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Tuberculosis: Culture-Free Technology for Diagnosis and Follow-Up of Patient

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Tuberculosis Situations Worldwide

Tuberculosis (TB) remains a major life-threatening, airborne infectious disease throughout the world. The World Health Organization (WHO) reports that approximately 1.7 billion people are currently infected by *Mycobacterium tuberculosis*, the pathogen, and 10 million new TB patients are identified every year. This pathogen results in approximately 1.5 million TB deaths per year, including infected patients living with HIV (PLHIV) [1]. The WHO set up a millennium developing goal (MDG) which aims for TB incidence to be less than 10/100,000 by 2035. However, this outcome requires a case reduction of over 15% every year, on average [2].

Mycobacterium tuberculosis is the major pathogen responsible for tuberculosis. Due to the zoonotic nature of the pathogen, it causes infectious diseases in many mammals and other animals, however humans are the major hosts. TB is a subacute or chronic granulomatous disease, mainly of the respiratory system (lungs), and may disseminate through blood vessels, resulting in a systemic infection. The clinical manifestations are cough, sputum, fever, general fatigue, weight loss, and other miscellaneous symptoms depending on the infection site. Bacteriological isolation of *M. tuberculosis* is essential for the definitive diagnosis of tuberculosis.

When TB is diagnosed, the patient will receive multidrug chemotherapy for at least 6 months. The major reason for the use of multiple antimicrobial agents is to prevent the development of drug resistance. Unfortunately, many drug-resistant strains already exist within communities, and some patients will be infected by drug-resistant *M. tuberculosis*. In drug-resistant TB, the ordinary treatment regimen is less effective or completely ineffective against *M. tuberculosis*. This is especially the case with so-called multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) strains, which are resistant to both isoniazid and rifampicin, the two major anti-tuberculosis drugs. The WHO estimates that approximately 480,000 MDR-TB cases are diagnosed every year, with a prevalence of 3.4% among total new TB cases. The treatment strategy should be different in pan-susceptible and drug-resistant TB [1].

The treatment of TB is basically empiric. A standard treatment regimen will be adapted to all new TB patients because drug susceptibility is unknown at the time of diagnosis. Therefore, monitoring of treatment effectiveness is of great importance. The treatment effectiveness will be monitored by observing the clinical conditions of the patient and by periodic laboratory examinations. This approach generally uses smear microscopy and culture examinations, but many practical problems exist, as described below.

Current Bacteriology for the Diagnosis and Follow-Up of Patients with Tuberculosis

To reach the MDG, a new technology for rapid and sensitive detection of *M. tuberculosis* at real clinical practise levels, especially in resource-limited settings, is required. This is particularly important in many developing countries where conventional culture examination methods are unavailable. In such areas, TB patients often remain undiagnosed and continue to spread the disease.

In the past decade, rapid and sensitive nucleic acid amplification test (NAAT) systems, such as Xpert MTB/RIF (Cepheid, US) [3], TB-LAMP (Eiken, Japan) [4], and TrueNat (Molbio, India) [5] have been endorsed by the WHO and introduced into clinical practise. However, the sensitivity of these tests has thus far been unable to match that of conventional liquid culture examination. The liquid mycobacterial culture may detect up to 80%-90% of active tuberculosis cases and has the highest diagnostic sensitivity. However, this method requires sophisticated laboratory settings, with significant bio-hazard measures and intensive maintenance in place. Such laboratory settings are costly and technically demanding, so the establishment of an efficient culture laboratory is not easy. Achievement of such clinical standards is also an issue of human resource development, and many NAAT systems are still under development because commercial tests are easier to handle.

As a gold standard of TB diagnosis, the liquid culture system remains essential. However, it is practically difficult to perform liquid TB culture for all presumed TB patients, especially in resource-limited settings and remote areas. People living outside the catchment area will not have affordable access to liquid culture because it requires fresh clinical specimens for testing. Even if the liquid culture is affordable for a presumed TB patient, it is time consuming. The typical liquid culture, MGIT (Becton Dickinson, USA), requires approximately two weeks for positive culture conversion. In paucibacillary cases, testing takes even more time, and a negative diagnosis requires six weeks. This is because of the slow-growing nature of *M. tuberculosis*. The pathogen takes approximately 12-24 h for one duplication event to occur, so detection at minimum limits takes time. By the time the results for this “gold standard examination” become available, the patient might pass away from the disease. This is the current reality of the situation in many instances.

Given the rapid diagnosis response time of NAAT, it is commonly used for initial diagnosis and even for the detection of certain anti-tuberculosis drug resistance estimations by gene mutations/indels. Unfortunately, NAAT technologies are not utilised for the follow-up of TB treatment outcomes because residual DNA will still be detected even though the bacteria are already dead. For MDR-TB and significantly drug-resistant *M. tuberculosis* (XDR-TB) infections in particular, the WHO recommends liquid culture as the standard follow-up method. Otherwise, conventional smear microscopy is the

only method for follow-up analysis of patients with TB in many settings.

Short Summary of recent Bacterial Biomarkers

To cope with the above-mentioned problems relating to culture examination, several biomarkers have recently been developed. Alere Determine TB LAM Ag (Abbott, US), a diagnostic method using urine specimens, is already endorsed by WHO and in clinical use for the diagnosis of TB in PLHIV patients with less than 200/μL CD4 cells, yielding a 31% to 62% sensitivity level, irrespective of signs and symptoms of TB [6]. This technology detects lipoarabinomannan (LAM), a core cell wall component of mycobacteria, in urine specimens. LAM antigen commonly exists in the genus Mycobacterium, and is therefore not specific to *M. tuberculosis*. However, it can be used for the diagnosis and follow-up analysis of highly-presumed TB cases. Recently, Broger et al. reported that a new urine LAM detection kit named SILVAM TB-LAM (Fujifilm, Japan) increased the sensitivity of testing by approximately 25%-30%, compared to Alere Determine TB LAM Ag. This resulted in a 43.9%-87.1% detection rate in pulmonary and extra-pulmonary tuberculosis patients [7]. Thus, the detection of cellular LAM components as bacteriological biomarkers without the requirement for culture examination has proven to be an effective diagnostic tool for TB. Using the same LAM antigen, Kawasaki et al. recently published results of a very interesting study. The team used LAM antigen as a biomarker for the follow-up of treatment effectiveness [8]. Although the mechanisms of rapid clearance of cellular components are unknown, the amount of LAM antigen in the sputum was reflective of the number of live *M. tuberculosis* bacteria, thus a clear correlation was identified. The test indicated a fast reduction (within a week) of LAM antigen after effective anti-tuberculosis treatment. The authors suggested the use of LAM as a biomarker for treatment outcome observation. This method will be useful for the follow-up of anti-TB treatment without culture examination.

Recently, Sakashita et al. reported a similar but different culture-free diagnostic and follow-up system. They evaluated MPT64, which is a *M. tuberculosis* complex (MTC)-specific secretory antigen used in the diagnosis and follow-up treatment of TB patients [9]. MPT64 is a well-known antigen excreted through the ESX system of *M. tuberculosis*, and has already been utilised in several commercial MTC identification kits, such as Capilia TB-Neo (Tauns, Japan) and SD BIOLINE TB Ag MPT64 rapid (Standard Diagnostics, Korea). Therefore, the species specificity of MPT64 is quite high, at almost 100% [10]. MPT64 is a secretory antigen, so its secretion will assure the viability of MTC directly. These advantages, species specificity, and viability indication, are useful for the diagnosis of MTC infection in active TB and the follow-up analysis of anti-TB treatment effects without culture examination. The authors reported a similar diagnostic capacity of the system compared to conventional liquid culture examination, and also showed a clear decline of MPT64 after effective anti-TB treatment. Therefore, this system can be used for diagnosis and follow-up of TB treatment, and has the capacity to replace traditional culture examination methods.

Reasons for Technology Requirements

As described above, liquid and/or solid mycobacterial culture is the gold standard for TB diagnosis. The purpose of mycobacterial culture is to isolate and identify the live pathogen to prove MTC infection is present and to use the cultured bacteria for further examinations, including drug susceptibility testing. When a clinician encounters a presumed TB patient, he or she will examine appropriate clinical specimens to confirm the presence of MTC in the patient. The examination methods typically used are smear microscopy, culture, and NAAT. Smear microscopy is the most affordable method in most instances, but lacks the ability to differentiate MTC from other mycobacteria which commonly exist in the environment (non-tuberculosis mycobacteria: NTM). In many settings, smear microscopy is the only available examination method for tuberculosis, so it is used both for diagnosis and follow-up. However, smear microscopy has the potential to provide false-positive results, even if the MTC are dead. Therefore, it cannot distinguish between drug resistant strains and residual non-viable MTC. This is results in a limitation for quick remedial actions, and thus for better treatment protocols. Culture examination is essential for viability testing, but is time-consuming and result timeframes are often unacceptable lengthy. To resolve this problem, culture-free *M. tuberculosis* viability assessment technology is required. If rapid culture-free viability assessment is available at the clinical site, the clinician will be able to judge the effectiveness of the administered drugs in a very short period, so that the TB patient can receive more efficient treatment based on the results. Better and more effective treatment in the early stages of the disease should lead to better patient outcomes.

In other ways, utilisation of these rapid tests could be an early indicator of new drug effectiveness in clinical trials. The need to wait for culture examination results will be eliminated if the culture-free method is employed, and faster remedial action could be taken for the patients taking part in these trials. All of this will also help to accelerate the clinical trial phase of new drug development and bring new hope to refractory TB patients.

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