Molecular Diagnosis of Tuberculosis and Drug Resistance

Shou-Yean Grace Lin, MS, Edward P. Desmond, PhD*

KEYWORDS

• Tuberculosis • Molecular detection • Drug resistance • GeneXpert • Line probes • Pyrosequencing • Sequencing • Tuberculosis diagnostics

KEY POINTS

- Molecular drug susceptibility testing (MDST) provides rapid diagnosis of tuberculosis (TB) and drug-resistance detection with commendable sensitivity and specificity.
- In exceeding the performance of smear microscopy, MDST enables accurate diagnosis of TB, and improved patient management and TB control; MDST reduces unnecessary isolation or treatment, when a negative result for TB is obtained.
- The GeneXpert MTB/RIF assay provides rapid detection of multidrug-resistant TB. Because of the possibility of false detection of rifampin resistance, confirmation by sequencing is recommended, especially in regions where prevalence of resistance is low.
- Revealing mutation identity by sequencing offers opportunities to study drug minimum inhibitory concentrations for each mutation. Such information enables prediction of resistance levels, and may be helpful in formulating optimal regimens.
- Owing to false susceptibility and false resistance with MDST, culture-based drug susceptibility testing should follow. When resistance is detected, an expanded drug panel should be tested.

INTRODUCTION

A new era in tuberculosis (TB) control is beginning in the United States. In 2012, reported TB cases in this country were less than 10,000 for the first time since national reporting of TB began.[1] However, the global picture is different. TB continues to be the principal killer among infectious diseases. Multidrug-resistant TB (MDR TB) cases have almost reached a half million (450,000), 9.6% of which are extensively drug-resistant TB (XDR TB).[2,3] Rapid TB diagnosis and drug-resistance detection remains a sizable challenge in reducing TB-related morbidity and mortality, especially in

Neither author has affiliations with the companies whose products are discussed in this article.
California Department of Public Health, Richmond, CA, USA
* Corresponding author. Microbial Diseases Laboratory, California Department of Public Health, 850 Marina Bay Parkway, E164, Richmond, CA 94804.
E-mail address: Ed.Desmond@cdph.ca.gov

Clin Lab Med 34 (2014) 297–314
http://dx.doi.org/10.1016/j.cll.2014.02.005 **labmed.theclinics.com**
0272-2712/14/$ – see front matter

regions where coinfection with TB and human immunodeficiency virus (HIV) is prevalent. In combating TB, diagnosis of TB alone is inadequate; improving drug-resistance detection is an essential component for effective TB control and TB patient management.[2–7]

With the availability of molecular diagnostic tools, rapid diagnosis of TB and detection of drug resistance have made significant improvement in recent years.[8–10] The Centers for Disease Control and Prevention (CDC) guidelines published in 2009 recommended the use of nucleic acid amplification testing (NAAT) as standard practice for aiding in establishing TB diagnosis in persons with suspected TB.[11] Molecular assays that exceed the performance of acid-fast smear microscopy enable rapid diagnosis of TB and/or detection of drug resistance even for smear-negative patients.[8,12] As commercial products are now available at reduced prices in TB-endemic regions, molecular testing has become cost-effective and technically easy to implement worldwide.[5–7,13–15] While rapid identification of MDR TB is being realized, rapid detection of resistance to second-line drugs, and timely and effective treatment of patients with MDR/XDR TB, still present tremendous challenges.[2,16]

MICROBIOLOGY

Mycobacterium tuberculosis complex (MTBC) includes *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canettii, Mycobacterium caprae, Mycobacterium microti*, and *Mycobacterium pinnipedii*. Approximately 98% of human TB cases are caused by *M tuberculosis*. MTBC has a complex cell wall containing mycolic acids, and resists decolorization by acid alcohol when stained with carbol fuchsin or auramine (a fluorescent stain). Hence they are called acid-fast bacilli. *M tuberculosis* grows slowly in culture, requiring an average of 2 to 3 weeks of incubation before growth can be detected. Colonies on solid media are off-white or buff in color, with a rough appearance. The cellular morphology of TB bacilli grown in culture demonstrates cording or ropy clumps. The *M tuberculosis* genome contains 4,411,529 base pairs with high GC content (>65%).[17,18] *M bovis* causes 1% to 2% of human TB. *M bovis* differs from *M tuberculosis* by being niacin and nitrate negative, and inherently pyrazinamide resistant.

EPIDEMIOLOGY

Approximately one-third third of the human population can be demonstrated to have immunologic evidence of current or past infection with *M tuberculosis*. In 2012, it was estimated that there were approximately 8.6 million human cases worldwide with 1.3 million deaths.[3] The average global incidence rate was 122 new cases per 100,000 population per year. By contrast, in the United States, the TB incidence rate was 3.2 new cases per 100,000 population in 2012.[1] Serious concerns remain for the United States, however, because 63% of TB cases and 88% of MDR TB cases were foreign-born from countries where TB prevalence and drug-resistance rates are high.

CLINICAL PRESENTATION

Pulmonary TB, the most common form of the disease, is chronic and slowly progressive. Patients commonly experience cough, weight loss, night sweats, fever, and occasionally chest pain and dyspnea.[18] Early in the course of the disease, cough may be nonproductive; as inflammation and tissue necrosis ensue, sputum is usually produced. Pulmonary infiltrates and cavitary lesions may often be observed on chest radiographs. Dyspnea with parenchymal lung involvement is less common but may