CME:

A REVIEW OF LABORATORY DIAGNOSIS OF TUBERCULOSIS

Ngo Viet Quynh Tram
Dept. of Microbiology, Hue University of Medicine and Pharmacy, Vietnam

Summary

The consequences of tuberculosis on human society are immense. Tuberculosis remains a major cause of morbidity and mortality in many countries and a significant public health problem worldwide. Active tuberculosis is diagnosed by *detecting Mycobacterium tuberculosis* complex bacilli in specimens from the respiratory tract (pulmonary TB) or in specimens from other bodily sites (extra pulmonary TB). Rapid diagnostic tools are urgently needed to interrupt the transmission of tuberculosis and multidrug-resistant tuberculosis. Laboratory confirmation of TB and drug resistance is critical to ensure that people with TB signs and symptoms are correctly diagnosed and have access to the correct treatment as soon as possible.

There have been many advances in methodology for tuberculosis diagnosis and earlier diagnosis is of value clinically, and through the early institution of appropriate drug therapy is of public-health benefit. Although many new molecular diagnostic methods have been developed, acid fast bacilli smear microscopy (positive in only half of TB patients) and culture on Lowenstein-Jensen medium (results take weeks to obtain) are still the "gold standards" for the diagnosis of active TB and, especially in low-resource countries.

At present many of new techniques are only economically viable in the developed nations, it is to be hoped that recent advances will lead to the development of novel diagnostic strategies applicable to use in developing nations, where the burden of tuberculosis is greatest and effective intervention most urgently required.

Key words: *Tuberculosis, laboratory diagnosis.*

1. INTRODUCTION

Tuberculosis remains a major cause of morbidity and mortality in many countries and a significant public health problem worldwide. The emergence of drug resistant strains and particularly multidrug-resistant strains of *Mycobacterium tuberculosis*, has become a significant public health problem in a number of countries and an obstacle for an effective control of tuberculosis. Tuberculosis is the number one cause of death in people with HIV/AIDS [1].

In 2012, there were an estimated 8.6 million incident case of tuberculosis (range, 8.3 million - 9.0 million) globally, equivalent to 122 cases per 100,000 population [2]. Tuberculosis is still a major health problem in Vietnam. Vietnam ranks 12 out of the 22 highest TB-burden countries identified by the World Health Organization. In 2011 in Vietnam, 91,500 new cases of TB were identified, with almost 9,000 previous cases needing retreatment, and approximately 30,000 people died from the disease [1].

The early, rapid and accurate detection of TB and drug resistance relies on a well-managed and equipped laboratory network. Laboratory confirmation of TB and drug resistance is critical to ensure that people with TB signs and symptoms are correctly diagnosed and have access to the correct treatment as soon as possible [18].

Active tuberculosis (TB) is diagnosed by detecting *Mycobacterium tuberculosis* complex bacilli in specimens from the respiratory tract (pulmonary TB) or in specimens from other bodily sites (extra pulmonary TB). Although many new (molecular) diagnostic methods have been developed, acid fast bacilli (AFB) smear microscopy and culture on Lowenstein-Jensen medium are still the "gold standards" for the diagnosis of active TB and, especially in low-resource countries, the only methods available for confirming TB in patients with a clinical presumption of active disease. AFB smear microscopy is rapid and inexpensive and thus is a very useful method to identify highly contagious patients. Culture is used to detect cases with low

- Corresponding author: Ngo Viet Quynh Tram, email: qtramnv@gmail.com
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mycobacterial loads and is also requested in cases at risk of drug-resistant TB for drug susceptibility testing, or in cases where disease due to another member of the *Mycobacterium* genus is suspected. AFB smear microscopy and culture can also be used to monitor the effectiveness of treatment and can help to determine when a patient is less likely to be infectious. Two manuals are recommended for the laboratory diagnosis of TB [19],[14].

However, AFB smear is positive in only half of patients with subsequently culture positive for Mycobacterium tuberculosis. Although the sensitivity of the smear is improved by fluorescent staining, the test fails to distinguish between tuberculous and nontuberculous mycobacteria. Correct diagnosis of TB is needed to improve treatment, reduce transmission, and control development of drug resistance. In patients with active pulmonary TB, only an estimated 45% of infections are detected by sputum microscopy [1],[18],[11]. This test, first developed in the 1880s and basically unchanged today, has the advantage of being simple, but is hampered by very low sensitivity: it may only detect half of all cases with active infection. It is also very dependent on the skill of the technician, and a single technician can only process a relatively small number of slides per day [11]. Furthermore, a staggering three million people who present annually with suspected TB may not be properly diagnosed, because their infection (so-called smear-negative disease) cannot be detected by sputum microscopy [7].

Diagnosis based on culture is the reference standard but results take weeks to obtain[1],[2]. Mycobacterial culture with the Lowenstein-Jensen (LJ) medium after decontamination and concentration is the traditional method for identification, but it takes at least 3 weeks to allow for sufficient growth for biochemical or genotypic confirmation. Drug susceptibility testing (DST) on cultured specimens is the conventional method used to detect resistance to first- and second-line TB drugs. The BACTEC MGIT 960 culture system, which uses the modified Middlebrook 7H9 broth and a fluorescent signalling system, allows for earlier detection of growth, but it still takes at least 10 days [17].

There are specific epidemiological factors that present additional challenges to TB diagnosis. HIV infection is thought to be a major contributor to the increase in TB incidence across the world [14]. An estimated 9% of adults globally with newly diagnosed TB are HIV positive. HIV co-infection with TB presents challenges to effective diagnosis of TB and diagnosis can also be more difficult in children. The rapid rise of drug-resistant (DR) TB has further complicated TB diagnosis [2]. Tests that measure drug susceptibility are essential to monitor the spread of resistant TB strains, and ensure that patients are given effective treatment.

New diagnostic tests that are simple and robust enough to be used in the field, accurate enough to diagnose all infected individuals, and able to identify drug resistance are desperately needed, and represent an essential complement to new drug development efforts and to effective control and treatment programmes [16].

An individual who is suspected of having TB disease requires a complete medical evaluation, including the following [7]:

1. Medical history, including exposure, symptoms, previous treatment for TB, and risk factors.
2. Human immunodeficiency virus (HIV) screening.
3. Physical examination.
4. Tuberculin skin test (TST) or interferon gamma release assay (IGRA).
5. Chest radiography.
6. Bacteriologic examination (laboratory diagnosis).

2. LABORATORY DIAGNOSIS

A definitive diagnosis of tuberculosis can only be made by culturing Mycobacterium tuberculosis organisms from a specimen taken from the patient (most often sputum, but may also include pus, CSF, biopsied tissue, etc.). A diagnosis made other than by culture may only be classified as “probable” or “presumed”. For a diagnosis negating the possibility of tuberculosis infection, most protocols require that two separate cultures both test negative [3].

2.1. Sputum

Sputum smears and cultures should be done for acid-fast bacilli if the patient is producing sputum. The preferred method for this is fluorescence microscopy (auramine-rhodamine staining), which is more sensitive than conventional Ziehl-Neelsen staining. In cases where there is no spontaneous sputum production, a sample can be induced, usually by nebulized inhalation of a saline or saline with bronchodilator solution. A comparative study found that inducing three sputum samples is more sensitive than three gastric washings [3].

2.2. Alternative sampling

In patients incapable of producing a sputum sample, common alternative sample sources for diagnosing pulmonary tuberculosis include gastric washings, laryngeal swab, bronchoscopy (with bronchoalveolar lavage, bronchial washings, and/or transbronchial biopsy), and fine needle aspiration (transtracheal or transbronchial). In some cases, a more invasive technique is necessary, including tissue biopsy during mediastinoscopy or thoracoscopy [9].

2.3. Culture - Based Methods

Culture of *Mycobacterium tuberculosis* remains the gold standard for both diagnosis and drug sensitivity testing. Many types of cultures are available [5]. A culture of the AFB can distinguish the various forms of mycobacteria [12]. Conventional culture methods using Lowenstein-Jensen (LJ), Kirchner, or Middlebrook media (7H9, 7H10, and 7H11), while cheap and simple, have the major disadvantage of being very slow. LJ cultures take 20–56 days for diagnosis and four to six weeks after initial culture for drug sensitivity testing. 7H11 medium slightly accelerates the process, but requires antibiotics in the medium to prevent contamination and a CO_2 incubator. Diagnosis with 7H11 medium takes 17–21 days, Daylight Saving Time (DST) information is available three to six weeks later. Some more rapid culture methods have been developed and are commercially available, most of which are difficult to implement in the field due to the complexity of the technique or the required equipment. New automated systems that are faster include the MB/BacT, BACTEC 9000, Versa TREK, and the Mycobacterial Growth Indicator Tube (MGIT) [16],[12]. There are also some emerging simplified culture techniques that can reduce time to diagnosis or DST that seem more appropriate for use in resource-limited settings such as the Microscopic Observation Drug Susceptibility (MODS) assay culture may be a faster (7-9 days) and more accurate method [9],[12],[1].

The sensitivity of culture is limited by the need to have bacilli present in the sample to be cultured. HIV positive patients and children have difficulty in producing sputum and sputum culture will not detect extrapulmonary (EP) forms of TB. EP TB is very common in HIV positive patients and is rapidly fatal. Even in patients with active pulmonary TB, the bacilli may be protected in lung cavities or not present in a particular sputum sample, or may be lost in the decontamination treatment required to process sputum for mycobacterial culture. All these factors limit the usefulness of the technique [16].

2.4. Nucleic Acid Amplification Test (NAA test/Polymerase chain reaction:PCR)

The use of nucleic acid amplification (NAA) tests in non-specialised laboratories is technically challenging. These tests have been shown to be highly specific, but sensitive if starting from patient samples, low and highly variable and is difficult to assess. These tests can also be used from primary culture. Although this improves the sensitivity, the technique is then very slow [16].

The NAA test is useful for the rapid detection of *M tuberculosis* in respiratory specimens. The Enhanced Amplified MTD (Mycobacterium Tuberculosis Direct) test (Gen-Probe, San Diego, CA) detects *M tuberculosis*ribosomal RNA directly from AFB smear–positive and AFB smear–negative respiratory specimens from patients with suspected TB. The Amplicor MTB (Mycobacterium Tuberculosis) test (Roche Diagnostic Systems, Branchburg, NJ) detects *M tuberculosis* DNA in AFB smear–positive respiratory specimens[17],[8].

Interpretation of NAA test results should be correlated with AFB smear results [4]. Positive findings on the NAA test and a positive sputum AFB smear are strongly indicative of TB [4]. When NAA and sputum microscopy test results are discordant, physicians should exercise their clinical judgment in deciding whether to start anti-TB treatment while culture results are awaited [6]. When the clinical suspicion for TB is high, a positive NAA test result in smear-negative cases can be valuable for the early detection of TB in approximately 50% to 80% of cases [6]. Findings on the NAA test often remain positive after cultures become negative during therapy and can remain positive even after completion of therapy [15]; therefore, it should not be used for assessing infectivity or response to treatment [8].

Other mycobacteria are also acid-fast. If the smear is positive, PCR or gene probe tests can distinguish *M. tuberculosis* from other mycobacteria. Even if sputum smear is negative, tuberculosis must be considered and is only excluded after negative cultures [9].

Some polymerase chain reaction (PCR) based techniques are being validated for use on patient samples for rapid detection of rifampicin/isoniazid resistance**[**16**]**.

Our department routinely performs in house IS6110 nested PCR and 16S rDNA realtime PCR assays for molecular diagnosis and monitoring therapy of pulmonary tuberculosis. Tuberculosis pleuritis, pericarditis, and meningitis have been associated with low number of organisms but high mortality. Microscopic examination of fluid or tissue is rarely positive and culture yield is also low. Therefore, a sensitive, rapid and accurate test would be of tremendous benefit in the diagnosis of extra pulmonary TB. Recently, our PCR assays have been extended to detect *Mycobacterium tuberculosis* in extra-pulmonary specimens with satisfactory results.

2.5. Others

A number of strategies to detect and report the presence of *M. Tuberculosis* have been developed. Serology (detection of antibodies) has not produced any reliable, informative tests despite decades of work. Detection of antigens is a more promising approach, as it detects the presence of the organism and thus may be able to diagnose active infection.

There are also some tests being developed that detect immunological responses (interferon gamma assays). These tests are rather expensive and complicated to perform, and still need to be validated in endemic areas, and their interpretation is not clear **[**16**]**.

3. CONCLUSION

There is a need to urgently address deficiencies in the diagnostic service for tuberculosis. There have been many advances in methodology for tuberculosis diagnosis and earlier diagnosis is of value clinically, and through the early institution of appropriate drug therapy is of public-health benefit. Nevertheless, many diagnostic tests have given promising results initially only to prove less effective in routine use. This is frequently due to bias resulting from non-independent interpretation of test results. While, at present many of these techniques are only economically viable in the developed nations, it is to be hoped that recent advances will lead to the development of novel diagnostic strategies applicable to use in developing nations, where the burden of tuberculosis is greatest and effective intervention most urgently required.

REFERENCES

1. Ngô Viết Quỳnh Trâm, Nguyễn Thị Châu Anh, Lê Hữu Dũng, Lê Nữ Xuân Thanh, Piero Cappuccinelli (2011), "Nuôi cấy vi khuẩn lao và phát hiện lao đa kháng thuốc bằng phương pháp MODS'',Tạp chí Thông tin Y Dược, số 9: 13-16.
2. ATS, CDC, IDSA (2005). ''Controlling Tuberculosis in the United States: Recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America''. MMWR, 54(No. RR-12): 51p.
3. Biswajit Batabyal, Sukanta Chakraborty (2013). "A review of laboratory diagnosis of tuberculosis'', J Pharm Biol Sci, 1(1): 26-28.
4. Centers for Disease Control and Prevention (CDC) (2009). "Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis''. MMWR Morb Mortal Wkly Rep. ;58(1):7-10 [PubMed]
5. Drobniewski F, Caws M, Gibson A, Young D (2003). "Modern laboratory diagnosis of tuberculosis". Lancet Infect Dis; 3 (3): 141–147.
6. Guerra RL, Hooper NM, Baker JF, et al (2007). ''Use of the amplified Mycobacterium tuberculosis direct test in a public health laboratory: test performance and impact on clinical care''. Chest; 132:946-951 [PubMed]
7. In the WAC, see Chapter 246-101 (Notifiable Conditions) in the Title 246 (Department of Health) at http://apps.leg.wa.gov/wac/default.aspx?cite=246-101
8. Irene G. Sia, Mark L. Wieland (2011), ''Current Concepts in the Management of Tuberculosis'', Mayo Clin Proc. 86 (4): 348–361.
9. Kumar, Vinay; Abbas, Abul K.; Fausto, Nelson; Mitchell, Richard N. Robbins (2007). Basic Pathology. 8th ed, Saunders Elsevier: 516-522.
10. Laraque F, Griggs A, Slopen M, Munsiff SS (2009). ''Performance of nucleic acid amplification tests for diagnosis of tuberculosis in a large urban setting''. Clin Infect Dis. ;49:46-54 [PubMed]
11. Master, R.N (1992). "Mycobacteriology". Clinical Microbiology Procedures Handbook, 1: ASM, Washington D.C.
12. Moore D, Evans C, Gilman R, Caviedes L, Coronel J, Vivar A, Sanchez E, Piñedo Y, Saravia J, Salazar C, Oberhelman R, Hollm-Delgado M, LaChira D, Escombe A, Friedland J (2006). "Microscopic-observation drug-susceptibility assay for the diagnosis of TB". N Engl J Med; 355 (15): 1539–1550.
13. PATH (2013), "Supporting tuberculosis control in Vietnam - Building bridges to reduce TB

burden'' http://www.path.org/publications/files/CP_vietnam_tb_program_fs.pdf.
14. PT Kent, GP Kubica (1985).'' Public Health Mycobacteriology: A Guide for the Level III Laboratory''. US Department of Health and Human Services,. CDC, Atlanta, Georgia.
15. Schluger NW, Rom WN (1994). "Current approaches to the diagnosis of active pulmonary tuberculosis''. Am J Respir Crit Care Med. ;149:264-267 [PubMed]
16. Suresh Jaiswal, Jay Prakash Sah, Bhoopendra Sharma (2013). ''Standard Diagnostic Procedure for Tuberculosis: A Review''. Research and Reviews: A Journal of Life Sciences, Volume 3, Issue 3.
17. WC Yam (2006). ''Recent advances in Rapid Laboratory Diagnosis of Tuberculosis'' Medical Bulletin, Hong Kong; Vol. II, No. I.
18. World Health Organization (2013). Global tuberculosis report 2013, WHO Library Cataloguing-in-Publication Data.
19. World Health Organization (2004). The World Health Report 2003: Changing History. Geneva, Switzerland.