Molecular Laboratory Testing for Tuberculosis: Innovators, Early Adopters, or Laggards?

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(See the Major Article by Sohn et al on pages 970–6.)

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Despite the recent progress toward meeting the Millennium Development Goal target to halt and to reverse the tuberculosis epidemic by 2015, the global burden of tuberculosis remains significant. In 2012, there were an estimated 8.6 million new cases of tuberculosis and 1.3 million died from the disease—5 deaths every 2 minutes! Among these deaths, an estimated 170,000 were caused by multidrug-resistant (MDR) tuberculosis, a relatively high total compared with 450,000 incident MDR tuberculosis cases. Worldwide and in most countries with a high burden of MDR tuberculosis, less than one-third of the tuberculosis patients estimated to have MDR tuberculosis were actually detected in 2012 [1].

In 2012, a total of 9945 new tuberculosis cases were reported in the United States, representing a 5.4% decrease from 2011 and 62.7% decrease from 1992 when the tuberculosis resurgence peaked at 26,673 cases. While this is indeed an accomplishment, the fact that 17 states reported increased case counts from 2011 to 2012 is concerning. Primary MDR tuberculosis is reported in 1.1% of tuberculosis cases and fluctuated between 0.9% and 1.3%—stable but no progress. Thirty-one states reported ≥50% of tuberculosis cases in foreign-born persons and 11 states even reported ≥70%. The top 5 countries of origin for foreign-born persons with tuberculosis during 2007–2012 were Mexico, the Philippines, India, Viet Nam, and China [2]. According to the World Health Organization (WHO), more than half of the global MDR tuberculosis cases are from India, China, and the Russian Federation [1].

Today, laboratory testing in the microbiology field is experiencing more changes than ever before. Determining what assay will be most useful to the healthcare provider is a challenge, and rapid acceptance of the new technology by the medical community is an even greater one. In August 2013, the US Food and Drug Administration permitted marketing of the Xpert MTB/RIF (Xpert) assay (Cepheid, Sunnyvale, California) to detect DNA of the Mycobacterium tuberculosis complex and genetic mutations associated with resistance to rifampin (RIF) in unprocessed sputum and concentrated sputum sediments.

In this issue of Clinical Infectious Diseases, Sohn and colleagues [3] from the Montreal Chest Institute, Canada, evaluated the Xpert assay in a low-incidence, high-resource ambulatory setting, specifically, in a university hospital tuberculosis clinic for the detection of pulmonary tuberculosis on induced sputum samples, using mycobacterial cultures as the reference standard. During the duration of the study, 924 patients were evaluated for tuberculosis and 502 consecutive patients were enrolled in the study (most with abnormal chest radiographs; only 18% symptomatic). Reasons for not enrolling included language barrier and refusal of the patient. Four induced sputum samples were collected. The second sample was subjected to the Xpert assay and the other 3 samples were sent to the clinical microbiology laboratory for acid-fast bacilli (AFB) smear microscopy and culture using one liquid medium (Mycobacterium Growth Indicator Tube, BD). If the AFB smear was positive, a reflex nucleic acid amplification test (NAAT) was performed. A NAAT was performed on AFB smear-negative sputum samples by request only. Xpert results were not made available for clinical decision making.

Twenty-four subjects were identified with active tuberculosis by culture. Xpert had an overall sensitivity of 46% and a specificity of 100%. The sensitivity was 86% in the 7 AFB smear-positive subjects and 29% in the 18 AFB smear-negative, culture-positive subjects. Most participants with culture-positive tuberculosis had minimal disease: only 7 of 25 (28%) culture-positive subjects were AFB smear-positive, only 12 (44%) had symptoms at...
presentation, and 2 subjects had no radiographic abnormalities at all. In addition, a longer period to culture positivity was noted for Xpert-negative, culture-positive subjects (28 days) compared to 14 days in Xpert-positive, culture-positive subjects.

The median time to treatment initiation was 1 day for smear-positive cases and 26 days for AFB smear-negative cases. For 13 of the 18 AFB smear-negative cases, Xpert was negative and therefore would have not influenced treatment decisions. For the remaining 5 subjects who were AFB smear-negative but Xpert-positive, treatment would potentially have been started a median of 12 days sooner, if results had been shared with the healthcare providers.

As the authors noted, their findings may suggest limited potential impact of Xpert testing because of the high-resource, ambulatory, tertiary care setting caring for subjects with minimal disease, in part detected as a result of active immigration screening, and to expedite diagnosis beyond what is achieved with the existing, well-performing diagnostic algorithm. They also discussed that induced sputum as a sample type may have contributed to the low sensitivity.

A review of commercially available NAATs demonstrated an overall sensitivity of 96% and 66% for AFB smear-positive respiratory samples and AFB smear-negative samples, respectively [4]. The MTD assay (Hologic Gen-Probe, San Diego, California), the most widely used assay in the United States, had sensitivities of 97% and 76%, respectively. Performance characteristics for a laboratory-developed NAAT had similar values (99.6% and 75.4%, respectively) [5]. Marlowe et al published data from the western United States using the Xpert assay and found sensitivities of 98% and 72%, respectively, for processed and concentrated sediment [6]. Could the unexpected low yield by Xpert testing be due to the fact that non-concentrated and potentially diluted specimens (induced sputa) were used?

Tortoli et al [7] in Italy evaluated the Xpert assay in almost 1500 extrapulmonary specimens, including almost 500 pediatric samples. In comparison with the reference standard consisting of combination of culture and clinical diagnosis of tuberculosis, an overall sensitivity and specificity of 81.3% and 99.8% were found for the Xpert assay, whereas the sensitivity of microscopy was 48%. For biopsies, urines, pus, and cerebrospinal fluids, the sensitivity exceeded 85%, whereas it was slightly <80% for gastric aspirates. However, it was <50% for fluids such as peritoneal, synovial, and pericardial fluids. Because the bacterial load is usually lower in these types of samples, so was the sensitivity; however, it was still higher than in the Montreal study. All the samples in the Italian multicenter study were either digested and centrifuged or, in the case of sterile fluid, only centrifuged in contrast to the Canadian study. Because the authors used induced sputum rather than expectorated sputum or sputum sediments as recommended by the manufacturer, additional studies may be warranted to assess the validity of using unconcentrated induced sputum.

This study [3] investigates the Xpert assay in an ambulatory setting with onsite laboratory capabilities for AFB, NAAT, and culture and the Xpert as an add-on. As the study design compared an algorithm that already included a NAAT with adding the Xpert, it was not designed to assess the Xpert to an algorithm without a NAAT.

Because the Xpert does not require operation in a Biosafety Level 3 containment facility [8], enhanced benefits will be realized when it is used in closer proximity to the patient, enabling shorter turnaround times.

The Montreal study identified 2 samples with Xpert RIF resistance (8%); one was in concordance with the conventional phenotypic antimicrobial susceptibility testing result, the other was discordant. Interestingly, the Xpert result turned out to be true RIF resistant as, upon sequencing, the *rpoB* gene one detected a point mutation resulting in a Pro > Leu substitution at amino acid 511. This mutation is known to generate unreliable results when using conventional, especially broth-based, methods [9, 10]. One could assume that in both cases there would have been drug regimen adjustment made several weeks earlier to conventional antimicrobial susceptibility testing, although the authors did not elaborate. Of note, the Centers for Disease Control and Prevention (CDC) recommends that RIF-resistant strains be confirmed by rapid molecular detection of mutations associated with RIF and, additionally, with isoniazid drug resistance [11].

Another aspect of the potential benefit of the Xpert assay is the cost savings that may be realized when patients with suspected tuberculosis with negative Xpert results can be released from respiratory isolation much sooner compared with current guidelines requiring negative AFB smear results. Millman et al from the University of California, San Francisco published a cost-benefit analysis about rapid molecular testing for tuberculosis to guide respiratory isolation in the United States [12]. The authors used a hypothetical cohort of 234 individuals undergoing evaluation for presumed active tuberculosis annually; 6.4% had culture-positive tuberculosis. Compared to AFB smear microscopy, Xpert reduced isolation bed utilization from an average of 2.7 to 1.4 days per patient, leading to a 48% reduction in total annual isolation bed usage from 632 to 328 bed-days. Xpert would have saved an average of $2278 per admission, or $533 520 per year compared with AFB smear microscopy.

According to the CDC, a NAAT should be performed on at least 1 respiratory specimen from each patient with signs and symptoms of pulmonary tuberculosis for whom a diagnosis of tuberculosis is being considered but has not yet been established, and for whom the test result would alter case management or tuberculosis control activities [13]. In 2010–2011, the Association of Public Health Laboratories and the CDC conducted a survey among all US laboratories enrolled in
a mycobacteriology proficiency testing program. Of the 1444 laboratories identified, 656 (46%) responded. Of those that responded, 580 (88%) performed some level of tuberculosis service in-house and were included in the further analysis. Regarding NAAT capabilities, 85 (15%) laboratories could perform NAAT in-house [14]. Although this is a low number, it would be more meaningful to know how many tuberculosis patients have a NAAT result than the number of laboratories performing NAAT in-house. Fortunately, the US Healthy People 2020 initiative [15] set a benchmark (IID-32) of 77% for culture-confirmed tuberculosis patients with a positive NAAT result reported within 2 days of specimen collection. It will be interesting to observe the progress toward this goal.

In 2009, the Florida Department of Health went one step further when it implemented a new algorithm for screening every highly infectious, NAAT-positive tuberculosis patient for RIF and isoniazid (INH) resistance. This universal screening process rapidly validates an initial treatment regimen before the patient experiences treatment failure. As a result of implementing this protocol, Florida tuberculosis patients were more accurately diagnosed, ensuring appropriate treatment, minimizing transmission, and providing the patient the best chance of being cured. With an earlier diagnosis of MDR tuberculosis, the patient receives adequate treatment, thus shortening the time period of transmitting and lessening the likelihood of developing MDR tuberculosis when only mono-resistance to INH or RIF is initially detected or if MDR tuberculosis is not suspected at all. On a side note, the Association of State and Territorial Health Officials awarded the Florida Department of Health for its MDR tuberculosis program with its 2012 Vision Award-Achieving Excellence in Public Health Through Innovation [16].

There are a few take-home messages for the reader: (1) A NAAT should be performed on at least 1 respiratory specimens from each patient with signs and symptoms of pulmonary tuberculosis; (2) the detection of 2 RIF-resistant tuberculosis cases (perhaps not expected) in this small cohort demonstrates the importance for rapid molecular RIF resistance and ideally MDR tuberculosis screening; and (3) until un-concentrated induced sputum is thoroughly validated as a sample type for the Xpert assay, do not use induced sputum without a concentration step.

The late Everett Rogers studied, as a son of a farmer in his native Iowa, how some farmers adopted new tools, seeds, and methods faster than others. In 1962, he published his original findings about the classification and frequency of the 5 adopter categories: innovators (2.5%), early adopters (13.5%), early majority (34%), late majority (34%), and laggards (16%) in his book entitled Diffusion of Innovations [17]. Even with the low sensitivity in the Montreal study, this should not give reason to slow down the implementation of NAAT and RIF/MDR tuberculosis screening of patients with signs and symptoms of pulmonary tuberculosis in the United States. To which of Rogers’s categories do you or your program belong—innovators, early adopters, or laggards?

Note

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References