Rapid diagnosis of extrapulmonary tuberculosis using nucleic acid amplification tests: what is the evidence?

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Extrapulmonary tuberculosis poses diagnostic challenges

Globally, tuberculosis (TB) continues to be a major infectious threat to human health [1], especially in the context of the HIV epidemic. Although pulmonary TB is the most common form of the disease, extrapulmonary disease occurs frequently in persons with HIV infection, and in children. TB pleuritis, lymphadenitis and meningitis are the most common forms of extrapulmonary disease, with pleural effusion being the most common extrapulmonary presentation. Some forms of extrapulmonary disease, especially TB meningitis, can cause life-threatening illness and is associated with high case fatality.

Because of the paucibacillary nature of extrapulmonary disease, the diagnosis of extrapulmonary TB is not easy to establish, especially in developing countries. Symptoms and signs of extrapulmonary disease are vague and nonspecific. Smear microscopy, the most commonly used TB test, lacks sensitivity for extrapulmonary disease. Culture and histopathology are more accurate but are not widely available in resource-limited settings. Also, procedures such as pleural or lymph node biopsy are invasive and not easy to implement in peripheral healthcare settings. Owing to these difficulties, misdiagnosis of extrapulmonary TB is common and may result in under- or overtreatment.

Nucleic acid amplification tests for the diagnosis of TB

As a result of the known limitations of conventional tests such as smear and culture, nucleic acid amplification tests (NAATs) have emerged with the intended goal of enabling clinicians to make a rapid and accurate diagnosis. PCR is the best-known NAAT platform. All NAATs amplify target nucleic acid regions that uniquely identify

the *Mycobacterium tuberculosis* complex. An important advantage of NAATs is the rapidity by which the results can be obtained – within hours from receipt of the specimen. Another advantage is their theoretical potential to amplify even minute amounts of nucleic acid.

NAATs are often inhouse (‘home-brew’) PCR-based assays. Commercial NAATs for TB include the GenProbe Amplified *M. tuberculosis* Direct[®] (AMTD) test (GenProbe Inc., San Diego, CA, USA), Roche Amplicor[®] MTB and Cobas Ampli-cor[®] tests (Roche Molecular Diagnostics, Pleasanton, CA, USA), Abbott LCx[®] test (now discontinued; Abbott Laboratories, Abbott Park, IL, USA), BD-ProbeTec ET[®] test (BD Diagnostics, Sparks, MD, USA) and Eiken Loop-mediated Isothermal Amplification[®] (LAMP) test (Eiken Chemical Co., Tokyo, Japan). While the AMTD and Amplicor tests have been around for a while, the BD-ProbeTec ET and LAMP tests are relatively new. Work is also ongoing to develop a fully automated NAAT for TB. For example, the GeneXpert[®] System (Cepheid, Sunnyvale, CA, USA) utilizes real-time PCR to amplify and detect target DNA. The system is fully automated and integrates all the steps, including sample preparation, DNA amplification and detection. Evidence on the use of NAATs for pulmonary TB has been previously summarized [2–4].

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NAAT for the diagnosis of extrapulmonary TB

While none of the commercial NAATs are US FDA approved for use in extrapulmonary specimens (such as pleural or lymph node fluids), several research studies have explored the use of NAATs for TB meningitis, pleuritis and lymphadenitis. Three recent meta-analyses have summarized the evidence (Table 1) on the performance of NAATs for these forms of TB [5–7]. Together, these meta-analyses synthesize the results of nearly 140 studies and therefore provide the best current evidence on these tests.

Table 1. Recent meta-analyses on the accuracy of nucleic acid amplification tests for extrapulmonary tuberculosis.						
Meta-analysis (year)	Site of extrapulmonary TB (specimen used)	Studies included in the review	Type of NAAT evaluated	Key findings about NAAT performance	Inferences and implications	Ref.
Pai et al. (2003)	TB meningitis (cerebrospinal fluid)	49	Commercial NAAT and inhouse PCR	High specificity; sensitivity was lower and variable	Commercial NAAATs have a potential role in confirming TBM, although their overall low sensitivity precludes the use of these tests to rule out TBM. Clinical applicability of inhouse tests is unclear because of inconsistent results from various studies	[6]
Pai et al. (2004)	TB pleuritis (pleural fluid)	40	Commercial NAAT and inhouse PCR	High specificity; sensitivity was lower and variable	Commercial NAAATs may have a potential role in ruling in TB pleuritis. However, these tests have low and variable sensitivity and may not be useful in ruling out the disease. Clinical applicability of inhouse tests is unclear because of inconsistent results from various studies	[5]
Daley et al. (2007)	TB lymphadenitis (lymph node aspirate or tissue)	49	Commercial NAAT and inhouse PCR	Estimates of sensitivity and specificity of NAAT were highly variable across studies	Studies on NAAATs for TB lymphadenitis produce highly variable and inconsistent results, precluding the determination of clinically meaningful estimates of accuracy. Because both false-positive and false-negative results are possible, NAAAT will need to be applied in conjunction with conventional methods and interpreted in the context of clinical suspicion	[7]

NAAT: Nucleic acid amplification test; TB: Tuberculosis; TBM: TB meningitis.

Overall, current evidence summarized in Table 1 suggests that NAATs cannot replace conventional tests such as microscopy, culture and biopsy for extrapulmonary TB. If used at all, they should be used and interpreted in conjunction with conventional tests and clinical data. NAATs seem to have high specificity and positive predictive value, and these characteristics may confer some value in terms of their ability to rule in TB. For example, a positive NAAT result in a child with a reasonably high pretest probability of TB meningitis is confirmatory of TB. NAATs, however, have relatively lower (and highly variable) sensitivity and negative predictive value in all forms of extrapulmonary TB. A negative NAAT result, therefore, does not rule out the diagnosis of TB. Therefore, a negative NAAT result in a patient with a high index of clinical suspicion should not preclude continued investigation and work-up.

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A key finding in all three meta-analyses was the tremendous variations (heterogeneity) in NAAT accuracy across studies, especially with inhouse PCR tests. Thus, it is hard to come up with clinically meaningful recommendations about the use of NAATs in routine practice.

In general, because all NAATs amplify dead and nonviable mycobacteria, they should not be used in patients who are being treated. Finally, to ensure reliability and reduce risk of contamination, NAATs should only be performed in laboratories that have appropriate quality assurance systems in place.

Future perspective & research directions

A major limitation of existing NAAT studies is the fact that diseases such as TB meningitis and TB pleuritis do not have a gold standard. Therefore, study results may be influenced by the reference standard. The lack of a diagnostic gold standard remains a serious issue for evaluating new diagnostics, especially in HIV-infected persons and in paucibacillary disease (e.g., extrapulmonary TB and pediatric disease) [8]. The true accuracy of NAAT may actually be higher than reported when using an imperfect reference standard.

Research work is needed to enhance the sensitivity of NAATs for extrapulmonary TB, without sacrificing the high specificity.

However, even if sensitivity were to be improved, a key concern that will remain is the cost and feasibility of newer molecular technologies in developing countries. Commercial NAAT kits are expensive (US\$25–50 per test), but are commonly used in developed countries. However, many developing countries cannot afford NAATs at all, or still use inhouse PCR assays, which although poorly standardized and unreliable, are considerably less expensive. Thus, the poorest countries that have the highest number of TB cases are least likely to benefit from expensive technologies.

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Clearly, efforts are necessary to simplify NAATs and develop technologies that can be easily implemented in developing countries. Realizing this need, agencies such as the Foundation for Innovative New Diagnostics (FIND), WHO, Special Programme for Research and Training in Tropical Diseases (TDR) and Stop TB Working Group for New Diagnostics have launched several initiatives to make technologies for detecting TB and other neglected diseases affordable and accessible for developing countries [9,10]. In fact, the Stop TB Partnership has created a new Retooling Task Force to oversee the adoption and implementation of new tools for TB, including new diagnostics, vaccines and drugs. Several new diagnostics are currently in the TB pipeline (reviewed in [11–14]) and extensive field evaluations are underway. It remains to be seen if promising new NAAT versions such as the GeneXpert and LAMP [15] tests will survive the rigors of real world field evaluations.

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