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Laboratory Diagnosis of Tuberculosis - Latest Diagnostic Tools

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1. Introduction

Early diagnosis of tuberculosis and drug resistance improves survival and by identifying infectious cases promotes contact tracing, implementation of institutional cross-infection procedures, and other public-health actions. There have been many advances in methodology for tuberculosis diagnosis [1-3].

For every stages of diagnosis, there are new approaches. New tests are available by level of laboratory and phase of application.

2. Microscopy

Microscopy has been a diagnostic tool for TB for over a century, and still currently the most rapid diagnostic method. Standard light microscopy (LM) and fluorescent microscopy (FM) are common methods. The recent development of light emitting diodes (LED), with the appropriate fluorescent light output for FM and low power consumption, has led to the development of simple, robust LED FM microscopes, requiring minimal mains or battery power and no dark room requirement. The WHO has recommended rolling it out as an alternative to LMs in resource-limited settings, based on studies that have shown comparable performance of LM and standard FM systems [4,5].

3. Culture and drug resistance testing

3.1. Phenotypic methods

Significant effort has been invested into further development of simple, alternative phenotypic methods such as the nitrate reductase assay (NRA), thin-layer agar (TLA), colour test (Color Test), the microscopic observation drug susceptibility assay (MODS), the colorimetric redox indicator (CRI) method and phage-based assays, most of which can be set up directly on specimens [6,7,8]. These methods can detect MTB and resistance to INH and RMP. While MODS, NRA and CRI have been endorsed by the WHO, current evidence was considered to be insufficient for recommending the use of TLA or phage-based assays [8].

MODS is an extensively validated method that has almost perfect agreement with conventional DST for INH, RMP and MDR-TB (100%, 97% and 99%). The results are available within a median of 7 days; the method is cheap, non-commercial and works well on all types of primary specimens as well as on isolates. However, it requires relatively long, detailed staff training. [6,7,9,10]

TLA recently demonstrated a good performance of the MDR-/XDR-TB colour test for the identification of MTB complex and detection of resistance to INH, RMP and ciprofloxacin in cultures [11].

3.2. Genotypic methods

Molecular techniques are aimed at the nucleic acid of the mycobacterium as the analyte. Ribosomal rRNA is useful genetic target for the identification of organisms, since it often contains specific sequences and is present in the cells and media in high quantity due to the growth of the mycobacteria. There are various applications of molecular techniques for the detection and identification of MTB.

PCR is the common format of nucleic acid amplification tests (NAAT); other methodologies include ligase chain reaction, strain displacement amplification, loop-mediated isothermal amplification (LAMP) and transcription mediated amplification. More recently, real-time (RT) PCR technologies based on fluorescent- probe detection or melting-curve analysis have been developed [12-16].

These molecular techniques also aimed detecting resistance genes. Example includes; DNA probe and DNA sequencing of MTB gene such as catalase (*katG*) or RNA polymerase (*rpoB*). Mutations in these genes have been associated with resistance to isoniazid and rifampicin respectively. The using of molecular primers in real- time PCR reaction can differentiate between the presence of the wild- type sequence and mutated sequence associated with drug resistance. Molecular tests are rapid (within few hours), highly sensitive and specific, but expensive, requires expertise and may not differentiate active infection as DNA from a dead organism during antibiotic treatment can be detected and amplified by PCR [17]. Genotypic methods are not routinely used in the mycobacterium laboratory; they are essentially for research purposes [18,19].

Line-probe assays and XPERT MTB/RIF: Line probe assays (LPAs) are actual molecular tests. Three main LPAs for the rapid diagnosis of TB and/or rapid detection of RMP resistance and MDR-/XDR-TB are currently available on the market: INNO-LiPA Rif. TB (Innogenetics, Belgium), GenoType®MTBDR/MTBDR*plus* and Geno-Type® MTBDR*sl* (both Hain Lifescience, Germany). These assays are based on the targeted amplification (PCR) of specific fragments of the MTB genome, followed by hybridisation of PCR products to oligonucleotide probes immobilised on membranes. INNO-LiPA Rif TB detects only RMP resistance, GenoType MTBDR/MTBDR*plus* detects both RMP and INH resistance, and GenoType MTBDR*sl* detects resistance to fluoroquinolones, injectable second-line drugs and ethambutol. These tests are designed for detection the MTB isolates in respiratory specimens. Xpert® MTB/RIF (Cepheid Inc, USA) is a fully automated RT-PCR based assay for the detection of TB bacteria and resistance to RMP in direct clinical specimens [20].

4. Lysis-centrifugation blood culture system

The recovery of mycobacterium from peripheral blood and bone marrow samples may be improved by lyses- centrifugation blood culture method. In this method, blood is put into a tube containing an anticoagulant and an agent to effect rupture of both erythrocytes and neutrophils. Following centrifugation of the tube, the sediment is inoculated into the appropriate culture media. This method has increased both the yield and shortened the time of recovery of mycobacteria from blood cultures [21].

5. Phage Amplification Technique (PAT)

This is a bacteriophage based test to detect MTB in sputum. Non-pathogenic mycobacteria (sensor cells) were used for control bacteria in the test. The phage replicate, infect and lyses the sensor cells leaving zones of clearing (holes) in the agar. The areas of clearing indicates that the patient sputum contain viable MTB. It is fast with a turnaround time of 2 days. It is cheap, requires few equipments, sensitive (detection as low as 100 tubercles per ml of sputum). It can be adapted for sensitivity testing. Limitations are applicability to sputum specimen only and technically demanding [22].

6. Immunological methods

Immunodiagnostic tests can provide indirect evidence current or past infections of MTB. Exception of tuberculin skin test, immunodiagnostic tests are of limited application due to cross reactivity and poor sensitivity.

6.1. Detection of antibodies

Although the detection of antibodies against MTB in the blood is a relatively simple and cost-effective method, recent meta-analyses and systematic reviews concluded that commercial serological tests provided inconsistent results [23,24]. As the overall test performance and data quality of these assays were poor, the WHO currently recommends against their use for the diagnosis of pulmonary and extrapulmonary TB.

Antibodies against lipoarabinomannans, A60, 38Kd and 16 Kd are mostly studied [25].

6.2. Detection of antigens

Lipoarabinomannan (LAM) was identified as a promising target for antigen detection for TB diagnosis due to its temperature stability and could be detected in urine. LAM-based assays are currently being developed by a number of commercial companies, and preliminary results indicate their potential applicability in the rapid diagnosis of TB by detecting LAM in a variety of body fluids, including urine [26]. LAM-based assays are included in the WHO TB diagnosis re-tooling programme [27] and form a part of a Foundation for Innovative New Diagnostics (FIND) funded TB Project [19,28].

MTB antigen detection provides direct evidence of TB. Such as LAM, 65Kd, 14 Kd antigens were widely used. It is very quick and easy to perform. Main limitation is low sensitivity (detect high levels of antibody). It does not rule out TB in patients with poor antibody response as in HIV and malnutrition and not specific due to cross reactivity with other species of mycobacteria in the environment [26].

7. Interferon-Gamma Release Assays (IGRA)

Because of the difficulties with the tuberculin test interpretation, the interferon-gamma assay test was developed. Two available formats of the interferon-gamma release assays are; the QuantiFERON-TB Gold and T Spot-TB test. The IGRA assay is based on the ability of the MTB antigens, which includes the Early Secretory Antigen Target 6 (ESAT-6) and Culture Filtrate Protein 10 (CFP-10) to stimulate host production of interferon -gamma. These antigens are not present in NTM or in BCG vaccine, so, these tests can distinguish latent TB infection from BCG immunization and NTM infections. Requiring a single visit to draw a blood sample and result available within 24 hours are main advantages. It does not boost immune response measured by subsequent tests which can happen with tuberculin skin test. It does not cause to readers bias as in tuberculin skin test and not affected by prior BCG vaccination. Blood must be processed within 12 hours while leukocytes are still viable. There are limited data for sensitivity of IGRAs in children younger 17 years of age and immunocompromised patients e.g. HIV/AIDS, diabetics, treatment with immunosuppressive drugs [29].

8. The future of TB diagnostics

The rapid technological evolution in the laboratory diagnosis of TB, especially in the application of molecular biology has diminished the time required for identification and susceptibility testing. Continuous effort endeavor for increasing reproducibility, improvement of performance and cost containment. WHO founded an organisation (FIND-Foundation for Innovative New Diagnostics) for researching fast, reliable and inexpensive tests as given Table 1 [28].

	Concept phase	Feasibility phase	Development phase	Evaluation phase	Demonstration phase	Implementation phase
Reference laboratory level	-	-	-	-	-	Liquid culture & DST Rapid speciation Line probe assay (1st line drugs)
District/peripheral level	-	Rapid colorimetric DST	-	Line probe assay / 2 nd line drugs)	LAMP TB	LED fluorescence microscopy Xpert MTB/RIF
Community level	LFI sensitivity increase	Antibody detection Antigen detection	Beta lactamase detection	-	-	-

LFI sensitivity increase: Alternative quantitative fluorescence (LFI) sensitivity increase

Table 1. WHO projects for TB diagnostic tests. LAMP TB: Loop mediated isothermal amplification (LAMP) for TB

9. Assays being developed/evaluated

Another new approach to diagnosis of TB is biosensing technologies. Variety of portable, rapid, and sensitive biosensors with immediate “on-the-spot” interpretation have been developed for MTB detection based on different biological elements recognition systems and basic signal transducer principles. Combination of nanotechnology and biosensing technology has very promising.

Transrenal DNA detection provides a challenging new target for molecular TB diagnosis. No commercial assays are currently available, largely due to the difficulties in the development of TB detection/read-out assays.

Combined high-resolution melting (HRM) curve analysis using a closed-tube RT-PCR is potentially an ideal screening method with a positive predictive value (PPV) of 100% and neg-

ative predictive value (NPV) of at least 99.9%, for screening large specimen numbers in any TB laboratory.

New amplification methodologies and refinements of 'molecular beacon' approaches, such as linear-after-the exponential PCR, offer future improvements, particularly in drug resistance analysis [30-32].

MTB urease is a bacterial virulence factor. Isotopically labelled urea as substrate, Urea tracer has detected in exhaled breath using portable infrared spectrofotometer. Signal correleted with bacterial load. [33]

A biophotonic detection platform has been developed that utilizes reporter enzyme fluorescence to detect β -lactamase produced by MTB. This innovative new technology is now being adapted for point of care (POC) use [28]

10. Conclusions and further work

This is an exciting time for new TB diagnostics. This is in part a reflection of the funding and application of good science, a clear understanding of unmet needs, a commercial sector that is considering new approaches to a global market, and the complexity of and limited progress in new drug and vaccine development, which has encouraged more academic and industrial partners to participate in diagnostic development.

Overall, the technology for the diagnosis of TB and RMP resistance in pulmonary specimens is well advanced, with high specificity and increasingly high sensitivity. Rapid, high-specificity molecular assays for TB identification and drug resistance cannot replace the standard diagnostic methods, such as microbiology, clinical and radiological assessments, and conventional DST for active TB in pulmonary (particularly sputum smear-negative) and extra-pulmonary TB specimens. Implementation of all of these tools in routine laboratory practice requires the implementation of appropriate quality assurance systems.

The performance of molecular tools of extra-pulmonary specimens varies and should be considered separately for each specific specimen type. Evidence for the use of these assays to identify TB and detect drug resistance in TB-HIV co-infected individuals is limited. There is a need for designed studies among children, including HIV-positive children. There also remains a need to increase the sensitivity of TB detection among all patients, but especially among immunocompromised patients and children [20].

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