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GP Forum

Diagnosis of pulmonary tuberculosis : recent advances

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India continues to have the highest number of TB cases in the world, and over 2 million cases are reported in India every year. The private sector in India is an important source of TB care with over 50% of TB patients seeking TB treatment in the private health sector. Early and accurate diagnosis is the first critical step in controlling TB. All persons with cough lasting two weeks or more, or with unexplained chronic fever and/or weight loss should be evaluated for TB. For pulmonary TB, Sputum is the most critical sample for laboratory testing. Although blood is a popular sample in the Indian private sector, there is no accepted, valid blood test for pulmonary TB. There are three accepted, validated sputum tests for active TB: Sputum smear microscopy for acid-fast bacilli; molecular or nucleic acid amplification test (NAAT); and culture. Chest radiography is useful but is not specific for the diagnosis of pulmonary TB. Therefore, chest radiography cannot provide a conclusive diagnosis on its own, and needs to be followed by sputum testing. If sputum smears and NAATs are negative, and TB is still suspected, cultures are the most sensitive tests available for TB.Culture is therefore very useful in diagnosing smear-negative TB, and drug-resistant TB. Blood-based antibody tests (eg, IgG/IgM tests) and interferon-gamma release assays (eg, TB Gold) are not accurate and should not be used for pulmonary TB diagnosis. In fact, use of serodiagnostic tests for TB is banned by the Government of India.

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Key words **: Tuberculosis, diagnosis, India, serological tests, test accuracy, case finding.**

The DOTS strategy for tuberculosis (TB) control has been a successful public health intervention[1]. Yet TB remains a problem of enormous magnitude that does not receive the attention it deserves. The statistics are compelling - nearly 9 million new cases and 1.5 million TB deaths every year[2]. Although many countries have met the Stop TB Partnership's targets of 70% case detection and 85% cure rate by 2005 (70/85 targets), TB incidence is still not falling or not falling as quickly as expected. One important reason is thatTB patients are not diagnosed and cured quickly enough. When TB patients are not diagnosed and cured quickly, they may unknowingly spread their infection to their families and communities – further exacerbating the epidemic. Without better tests and approaches for TB diagnosis, TB cannot be eliminated by 2050.

India continues to have the highest number of TB cases in the world, with over 2 million active TB cases every year[3]. Drug resistant TB is a growing concern, especially in hot-spots like Mumbai[4]. Studies have shown considerable delays in TB diagnosis, and patients often move from one healthcare provider to another before they are diagnosed and put on TB treatment[5]. About half of all TB cases are treated in the private sector in India[6]. This means general practitioners have a key role to play in early and prompt diagnosis of pulmonary TB. This article provides an overview of the internationally recommended approaches for TB diagnosis, and recent advances in this area. It will also provide an overview of a recent Indian governmental order on banning TB serological tests.

Internationally Accepted Methods for TB Diagnosis :

All persons with otherwise unexplained productive cough lasting two weeks or more, or with unexplained chronic fever and/or weight loss should be evaluated for TB[7]. In addition, clinically vulnerable populations such as people living with HIV, household contacts of TB cases, malnourished children, diabetics, and tobacco users should also be considered for intensified TB screening.

For pulmonary TB, sputum is the most critical sample for laboratory testing. Although blood is a popular sample in the Indian private sector, there is no accepted, valid blood test for pulmonary TB. Therefore, blood samples are of no value. For extrapulmonary TB, it is important to collect specimens from the site of the disease. For example, for suspected TB lymphadenitis, it is important to do a biopsy or fine needle aspiration. For pleural effusion, pleural tap and/or biopsy are important. For TB meningitis, cerebrospinal fluid (CSF) is the specimen of choice.

All patients (adults, adolescents, and children who are capable of producing sputum) suspected of having pulmonary TB should have at least two sputum specimens submitted for microscopic examination and/or a World

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Health Organisation (WHO) approved molecular test in a quality-assured laboratory[7]. Where feasible, 2 sputum specimens can be collected on the same day, a minimum of one hour apart, to minimise losses to follow-up[8].

There are 3 accepted, validated sputum tests for active TB: Sputum smear microscopy for acid-fast bacilli (AFB); molecular or nucleic acid amplification test (NAAT); and culture (Table 1). Chest radiography is useful and can be performed along with sputum investigations, but is not specific for the diagnosis of pulmonary TB. In other words, many lung diseases and infections can cause radiological abnormalities. Therefore, chest radiography cannot provide a conclusive diagnosis on its own, and needs to be followed by sputum testing. Therefore, all persons with chest radiographic findings suggestive of TB should have sputum specimens submitted for microbiological examination (eg, sputum smears or WHO -approved molecular tests)[7].

At a minimum, all patients with suspected pulmonary TB should be offered two smears for AFB. Sputum smears detect highly infectious patients and has high specificity. However, sensitivity of sputum AFB is modest (about 50-60%), although higher sensitivity can be obtained by performing fluorescence staining and with the use of light-emitting diode (LED) microscopy (Fig 1). LED microscopy is approved by the WHO.

NAATs amplify and detect DNA of *Mycobacterium tuberculosis*. They are more sensitive than sputum smears and can help diagnose TB rapidly. NAATs can either be in-house ("home-brew") tests or commercial kits. In-house PCR is known to produce highly inconsistent results and should be avoided[9]. While there are many commercial NAATs on the market, the biggest recent advance is the development and WHO endorsement of Xpert® MTB/RIF (Cepheid Inc, Sunnyvale, CA, USA), an automated, cartridge-based NAAT that can detect TB, as well as drug-resistance, within 90 minutes (Fig 2)[10]. When compared to culture, Xpert has about 88% sensitivity and 98% specificity. For rapid detection of rifampicin resistance, the sensitivity is about 95% and specificity is 98%. So, this test is substantially more sensitive than sputum smear microscopy, and has great potential to increase the case detection rate.

In August 2012, the United States President's Emergency Plan for AIDS Relief, the United States Agency for International Development, UNITAID, and the Bill & Melinda Gates Foundation announced an agreement that has significantly reduced the cost of the Xpert technology by more than 40%. Funds provided by this partnership have reduced the cost of Xpert MTB/RIF cartridges from $16.86 to $9.98.This reduced price is available for the public sector in 145 high-burden and developing countries. The private sector in high TB burden countries is not eligible for this price. Efforts are currently underway to make the Xpert MTB/RIF test more affordable in the Indian private sector, by forming a consortium of private laboratories which will offer the test at lower prices.

If sputum smears and NAATs are negative, and TB is still suspected, cultures are the most sensitive tests available for TB. TB cultures can be done on solid or liquid media. Liquid cultures are more sensitive than solid cultures and produce results much faster (ie, within 2 weeks).

Table 1 — *Accepted, Validated Sputum Tests for Pulmonary TB*

Test type or platform	WHO endorsed?	Goal of testing (to detect)	Test description	Benchmarks (examples of current validated commercial versions)	Expected sensitivity	Expected specificity
Smear microscopy 	Yes	Active TB	Light-emitting diode fluorescence microscopy	Primo Star iLED™ (Carl Zeiss, Oberkochen, Germany) Lumin™ (LW Scientific, Lawrenceville, GA, USA)	>60% (compared to culture)	98% (compared to culture)
Liquid TB culture 	Yes	Active TB and drug susceptibility testing (DST)	Fully automated system for mycobacterial liquid culture and drug susceptibility testing	BACTEC MGIT® 960 [BD] BacT/ALERT® 3D [bioMérieux]	100% in smear-positive cases >75% in smear-negative cases	>99%
Nucleic acid amplification test (NAAT) 	Yes	Active TB and DST	Cartridge-based automated NAAT is a self-contained and fully automated technological platform that integrates sputum processing, DNA extraction and amplification, TB and MDR-TB diagnosis	Xpert MTB/RIF® [Cepheid]	For active TB: >98% in smear-positive patients and 60 - 70% in smear-negative For detecting drug-resistance to rifampicin: 95%	For active TB: >98% For detecting drug resistance to rifampicin: >98%

Fig 1 — Light-emitting Diode (LED) Fluorescence Microscopy for Acid-fast Bacilli

Fig 2 — Xpert® MTB/RIF (Cepheid Inc, Sunnyvale, CA, USA), an Automated 2-hour Test for TB and Rifampicin Resistance

Culture is therefore very useful in diagnosing smear-negative TB, and drug-resistant TB. Previously treated patients and patients who remain sputum smear-positive at completion of 3 months of treatment and patients who have failed, defaulted from, or relapsed following one or more courses of treatment should always be assessed for drug resistance, either using liquid culture or by doing a NAAT such as Xpert MTB/RIF for rifampicin resistance, or line probe assay (LPA) for INH and rifampicin resistance. LPAs are molecular tests (eg, Genotype MTBDRplus by Hain LifeScience, Germany) that detect mutations that confer drug-resistance and they are endorsed by the WHO. They have excellent accuracy for rifampicin resistance and good accuracy for INH resistance[11].

Role of Serological, Antibody-based Blood Tests for TB :

There are several serological tests on the market, based on antibody detection. For decades, researchers and industry pinned their hopes on serological antibody-detection methods for point-of-care test development. Indeed, dozens of serological rapid (lateral flow assays) and ELISA tests got commercialised, even though no international guideline recommended their use. Today, these tests are on the market in at least 17 of the 22 highest TB burden countries, and millions of patients in the private sector undergo serological testing[12]. The situation is particularly bad in India, where over 1.5 million serological TB tests were estimated to be performed in the private sector[13]. Unfortunately, TB serological tests are neither accurate nor cost-effective, prompting the WHO to issue a historic strong negative recommendation against their use in 2011[14]. The WHO policy states that, since the "the harms/risks [of commercial serodiagnostic tests] far outweigh any potential benefits (strong recommendation)...these tests should not be used in individuals suspected of active pulmonary or extrapulmonary TB, irrespective of their HIV status"[14].

The WHO policy was based on a published meta-analysis that synthesised evidence from 92 studies and concluded that commercial serological tests remain inconsistent and inaccurate, supported only by data of very low quality[15]. A cost-effectiveness study, also considered by WHO, found that serology results in more human suffering, secondary infections, and false-positive diagnoses than sputum smear microscopy, while increasing per-patient costs to the Indian TB control sector[16].

After this WHO policy, the Revised National TB Control Programme (RNTCP) published an advisory statement against the use of serological TB tests in India[17]. Subsequently, an expert committee convened by the Drug Controller General of India (DCGI) reviewed the evidence in December 2011, and unanimously recommended a ban on serodiagnostics for TB in India as these tests provide inconsistent and imprecise results. The DCGI recommendations were formally approved and notified in the Gazette of India by the Ministry of Health and Family Welfare on 7th June 2012. This governmental order bans the use, sale and import of all TB serodiagnostics test kits in India. Serodiagnostic tests for TB are defined immunological tests that detect antibody responses to TB antigens in blood/serum samples. They come in two platforms: (1) ELISA

tests that detect IgG, IgM and IgA antibodies, and (2) rapid strip or card tests (lateral flow assays; immunochromatography tests).The ban applies to all TB serological tests – ELISA as well as rapid diagnostic tests.The ban applies to all TB serological tests – Indian as well as imported. In light of the WHO policy and the Indian government ban, it is important for general practitioners to avoid using all antibody blood tests for TB[18].

Role of Other Blood Tests Like IGRAs for Active TB Diagnosis :

Blood tests like QuantiFERON-TB Gold In Tube ("TB Gold" by Qiagen/Cellestis, Australia) and T-SPOT.TB (by Oxford Immunotec, UK) are called interferon-gamma release assays (IGRAs). These tests do not detect antibodies in the blood. They detect T-cell interferon-gamma response after incubation with TB antigens and measure cellular immune response to TB antigens. They are meant for detection of latent tuberculosis infection (LTBI) and as a potential replacement for the Mantoux tuberculin skin test[19]. Like the Mantoux test, IGRAs cannot separate latent TB infection from active TB disease[20-22].

Tests like QuantiFERON-TB Gold should not be used to replace serological, antibody-detection tests. IGRAs were never meant for the diagnosis of active TB. In fact, a 2011 WHO policy strongly discourages the use of IGRAs for the diagnosis of active TB in low and middle income countries[23]. If tests like QuantiFERON-TB Gold are used for diagnosing active TB, it will result in very high rates of false-positive results and a lot of unnecessary anti-TB drug therapy[22]. This is because an estimated 40% of the Indian population are latently infected[24] and this will result in a large number of positive IGRA results. The same logic applies to the Mantoux test – it has no value for active TB diagnosis in adults in India. Therefore, general practitioners should avoid using tests like TB Gold and Mantoux for active TB diagnosis in adults. In children, these tests can provide supportive evidence, along with symptoms, history of contact, and chest x-rays.

Can serological TB tests be replaced with NAATs on blood samples? NAATs are not recommended for use on blood specimens. They are meant to be used on respiratory samples, and samples from the site of the disease in extrapulmonary TB. Overall, a key message for all practitioners is that there is no valid blood-based test for pulmonary TB. Sputum is the most critical sample to collect and test.

Improving TB Diagnosis and Case Finding in India :

To improve TB case finding in India, several efforts are needed in parallel. Indian practitioners and laboratories must adopt new tools that are accurate, validated, and WHO-endorsed, and replace suboptimal blood tests with sputum tests that can impact patient outcomes and reduce TB transmission in the community. The DCGI must tighten regulation of diagnostics in the country and ensure that the ban on TB serological tests is enforced. Private laboratories in India must respect the ban on serodiagnostics and avoid replacing antibody tests with tests like TB Gold.

The RNTCP has recently announced its National Strategic Plan (NSP), an ambitious plan for 2012-2017, that aims to provide universal access to quality diagnosis and treatment for the entire Indian population[3]. RNTCP alone cannot meet this goal of universal access. The private sector has a very important role to play in TB control[25]. The Indian Medical Association (IMA) has been engaged in TB control initiatives as a partner of the RNTCP and it should continue supporting the NSP to ensure that all patients in the private sector get high quality diagnosis and TB treatment.

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of delivery decreases the risk of neonatal hypoglycaemia. The Join clinic reported an incidence of 31% of respiratory distress syndrome in infants of diabetic mothers declining to an average of 5.5% in the same clinic with better glycaemic control[8,9]. In this study respiratory distress syndrome was observed in 8.6% of babies, which is comparable. Macrosomia was observed in 6.9% of babies. Comparatively the reported incidence of macrosomia in a literature is 25-40%[6]. Farooq *et al*[3] observed 36%. Early detection of GDM and instituting in time, appropriate management with better achievement glycaemic control might have contributed to such an observation in this study. One baby suffered neonatal sepsis in this study with the mothers suffering from bacterial vaginosis and premature prelabour rupture of membrane delivering at 34 weeks of gestation. A considerable portion of gestational diabetic women may continue to have glucose intolerance[10]. At the follow-up with 75 g glucose OGTT at 6-8 weeks postpartum, a statistically significant number of women were found to have impaired glucose tolerance.

In conclusion, GDM is a commonly occurring medical disorder in pregnancy, which should be timely diagnosed, appropriately managed and monitored in order to avoid foetomaternal complications.

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