Research Progress of Laboratory Diagnosis of TB

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Abstract: Early, rapid, and accurate identification of Mycobacterium tuberculosis is crucial to the treatment and management of the disease, and laboratory diagnosis is an important means for its diagnosis, treatment, and prevention control. Common methods include pathogenic methods based on bacterial smear and culture, molecular methods based on polymerase chain reaction (PCR), immunological methods such as tuberculin skin test and gamma-interferon (IFN-γ) release test, and the latest emergence of molecular methods, such as Xpert MTB/RIF and CRISPR technology have provided new perspectives for TB diagnosis. This review focuses on the main research advances in laboratory diagnosis of TB.

Keywords: TB; Mycobacterium Tuberculosis; Laboratory Diagnosis

Introduciton

Tuberculosis (TB) is a chronic wasting disease caused by Mycobacterium tuberculosis (M.tb). Due to its high incidence and mortality, TB has become one of the primary research objects in public health, infectious disease prevention and treatment[1]。Tuberculosis is one of the highest mortality rates from a single source currently, especially since the outbreak of novel Coronavirus in 2019, the shortage of medical resources make the increasing number of people infected with tuberculosis [2,3]。As a large country with tuberculosis infection, China has so great difficulties to the prevention and control of tuberculosis due to the epidemic of AIDS, mycobacterium tuberculosis drug resistance and the emergence of multi-drug-resistant tuberculosis bacteria.

Early, timely and effective treatment is crucial for TB patients, which relies on rapid and accurate diagnostic techniques. At present, the commonly used diagnostic methods in the laboratory include microscopic microscopy, bacterial culture, immunological examination, molecular biology examination and so on. Bacterial culture is the gold standard for tuberculosis diagnosis, but due to the slow growth of Mycobacterium tuberculosis, the isolation, identification and drug sensitivity testing of Mycobacterium tuberculosis usually take several weeks with a low sensitivity; although molecular diagnostic methods improve the detection sensitivity and specificity, most of them rely on large equipment and professional technicians; immunological diagnosis usually has poor specificity and high false positive rate due to the existing antigen or antibodies and other microorganisms[4]. None of these methods meet the clinical need for rapid and accurate detection. With the deepening of continuous research in recent years, the laboratory detection methods of tuberculosis have been continuously improved. This article reviews the current status and progress of laboratory tests for TB.

1. Bacteriological detection

1.1. Microscopy of traditional smear staining

Traditional microscopic detection of Mycobacterium tuberculosis after smear acid-fast staining is still a simple and

rapid method to confirm pulmonary tuberculosis. Cellule-Nielsen acid staining microscope as a classic MTB detection method is widely used, because of its simple, fast, high specificity, cheap, without special equipment and other advantages , but its also has the following disadvantages: such as low sensitivity, cannot distinguish between active and inactive tuberculosis, and also limited by sputum specimen is qualified or not and inspection personnel technical level[5].

In addition, there is also the fluorescent staining method represented by gold amine O-rhodamine. Compared with the traditional smear acid staining, this method makes the smear fluorescent staining fast, which greatly shortens the reading time, so is more suitable for the examination of a large number of specimens, and improves the sensitivity of microscopy[6]. Although used as a WHO-recommended highly specific method for detecting Mycobacterium tuberculosis, its difficult to use in poor area because the fluorescence microscopy is expensive[7].

1.2 Light-emitting diodes (LED) fluorescence microscopy

In recent years, some researchers combine fluorescence microscope technology with light-emitting diode (LED), developed a tool named LED fluorescence microscope, which compared with ordinary fluorescence microscope has many advantages, such as simple operation, long life, low price, no light and dark vision requirements, short reading time at the same time has high sensitivity and specificity[8]. This makes it have great application value in the basic hospitals. The WHO recommends using LED fluorescence microscope instead of conventional light microscope[9].

1.3 Culture of Mycobacterium tuberculosis

Isolation and culture of Mycobacterium remains the gold standard for detection of Mycobacterium tuberculosis. Its sensitivity is higher than smear staining microscopy, but due to the biological characteristics of the slow growth of Mycobacterium tuberculosis, the traditional solid culture method will take 4-8 weeks to detect the growth of Mycobacterium tuberculosis, but the positive rate is low and it is more difficult to distinguish whether it is Mycobacterium tuberculosis, so that it have to get the help of Mycobacterium species identification and drug sensitivity tests[10]. The principle of liquid culture of Mycobacterium is to use the liquid medium containing redox display agent for culture, when there is mycobacterial growth, the redox system reduces the colorless tetrazolium salt in the medium to a water-insoluble purple-red substance, which can be easily observed by visual observation. The subsequent development of liquid rapid culture systems, such as Mycobacterial growth indicator tube (MGIT) 960 system, which includes a growth system and an indicator system, has a higher degree of automation, a higher rate of positive isolation, a shorter time required for detection, and the ability to perform drug sensitivity tests. However, it is difficult to be widely used in developing countries with high prevalence of TB due to its high price[11].

2. Molecular biology testing

2.1 Polymerase chain reaction

Polymerase chain reaction PCR is the most common nucleic acid amplification method to determine whether infection is tuberculosis by testing the nucleic acid sequence specific to Mycobacterium tuberculosis. Compared with traditional examination methods, it has the advantages of speed, high sensitivity, strong specificity and no need for long bacterial culture. Some studies have shown that the positive rate of Mycobacterium tuberculosis by PCR is significantly higher than by smear microscopy. PCR amplification technology has been widely used since its inception in 1980, but it also has many disadvantages, such as high false positives, easy pollution, cumbersome operation, and the need for professional equipment and personnel[12].

2.2 Loop-Mediated Isothermal Amplification(LAMP)

Loop-mediated isothermal amplification(LAMP) is a novel isothermal amplification technique that allows nucleic acid amplification to detect MTB DNA fragments at 65℃ to achieve the diagnosis of TB. Based on this, another molecular detection method recognized by WHO named tuberculosis ring-mediated isothermal amplification (TB-LAMP) has been developed, and can even be used as an alternative method for smear microscopic examination, with high sensitivity, simplicity and speed[13,14].

2.3 Nucleic acid amplification of real-time rifampicin resistance

Mycobacterium tuberculosis and the rapid molecular detection system-Xpert MTB/RIF can directly detect the presence of Mycobacterium tuberculosis and the resistance to rifampin within 2h[15]. This method is based on semi-nested real-time PCR and uses the rpoB gene as the target gene. In recent years, Xpert has been widely used in MTB and rifampicin resistance testing in clinical specimens, with the advantages of simple operation, short time consuming, high sensitivity and specificity, and good biological safety, so it has been recommended by WHO as the preferred method for molecular drug susceptibility detection of MTB[16].

3. Immunological testing

3.1 Tuberculin skin test

Tuberculin skin test (TST) as a simple, low cost of mycobacterium tuberculosis infection diagnosis method is widely used in China, especially in the childhood tuberculosis diagnosis. However, due to the cross-reactivity of this method with the antigenic components of BCG (bacillus Calmette-Guérin, BCG) and non-tuberculous mycobacteria, its sensitivity and specificity are low, application in tuberculosis diagnosis is limited[17].

3.2 γ -interferon (IFN- γ) release test

The γ -interferon release assay (IGRA) utilizes that mycobacteria contain specific proteins, but BCG strains and most nontuberculous mycobacteria do not contain these proteins[18]. When the body is infected with tuberculosis, by adding polypeptide antigen of mycobacterium specific protein, it can stimulate the T cells infected with Mycobacterium tuberculosis to produce interferon γ, which can reflect the cell immune intensity of the cell, so as to determine whether infected with tuberculosis[19]. Including whole blood-based enzyme-linked immunosorbent assay and peripheral blood lymphocyte-based immune spot assay T-SPOT.TB. It has high specificity and sensitivity, and is not affected by body immunity and BCG vaccination. It has high diagnostic value for incubation period, atypical tuberculosis and extrapulmonary tuberculosis and HIV with tuberculosis infection[20].

In conclusion, there are so many laboratory methods for TB diagnosis currently, but all have their own advantages and disadvantages. Bacteriological method is the gold standard but long time consuming and low sensitivity; molecular diagnosis is fast and sensitive, which is the ideal clinical diagnostic method. Especially, with the development of CRISPR-Cas technology, more and more rapid diagnostic methods emerged, but also has the disadvantages of expensive instruments and reagents. At present, tuberculosis is still a global health problem, and the situation of tuberculosis prevention and control in China is very serious, so we need to complement various methods, in order to provide a strong basis for the diagnosis and treatment of tuberculosis.

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* National Natural Science Foundation of China (81972026)