegative children: weight loss 69%, fever 100%, cough 83%, night sweat 43%, fatigue 21%, tuberculosis contact 60%, malnutrition 57%, lymphadenopathy 88%, organomegaly 31%, positive TST 89%, elevated erythrocyte sedimentation rate 79%, and chest X-ray infiltration 100%. Consequently, the diagnosis of tuberculosis in children in resource-poor settings is largely dependent on a combination of a history of contact with a known tuberculosis patient, clinical signs and symptoms, and

special examinations, such as chest radiography and the TST when available. Edwards and colleagues observed a total of 91 tuberculosis cases younger than 15 **108** Based on these observations, several point-scoring systems, diagnostic classifications and diagnostic algorithms have been developed to support an objective diagnostic judgment. Marais *al et*months) and fatigue provided reasonable diagnostic accuracy in HIV-uninfected children (sensitivity 62.6%; specificity 89.8%; positive predictive value 83.6%). The performance was poorer in HIV-infected children than in the low-risk group, which offers a serious challenge in resource-poor settings with high HIV epidemics. weeks, documented deterioration of health (in the preceding 3 . tested such an approach and found that combining a persistent non-remitting cough lasting over 2However, given this set of sensitivity and specificity, the positive predictive value is calculated as only 24% in a patient population with a prevalence of tuberculosis of as high as 5%.

Tuberculosis in old ages

In low-prevalence situations, tuberculosis is a problem predominantly of the aged population and includes many more cases of clinical development in immunologically compromised subjects. This is why there are many tuberculosis cases with 'atypical' clinical presentation(s) in older persons. Elderly patients are more likely to have extrapulmonary tuberculosis, including miliary disease The proportion of bacteriologically confirmed pulmonary tuberculosis patients was higher in the elderly than in the younger patients as reported in a meta-analysis.

Fever, sweating and haemoptysis are less frequent in older patients, but dyspnoea is more frequent Laboratory findings, such as the TST-positive rate, serum total protein level and white blood cell counts, were lower in elderly patients. Also, cavity formation was less common in elderly patients, while lesions in the upper lung were similar for both age groups. The most common chest X-ray findings in the elderly or immunocompromized tuberculosis patients are lesion in the lower zone accompanied by basal effusion or thickening. Such atypical clinical presentation of tuberculosis in the elderly can often cause delay in diagnosis, which can be further complicated due to underlying illnesses.

ADVANCES IN THE IMAGING DIAGNOSIS OF TUBERCULOSIS:

None of the radiological abnormalities seen in pulmonary tuberculosis are pathognomonic for this disease. Nevertheless, several features are typical of tuberculosis. While the classification in 'primary' and 'reactivation' tuberculosis is still widely *en vogue*, evidence from genotype fingerprinting studies confirms that the radiographic feature in tuberculosis following recent and remote infection are very similar and that integrity of the immune system predicts the appearance of the patterns of active tuberculosis on chest imaging: immunocompromized individuals (e.g. those with advanced HIV infection) having the appearance of 'primary'

tuberculosis and immunocompetent individuals having the appearance of 'reactivation' tuberculosis.,

The conventional chest X-ray is still the most commonly used method for screening, diagnosis and follow up of treatment responses in patients with pulmonary tuberculosis. However, chest computed tomography (CT), in particular high-resolution CT, is more sensitive than conventional chest X-ray to identify early parenchymal lesions or mediastinal lymph node enlargements and to determine disease activity in tuberculosis.

Radiographic features on CT that are suggestive of active tuberculosis include cavitations and parenchymal abnormalities and/or centrilobular nodules and the *tree-in-bud pattern*. Recently, serial pulmonary [(18)F]-2-fluoro-deoxy-D-glucose positron emission tomography has been investigated as a promising non-invasive method to monitor disease activity and responses to anti-tuberculosis chemotherapy. Although highly expensive, this technique could be useful and even be cost-effective for the managment of patients with MDR and XDR tuberculosis in selected cases.

MICROBIOLOGICAL DIAGNOSIS: CONVENTIONAL METHODS

Remarkable efforts have been made globally to accelerate the development and expansion of new diagnostic technologies. However, tuberculosis case detection still remains dependent upon sputum smear and culture, radiography and clinical symptomatology, and currently 57% of global tuberculosis patients receive a bacteriological diagnosis. Therefore, efforts to improve the quality of existing methods are necessary, and there actually have been certain achievements in this direction.

Sputum smear examination by microscopy

One recent achievement in conventional tuberculosis microscopy is the recognition of the benefit of fluorescent microscopy for enhancing sensitivity over that of ordinary light microscopy without any loss in specificity. The fluorescence microscopy widely used in resource-rich countries has been accepted as more sensitive than ordinary microscopy, although with a concern of loss of specificity, especially under conditions in the developing world. Nonetheless, a recent literature review has confirmed that it may be also beneficial in the latter as well. This could be further improved by attaching a stronger light source called an ultra-bright (Lumin™, LifeEnergy®, Germany) light-emitting diode specificity.

Progress in culture examinations

Since the 1990s, a series of culture examination systems has been developed using liquid media for rapidly detecting *tuberculosis M.*. A systematic review demonstrates that these liquid cultures are more rapid and sensitive than solid medium cultures. days with Lowenstein Jensen solid medium. days with BACTEC 460, compared with 27.0 days by BACTEC MGIT960, and 15.0 The mean time to detection was 12.9 Thus, WHO recently endorsed the use of liquid

tuberculosis culture and drug susceptibility testing for *tuberculosis M.* in low-resource settings. The newly developed rapid liquid culture systems have unique sensing systems to detect a small amount of bacterial growth, such as by radioactivity or oxygen concentration changes, as quickly as possible. These systems can also be used for drug susceptibility testing as well as detecting *tuberculosis M.*. Novel diagnostic test using mycobacteriophages to identify *tuberculosis M.* days of turnaround time in the laboratory. They have a high specificity (range 83% to 100%), but lack sufficient sensitivity (range 21% to 88%) to substitute conventional culture techniques. from biological specimen require only 2 Still other systems based on phenotypes have been devised mainly for drug susceptibility testing. One such system is the microscopic observation drug susceptibility, where the characteristic growth of *tuberculosis M.* in the liquid medium in a well is checked under an inverted light microscope. In another system, bacterial growth is confirmed from the bacterial activity to reduce nitrate to nitrite in the liquid media, which is indicated by the change in colour of the media (nitrate reductase assay).

MICROBIOLOGICAL DIAGNOSIS: MOLECULAR METHODS

Nucleic acid amplification techniques

The *tuberculosis M.* day after obtaining sputum or bronchoalveolar lavage (BAL) fluid and can have important implication for the management of a patient. Unfortunately, NAAT amplification targets are not standardized and the diagnostic accuracy of the tests is highly heterogenous. -specific nucleic acid amplification tests (NAAT) performed on bronchopulmonary specimen are the most frequently used molecular tests for laboratory diagnosis of pulmonary tuberculosis. NAAT results can be available to the clinician within 1day.

The clinical value of in-house and/or commercial NAAT performed on respiratory specimens for diagnosis of pulmonary tuberculosis have recently been repeatedly reviewed in meta-analyses.

In individuals with positive AFB sputum smears, the sensitivity of NAAT to detect *tuberculosis M.* nucleic acid on these specimens is greater than 95%. When AFB are found on sputum or BAL smears, the presumptive diagnosis of tuberculosis can thus be rapidly confirmed. Apart from rare exceptions, a negative NAAT result in this situation strongly indicates the presence of a non-tuberculous Mycobacteria (NTM) species in this specimen.

In contrast, in individuals with negative AFB sputum smears, the estimated sensitivity of NAAT for the diagnosis of active tuberculosis is highly heterogeneous (especially when in-house assays are compared) and is not consistently accurate enough to be routinely recommended for the diagnosis of tuberculosis. In general, nested NAAT methods, and the use of *IS6110* as the amplification target are related to a higher diagnostic accuracy.

In individuals with a negative sputum smear, the specificity of NAAT for the

diagnosis of active tuberculosis has been 97% and 98% in an earlier meta-analysis and an independant recent study. A positive result in a *tuberculosis M.*-specific NAAT performed on a respiratory specimen is therfore highly indicative of pulmonary tuberculosis. However, in our experience far less than 50% of patients with smear-negative tuberculosis have a positive sputum or BAL NAAT result. False positive results are seen in individuals with a past medical history of tuberculosis and in patients with bronchogenic carcinoma.

LINE PROBE ASSAYS:

Line probe assays are NAAT to detect common genomic mutations responsible for antibiotic resistance from a biological probe or culture by DNA hybridization (GenoType MTBDR assay, Hain Lifescience, Nehren, Germany or INNO-LiPA Rif. TB kit, Innogenetics, Zwijndrecht, BelgiumIn brief, the tests involve DNA extraction, multiplex NAAT, solid phase reverse hybridization on the test strip and detection of the resistance mutations. The Genotype MTBDR*plus* assay detects several mutations in the *rpoB* gene, in the *katG* gene and the *inhA* gene promoter regions. In a meta-analysis, the pooled sensitivity in resistance detection on clinical specimen for rifampicin was similar to conventional DST following culture. However, the pooled sensitivity for isoniazid-resistance testing was less optimal at 85% (72–92%). The latest version of the line probe assays, the Genotype MTBDR*sl* assay in addition can detect genetic mutations that are related to drug resistance of strains of *tuberculosis M.*, including those for fluoroquinolones and injectable drugs (amikacin or capreomycin) enabling the rapid diagnosis of XDR tuberculosis in >85% of all cases, including direct testing on clinical specimen.

IMMUNOLOGICAL DIAGNOSIS:

Avances in serology for antibody/antigen detection

There has been a long history of developing systems to diagnose tuberculosis based on the serological reaction, that is, detection of a specific antibody. Currently, the development of such systems is very urgently needed due to the pressure for strengthening earlier diagnosis of diseases in the paucibacillary stage, including pulmonary tuberculosis with negative sputum smears of adults, extrapulmonary tuberculosis, childhood tuberculosis and tuberculosis patients with HIV coinfection. Furthermore, WHO/TDR evaluated commercially available tuberculosis tests with regard to their performance, reproducibility and operational characteristics. They used 355 well-characterized archived serum samples to evaluate 19 rapid tuberculosis tests at one laboratory. The sensitivity of these rapid tests ranged from 1% to 60%; the specificity, from 53% to 99%; and in general, tests with high specificity had very low sensitivity. Test performance was poorer in patients with sputum smear-negative tuberculosis and in HIV-positive patients. Again they concluded that none of the assays performed well enough to replace microscopy.

Currently available serological tests cannot be recommended for the diagnosis of tuberculosis.

Advances in cellular immunodiagnosis

The TST and interferon-γ release assays (IGRA) evaluate *in vivo* (TST) and *ex vivo* (IGRA) the presence of persistent mycobacteria-specific T cell responses. They are indirect marker for past or present infection. TST and IGRA performed on peripheral blood alone cannot distinguish between individuals with LTBI, active tuberculosis or past tuberculosis.

Tuberculin skin test

The TST was developed by the Austrian paediatrician Clemens v. Pirquet as an 'allergy-test for the diagnosis of tuberculosis in children'. It has been the standard test for the immunodiagnosis of tuberculosis since the beginning of the 20th century.

The specificity of the TST is dependent on the BCG vaccination status and the immune status of the individual who is tested. Cross-reactivity of antigens may result in a positive TST reaction after exposure to NTM or following *bovis M.* BCG vaccination. mm are likely related to tuberculosis or LTBI, TST induration reactions exceeding 15 irrespectively of the BCG vaccination status. mm, the specificity of the TST increases with the increase of the cut-off used to define a positive induration. In combination with culture filtrate protein (CFP)-10 antigen to increase diagnostic sensitivity, such a skin test could overcome some of the obstacles currently related to the use of the TST. If clinical trials show superiority to the TST this test could be made widely available for the diagnosis of LTBI in resource-limited settings where the use of IGRA is prohibited by their costs and demands for an established laboratory infrastructure.

Interferon-γ release assays

Introduction of IGRA into clinical practice is regarded by many as the most important development in the diagnosis of *tuberculosis M.* infection over the last decade. IGRA is a coupling of the discovery of antigens ESAT-6 and CFP-10, which are relatively specific to *tuberculosis M.* and the development of simplified technologies of measuring interferon-γ. There are two commercialized systems for the latter technology. QuantiFERON-Gold (QFT-G) (Cellestis Ltd, Carnegie, Australia**2** measures interferon-γ in IU/mL using an enzyme-linked immunosorbent assay (ELISA) and T-SPOT.TB (Oxford Immunotec Ltd, Abingdon, UK counts the cells releasing interferon-γ visualized as spots with the enzyme-linked immunospot (ELISPOT) technique. During the last several years, these systems have been approved in various countries and the findings of their diagnostic performance have been accumulated and characterized. The QFT-G test is now available as an 'in tube' version (QFT-G-IT), which also includes, in addition to ESAT-6 and CFP-10, the antigen TB7.7. We present a summary of the performance of these systems in various settings, based on review and meta-analysis. IGRA were originally intended to diagnose LTBI, but because there is no gold standard of tuberculosis infection, the active disease is usually used as a surrogate for the infection when quantifying sensitivity.

Specificity is measured in subjects with low risk of *tuberculosis M.* infection, for example, healthy young subjects without known contact with tuberculosis patients.

As indicated in **Table**-1, the specificity of IGRA is consistently high and obviously superior to TST, whereas sensitivity is rather variable between studies. This variability may be greatly ascribed to the difference in patients' characteristics in terms of tuberculosis disease condition, age, extent of immunosuppression due to underlying illnesses, etc. However, IGRA generally perform better than TST in its sensitivity. Comparing QFT-G and T-SPOT.TB, T-SPOT.TB seems to be more sensitive than QFT-G, and vice versa for specificity. This comparison is clearer when they are compared head to head in the same subjects. The same is also true for the comparison between QFT-G and QFT-G-IT, where the latter exhibits higher sensitivity in head-to-head comparison, perhaps due to addition of the third antigen TB7.7, while the difference was in an opposite direction in the comparison between the different subject groups, as seen in series 1 and series 2 in **Table**-1.

Table 1. Summary sensitivity and specificity of IGRA (meta-analysis)

Series	Diagnostics	Subject	No. studies	Summary	Range
Sensitivity					
1	QFT-G	TB patients, adult	21	0.80 (0.78–0.82)	0.62–0.95
2	QFT-G-IT	TB patients, adult	6	0.74 (0.69–0.78)	0.64–0.93
3	QFT-G/G-IT	TB patients, child	9	0.82 (0.75–0.87)	0.53–1.00
4	QFT-G/G-IT, T-SPOT.TB	HIV-infected TB patients	5	0.70 (0.60–0.79)	0.63–0.85
7	T-SPOT.TB	TB patients	13	0.90 (0.86–0.93)	0.83–1.00

Series	Diagnostics	Subject	No. studies	Summary	Range
8	TST	Healthy subjects	20	0.77 (0.71–0.82)	0.57–1.00
Specificity					
1	QFT-G/G-IT	Healthy young adults	12	0.98 (0.97–0.99)	0.92–1.00
2	QFT-G/G-IT	Healthy young adults, BCG(−)	8	0.99 (0.98–1.00)	0.95–1.00
3	QFT-G/G-IT	Healthy young adults, BCG(+)	8	0.96 (0.94–0.98)	0.89–0.99
4	T-SPOT.TB	Predominantly BCG vaccinated	8	0.93 (0.86–1.00)	0.85–1.00
5	TST	BCG not vaccinated	6	0.97 (0.95–0.99)	0.93–1.00
6	TST	BCG vaccinated	6	0.59 (0.46–0.73)	0.35–0.79

- Figures in parentheses in column 'Summary' indicate 95% confidence limits. For specificity, several studies under series 1 are included in series 2 or 3.
- IGRA, interferon-gamma release assay; QFT-G, QuantiFERON-TB Gold; QFT-G-IT, QuantiFERON-TB Gold In-Tube; TB, tuberculosis; TST, tuberculin skin test.

The performance of the IGRA for the immunodiagnosis of *tuberculosis M.* infection has been investigated in immunocompromised hosts, such as in HIV-infected, elderly, chronic-renal-failure patients and those taking corticosteroids or TNF-alpha blockers.

- In general, the responses to IGRA (T-SPOT.TB>QFT-G-IT) are more frequently present in individuals from these patient

groups when compared with the TST. This is commonly interpreted that these assays are superior to the TST to detect LTBI.

Apart from the performance using tuberculosis patients as surrogates of *tuberculosis M.* infection, there are arguments concerning the discordance between IGRA and TST in those suspected of recent infection. However, it is now considered that IGRA may reflect the dynamics of infection immunity more sensitively, so that the interferon-γ level may fluctuate above and below the cut-off. Similar concern is raised about the predictability of the future clinical development according to the IGRA response level, which is the main purpose of testing contacts for possible latent infections. One report suggests the higher risk of developing tuberculosis in cases with higher response at the time of infection. This should be further confirmed and the discussion should be expanded to the level of response that persists after many years of infection.

For the diagnosis of tuberculosis in non-immunocompromised hosts the best use of IGRA is to rule out active tuberculosis, as the negative predictive value for tuberculosis is higher than 95% if combined IGRA and TST test results are negative.

. This article has overviewed such changing aspects of each of the established diagnostic techniques as summarized in **Table**-2.

Table-2**.** Comparison of established methods for the diagnosis of active tuberculosis

	Method	**Advantage**	**Disadvantage**	**Duration**	**Clinical significance**
Medical history	Conversation, review of medical records	Information on the individual risk for TB	Risk for tuberculosis may not be obvious, clinical symptoms may be non-specific	<h 1	Very important
Clinical examination	Physical examination	Identification of the severity of the illness	Clinical signs may not be obvious or specific	<h 1	Very important

	Method	Advantage	Disadvantage	Duration	Clinical significance
Imaging	CXR	Inexpensive, rapid, low exposure to radiation	Wide spectrum of differential diagnoses	Minutes	Standard diagnostic
	Chest computed tomography	Superior quality compared with CXR (higher resolution). May identify minimal active lesions	Images are not pathognomonic for TB. Higher exposure to radiation compared with CXR	<h 1	Improves differential diagnostic ability, improves the evaluation of treatment success
TST	Intracutaneous injection of tuberculin	Inexpensive, widely available	Positive test results in individuals with a history of BCG vaccination. Reduced sensitivity in immunocompromised individuals. Reading of the test result requires a second visit	h 48–72	Standard procedure for the diagnosis of LTBI in non-BCG-vaccinated individuals from countries of low incidence of tuberculosis

	Method	Advantage	Disadvantage	Duration	Clinical significance
IGRA	Quantiferon-Gold-IT assay: IFN-γ-release in whole blood following *ex vivo* stimulation with ESAT-6, CFP-10 and TB 7.7	Very high specificity (T-SPOT.TB< QTF-G-IT) High sensitivity (T.Spot.TB >QTF-G-IT) for LTBI	IGRA responses from peripheral blood do not allow a discrimination of active TB and LTBI. More expensive than the TST	day 1	Substitutes TST in routine clinical practice, especially where BCG vaccination is prevalent
	T-SPOT.TB assay: IFN-γ-release by peripheral blood mononuclear cells following *ex vivo* stimulation with ESAT-6 and CFP-10	Can be adapted to comparatively assess immune responses in the periphery (blood) and at the site of disease (e.g. BAL)	Requires appropriate lab-facility	day 1	Local immunodiagnosis of MTB-specific cells in the BAL, pleural effusion, peritoneal fluid or CSF can distinguish active TB from LTBI
Microscopy	Staining for acid-fast bacilli (Ziehl–Neelsen or Kinyoun method)—	Inexpensive, rapid, low technical demands	No differentiation between MTB and NTM species	h 2	Standard diagnostic procedure

	Method	Advantage	Disadvantage	Duration	Clinical significance
	Fluorescence				
NAAT	Identification of MTB-specific genomic sequences	Differentiates MTB from NTM. Very high specificity, very high sensitivity when acid-fast bacilli are seen on sputum smears. Rapid amplification of genes that are related to drug resistance forms the basis of line probe assays	Diagnostic accuracy of 'in-house' methods may be highly variable. Limited sensitivity in smear-negative cases	days 1–2	Requires special laboratory facilities. Not sensitive enough in smear-negative cases
Culture	Growth of MTB on solid or in liquid media	Definitive proof of active disease	Results are not readily available	weeks 2–6	Gold standard for active tuberculosis

	Method	Advantage	Disadvantage	Duration	Clinical significance
Histology	Caseating granuloma in biopsy specimen	Very supportive of active TB	Histology does not distinguish TB (or NTM infection) from other granulomatous diseases (except with presence of stainable AFB)	days ~1–2	Important when sputum acid-fast bacilli smear are negative
Serology	Identification of MTB-specific antibodies	High specificity	Low sensitivity	h 2	Currently not advocated
Other	Adenosine deaminase on pleural or cerebrospinal fluid	Inexpensive, high diagnostic accuracy	Not indicated for active TB	h 2	Inexpensive and useful for the rapid diagnosis of pleural or meningeal tuberculosis

- AFB, acid-fast bacilli; BAL, bronchoalveolar lavage; BCG, Bacille Calmette Guerin; CFP-10, culture filtrate protein-10; CSF, cerebrospinal fluid; CXR, chest X-ray; ESAT-6, early secretory antigenic target-6; IFN-γ, interferon-γ; IGRA, interferon-γ release assay; LTBI, latent tuberculosis infection; MTB, *Mycobacterium tuberculosis*; NAAT, nucleic acid amplification techniques; NTM, non-tuberculous mycobacteria; TST, tuberculin skin test.

CONCLUSION:

The good combination of diagnosis and treament is the most critical element of tuberculosis control and it will remain so until the advent of novel vaccines or drugs powerful enough to prevent development of tuberculosis perfectly. In the middle of the 20th century the treatment of tuberculosis

made a revolutionary progress with the development of a series of chemotherapeutics, while only very little change was seen in the diagnostics. This caused disruption of the above combination leading to a low case detection rate in contrast with a fairly high treatment success rate as we see today worldwide. However, tuberculosis control is not possible, if the diagnosis of active cases is delayed as *tuberculosis M.* continues to be transmitted from cases to contacts. In addition, false positve diagnosis of LTBI has caused unnecessary burden to individuals and healthcare systems. The urgent need for innovation in diagnostics is obvious.

However, it is good to see that the changes in diagnostics have started towards the end of the last century, assisted by the progress of biotechnology and the late riser's alertness to the problem. The balance between developments in the diagnosis and in the treatment of tuberculosis has changed. Recent diagnostic advances overweigh the inefficient progress of new drug development against tuberculosis by far. Today, we have the technology to rapidly identify individuals with smear-positive MDR or XDR tuberculosis, but we do not have the drugs to treat these patients adequately.**8**,

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