REVIEW ARTICLE

ROLE OF MOLECULAR TESTS FOR DIAGNOSIS OF TUBERCULOSIS IN CHILDREN

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Introduction

The most common method for diagnosing tuberculosis (TB) worldwide is sputum smear microscopy. Following recent breakthroughs in TB diagnostics, the use of rapid molecular tests to diagnose TB and drug-resistant (DR) TB is increasing. The various tests used for bacteriological diagnosis of TB are discussed and role of molecular tests to diagnose TB in children are analysed.

Bacteriological Diagnosis of TB in children

1. **Isolation of acid fast bacilli (AFB) on smear:** Sputum smear microscopy has been the primary method for detecting TB and monitoring treatment response in most resource-constrained countries for decades. While inexpensive and requiring minimal biosafety standards, microscopy is not a sensitive test, particularly in people living with HIV and in children: it provides no information on the viability and drug susceptibility of the bacilli, and it cannot distinguish between *Mycobacterium tuberculosis* complex and non-tuberculosis mycobacteria. (1,2)
2. **Culture:** Though diagnosis based on culture is considered the reference standard, results take weeks to obtain and testing requires a well-equipped laboratory, highly trained staff, and an efficient transport system to ensure viable specimens. Culture is also critical for monitoring patients' response to treatment for DR-TB. The conventional culture are media Middlebrook 7H10, selective Middlebrook 7H11 [S7H11], and Lowenstein-Jensen media. The BACTEC system (Johnston Laboratories,Inc.,Cockeysville,Md.) has been reported to be valuable for the rapid detection of clinically important mycobacteria. The system uses Middlebrook 7H12 Broth medium containing 14C-labeled Palmitic acid for the radiometric detection of mycobacterial growth and has been shown to be more successful in detecting mycobacteria than conventional methods involving only one medium. An average of 18 days is required by the BACTEC method for complete recovery and drug susceptibility testing of M. tuberculosis, as compared with 38.5 days for the conventional methods. (3)

3. Nucleic Acid Amplification tests (NAAT):

a. **GeneXpert:** More recently, the World Health Organization (WHO) endorsed the Xpert® MTB/Rif assay (GeneXpert) for the diagnosis of TB. (4,5) Xpert® MTB/Rif relies on DNA-PCR technique for detection of TB and rifampicin resistance related mutations simultaneously. It is the first molecular assay for TB detection to be fully automated and to integrate all the steps required for PCR-based DNA test. It gives results within 3 hours. The test has also been reported to be highly accurate for diagnosis of pulmonary TB. Patients with presumptive HIV associated TB who are negative on smear examination are the most likely to benefit from Xpert® MTB/Rif.

b. **AMPLICOR MTB Test:** The Amplicor Mycobacterium Tuberculosis Test (Amplicor, Roche Diagnostics) is approved for the detection of *M. tuberculosis* complex bacteria in AFB smear-positive respiratory specimens from patients suspected of having TB. This test uses the polymerase chain reaction (PCR) to amplify a portion of the 16S rRNA gene that contains a sequence that hybridizes with an oligonucleotide probe specific for *M. tuberculosis* complex bacteria. The Amplicor test displays a sensitivity of >95% for detecting *M. tuberculosis* bacteria in respiratory specimens from AFB-smear positive TB suspects and a sensitivity of 60% to 70% for detecting *M. tuberculosis* bacteria in respiratory specimens from AFB-smear negative TB suspects. The sensitivity of the Amplicor test for all of the specimens and for extrapulmonary, smear-positive, and smear-negative specimens was 86, 83, 94.5, and 74%, respectively. (6)

c. **Line Probe Assays (LPA):** As per recent WHO objectives to reduce the time for culture, identification and drug resistance detection to as short as 2 days, LPA is used. Molecular line probe assay (LPA) technology for rapid detection of multi-drug resistant tuberculosis (MDR-TB) was endorsed by the WHO in 2008. Line probe assays are tests that use PCR and reverse hybridization methods for the rapid detection of mutations associated with rifampicin and isoniazid drug resistance. Line probe assays are designed to identify *M. tuberculosis* complex and simultaneously detect mutations associated with drug resistance. Commercially available line-probe assays include the INNO-LiPA® Rif. TB kit (Innogenetics N.V., Ghent, Belgium) targeting rpoB and GenoType® MTBDRplus (Hain Lifescience, Nehren, Germany) targeting rpoB, katG and inhA. (7-10)

Recommendations regarding use of NAAT for Diagnosis of TB

Center for Disease Control (CDC): CDC recommends that NAAT be performed on at least one (preferably the first) respiratory specimen from each patient suspected of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities. NAAT does not replace the need for culture; all patients suspected of TB should have specimens collected for mycobacterial culture.(11) Although an Xpert MTB/RIF assay may result positive for MTB and negative for RMP resistance, it has high negative predictive value for ruling out RMP resistance. However, growth-based DST to first-line TB drugs is still necessary.

World Health Organization (WHO) (12,13): WHO's current policies and guidance recommend that Xpert MTB/RIF be used as an initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB (strong recommendation, moderate quality of evidence). It states that

1. Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in adults and children suspected of having MDR-TB or HIV-associated TB (strong recommendation).
2. Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in all adults and children suspected of having TB (conditional recommendation).
3. Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test in testing cerebrospinal fluid (CSF) specimens from patients suspected of having TB meningitis (strong recommendation).
4. Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture, or histopathology) for testing specific non-respiratory specimens (lymph nodes and other tissues) from patients suspected of having extrapulmonary TB (conditional recommendation).

These recommendations are based on a review of 16 studies (12 published and 4 unpublished). The overall pooled sensitivity of Xpert MTB/RIF compared against culture as a reference standard in children presumed to have TB was 66% in 10 studies where expectorated sputum or induced sputum was used (95% CI, 52–77%); the pooled sensitivity was 66% in 7 studies where samples from gastric lavage or aspiration were used (95% CI, 51–81%). The pooled specificity of Xpert MTB/RIF compared against culture as the reference standard was at least 98%, with narrow confidence intervals.

The pooled sensitivity of Xpert MTB/RIF in culture-negative specimens from children compared against clinical TB used as the reference standard was very low at 4% for samples of expectorated or induced sputum (8 studies), and 15% for samples from gastric lavage or aspiration (3 studies), both sensitivities had wide confidence intervals. It is likely that the apparently poor performance of Xpert MTB/RIF was the result of a reference standard for clinical TB that lacked specificity. The sensitivity of Xpert MTB/RIF to detect rifampicin resistance in specimens from children was 86% (95% CI, 53–98%).

Cochrane Review (14): The authors concluded that in adults thought to have TB, with or without HIV infection, Xpert® MTB/RIF is sensitive and specific. Compared with smear microscopy, Xpert® MTB/RIF substantially increases TB detection among culture-confirmed cases. Xpert® MTB/RIF has higher sensitivity for TB detection in smear-positive than smear-negative patients. Nonetheless, this test may be valuable as an add-on test following smear microscopy in patients previously found to be smear-negative. For rifampicin resistance detection, Xpert®MTB/RIF provides accurate results and can allow rapid initiation of MDR-TB treatment, pending results from conventional culture and DST.

As per a metaanalysis by Walusimbi et al (2013) (15), the pooled sensitivity and specificity for detection of smear-negative pulmonary tuberculosis were 67% and 98% for Xpert® MTB/RIF, 73% and 91% for Microscopic Observation Drug Susceptibility assay (MODS), and 61% and 69% for WHO 2007 algorithm, respectively. The sensitivity of Xpert® MTB/RIF reduced from 67% to 54% when sub-group analysis of studies with patient HIV prevalence ≥30% was performed.

Conclusion

Though Xpert® MTB/RIF has been recommended as an initial diagnostic test rather than conventional microscopy, culture and DST in adults and children suspected of having MDR-TB or HIV-associated TB and even in all patients with TB, the sensitivity of Xpert® MTB/RIF seems to be lower in smear-negative pulmonary TB and even in extrapulmonary TB. It also may be extremely low in samples such as gastric lavage or lymph node tissue. Thus, Xpert® MTB/RIF may be used along with conventional microscopy and culture in children for diagnosis of TB and not as a replacement for conventional microscopy and culture DST.

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Conflict of Interest : None

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