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Mini Review

Biological Tools for the Diagnosis of *Mycobacterium tuberculosis* Infections

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Abstract

The tubercle bacilli was first discovered in 1882 by Robert Koch. Since this period, biological diagnosis of Tuberculosis (TB) has evolved considerably. New culture media were used and various means for detecting the growth and molecular methods to directly amplify the genetic material of microorganisms were identified and added to microscopic examination. There is also the development of indirect diagnostic methods, mainly for TB latent infection.

Keywords: *Mycobacterium tuberculosis*, diagnostic, biology

Introduction

Tuberculosis remains a major worldwide public health threat with around 10 million people newly infected and about 2 million death in 2017. Biology plays a key role in detecting patients suffering from the disease with the final goal to treat them. Along with this objective, resistance profile of the strains can be determined in order to provide patients with the most efficient treatment. Also, biological tools are at great important in monitoring the evolution of the disease.

Phenotypic Methods

Smear microscopy

After the isolation of *Mycobacterium tuberculosis*, the first decisive innovation made by Robert Koch, was to develop the method allowing to stain the bacterium. This method was finalized by Ziehl and Neelsen, leading to one of the two most commonly used methods for staining mycobacteria [1,2]. These techniques reveal the ability of mycobacteria to retain their color after the action of acid and alcohol used as bleaches, hence the initial term "Acid-Fast Bacilli" (AFB). The second method using a fluorochrome has the advantage of allowing a faster reading of the slides. It requires a fluorescence microscope made of devices comprising Light Emitting Diodes (LEDs) adapted to an ordinary microscope or new fluorescent microscopes LED type, which are cheaper alternatives to the traditional mercury fluorescence microscope. Microscopic examination of smears allows rapid and reliable identification of patients with pulmonary tuberculosis even if it is less sensitive than other methods [3]. This technique is also less specific as *M. tuberculosis* has the same appearance as Non-Tuberculous Mycobacteria (NTM).

Culture

Mycobacterial culture allows to confirm of the diagnosis of tuberculosis and is more sensitive than microscopy. Indeed, 10 bacilli/ml may be sufficient for the positivity of the culture [3]. After decontamination and fluidification, to eliminate other contaminating organisms, the sample is centrifuged and the pellet inoculated on different media. Is it possible to use solid media (Lowenstein-Jensen, 7H10 or 7H11 Middlebrook medium), Middlebrook 7H9 based liquid medium (MGIT, BacT/ALERT MP) and micro colony detection methods with the use of thin layer TLA agar [4] [5]. In case of growth either on a solid or liquid medium, the microorganism must be identified by phenotypic or genotypic methods. Instead of a precise determination of the specie, some simple phenotypic tests can allow to differentiate *M. tuberculosis* complex from mycobacteria other than tuberculosis (i.e. niacin test, detection of MPT64 antigen).

Drug Susceptibility Testing (DST)

Phenotypic DST consist in the assessment of growth or metabolic activity of an isolate vis-a-vis an anti-tuberculosis drug, determining its susceptibility or resistance. The reference method (by proportion) was developed by Canetti et al. [6] on solid culture medium and then adapted on liquid media . This method is reliable for first-line anti-TB drugs (rifampicin, isoniazid, pyrazinamid and ethambutol and some second line drugs like aminoglycosides, polypeptides and fluoroquinolones. However, DSTs of other second-line drugs are much less reliable and reproducible (para-amino-salicylic acid, ethionamide, prothionamide, and cycloserine). Alternative methods can be used for DST; it is the case for Microscopically Observed Drug Sensitivity (MODS) [7].

Molecular Methods

Mycobacterial nucleic acids (DNA or RNA) can be amplified and detected by different methods which can also allow to detect drug resistance by the identification of genetic mutations responsible for or associated with resistance. A number of tests and platforms have been developed to perform these genotypic DSTs. Most of these tests have been endorsed by the World Health Organization (WHO).

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Semi-automated method

Truenat MTB is a chip-based nucleic acid amplification test used for the detection of *M. tuberculosis* in clinical specimens. The test involved specimen processing using Trueprep-MAG, a nanoparticle-based protocol and real-time PCR performed on the Truelab Uno analyzer [7].

Automated methods

Loop-Mediated Amplification (LAMP): LAMP is an isothermal nucleic acid amplification method in which reagents react under isothermal conditions. The large amount of DNA produced, associated to the high specificity of the reaction, allow to detect amplification by visual inspection of fluorescence or turbidity, without the need for gel electrophoresis or instrument detection of the labeled probes [8]. Samples processing can be completed in around 40 minutes.

Real-time PCR (Xpert MTB/RIF): This test which is used to diagnose tuberculosis and resistance to rifampicin is based on real-time PCR, targeting specific nucleic acid sequences in the *M. tuberculosis* complex genome, while at the same time providing information on the most common mutations related to rifampicin resistance. The assay is a highly automated test managed in a closed system with one cartridge per sample. Each instrument, depending on the number of modules available, can process from 1 to 32 samples at a time, with a treatment time of just under 2 hours [9].

Line Probe Assay (LPA): The two molecular techniques commercially available (GenoType MTBDRplus and INNO-LiPA Rif) have the advantage of giving fast results, in a few hours [10]. Moreover, the GenoType MTBDRplus makes it possible to determine whether the mutations are observed in the katG gene or the inhA gene promoter: the mutations in the katG gene lead to a high level resistance to isoniazid when mutations in the inhA gene promoter result in resistance to isoniazid and ethionamide, but certainly not a high level of isoniazid.

Interferon Gamma Release Assays (IGRA)

These *in vitro* tests of cellular immunity are used pour the diagnostic of latent tuberculosis. They measure interferon γ released by sensitized T cells after stimulation by *M. tuberculosis* antigens, the new versions using antigens more specific to *M. tuberculosis* including Early-Secreted Antigenic Target (ESAT)-6, Culture Filtrate Protein (CFP)-10, and TB7.7. [9]. Two commercial kits are available: the T-SPOT.TB test and the QuantiFERON-TB GOLD assay.

Other Biological Tests

Liporabinomannan is a wall glycoprotein antigen of M. tuberculosis, which can be excreted and detected in the urine of TB patients. Its sensitivity is higher in case of disseminated infection, a situation mainly observed in case of advanced HIV infection with a CD4 count <200 [10].

Conclusion

Various biological methods are available for the diagnostic of tuberculosis with different characteristics and variable performance. Therefore, it is necessary to develop specific algorithms allowing their optimal use.

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