

## Editorial

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### The accuracy and reliability of nucleic acid amplification tests in the diagnosis of tuberculosis

Accurate and early diagnosis of tuberculosis is a critical part of the management and control of the disease. Diagnostic work up for tuberculosis involves the detection of acid-fast bacilli in clinical samples by microscopy (smear) and culture. These conventional tests are not always helpful in making the diagnosis. Microscopy, although rapid and inexpensive, has only modest sensitivity and specificity. Mycobacterial cultures, although very specific, might be negative in 10%–20% of cases, and the results are often not available for weeks. In the context of these limitations, nucleic acid amplification (NAA) tests have emerged with the intended goal of enabling clinicians to make a rapid and accurate diagnosis. Polymerase chain reaction (PCR) is the best known and most widely used NAA test. All NAA tests amplify target nucleic acid regions (DNA or RNA) that uniquely identify the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex. Because NAA tests can be used directly on clinical specimens (such as sputum), they are also called 'direct amplification tests' (DAT).

In theory, PCR tests are exquisitely sensitive—they can amplify even a single copy of the target genomic sequence. Their specificity is also expected to be high because they amplify genomic targets that are highly specific to the *M. tuberculosis* complex. NAA tests are rapid—results are usually obtained within 6–12 hours. Because of these potential advantages, the introduction of NAA tests was hailed as a major breakthrough in the diagnosis of tuberculosis. Have these tests lived up to their reputation? How accurate and reliable is their clinical performance? It is important to examine the evidence from recently published meta-analyses and systematic reviews on the validity and role of NAA tests for the diagnosis of tuberculosis.

NAA tests are categorized as commercial or in-house ('home-brew'). Commercial kits include the Amplicor® MTB tests (Roche Molecular Systems), the Amplified *Mycobacterium tuberculosis* Direct Test® (MTD) (Gen-Probe Inc), the LCx® kit (Abbott Laboratories), and the BD ProbeTec ET assay (BD Diagnostic Systems). The costs of commercial NAA tests vary (list price US\$ 25–50 per test). In-house tests are laboratory-developed PCR assays where the investigators put together their own PCR protocols. In-house assays, therefore, vary greatly in their design and laboratory methods. The cost of an in-house PCR is about US\$ 15–20 per test. These cost estimates do not include the cost of buying and maintaining PCR equipment. All NAA tests require a specialized and sophisticated laboratory infrastructure, and skilled personnel for optimal performance.

The accuracy and reliability of NAA tests for tuberculosis have been extensively studied since the early 1990s. Accuracy refers to test performance characteristics such as sensitivity and specificity. Reliability refers to repeatability (variability when the test is repeated). As hundreds of studies have evaluated NAA tests, it is now possible to determine their overall performance using meta-analyses and systematic reviews.<sup>1–5</sup> Table I presents the results of some recent meta-analyses and reviews on the accuracy of NAA tests. Because these meta-analyses and reviews synthesize data

TABLE I. Results of recent meta-analyses and systematic reviews on the accuracy of nucleic acid amplification (NAA) tests for tuberculosis (TB)

Meta-analysis/ systematic review (year)	Number of studies included	Type of tuberculosis	Type of NAA tests	Principal findings about the accuracy of NAA tests	Conclusions
Sarmiento <i>et al.</i> (2003) <sup>1</sup>	50	Smear-negative pulmonary TB	Commercial tests and in-house PCR	Highly variable sensitivity and specificity estimates, sensitivity lower than specificity	Not consistently accurate enough to be routinely recommended for the diagnosis of smear-negative pulmonary TB
Flores <i>et al.</i> (2004) <sup>2</sup>	84	Pulmonary TB	In-house PCR	Highly variable sensitivity and specificity estimates, sensitivity lower than specificity	Accuracy of in-house PCR poorly defined because of variability in accuracy estimates
Piersimoni and Scarpato (2003) <sup>3</sup>	>40	Pulmonary and extrapulmonary TB	Commercial tests	High specificity, sensitivity was lower and variable	NAA tests have to be performed in conjunction with smears/cultures. Clinical value depends on pre-test probability.
Pai <i>et al.</i> (2003) <sup>4</sup>	49	TB meningitis (TBM)	Commercial tests and in-house PCR	High specificity, sensitivity was lower and variable	Commercial NAA tests have a potential role in confirming TBM, although their overall low sensitivity precludes their use to rule out TBM. Clinical applicability of in-house NAA tests is unclear because of inconsistent results from various studies.
Pai <i>et al.</i> (2004) <sup>5</sup>	40	TB pleuritis	Commercial tests and in-house PCR	High specificity, sensitivity was lower and variable	Commercial NAA tests 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 in-house NAA tests remains unclear because of inconsis- tent results from various studies.

PCR polymerase chain reaction

from over 200 primary studies, and because their results are highly consistent with each other, they provide us with the best available evidence.

With respect to accuracy, the following are the main findings of the meta-analyses (Table I). An overwhelming majority of the studies on NAA tests reported very high estimates of specificity.<sup>1-5</sup> This finding has been reported for both pulmonary and extrapulmonary tuberculosis. Sensitivity estimates, in contrast, have been much lower and highly inconsistent (variable).<sup>1-5</sup> In particular, sensitivity estimates have been lower in paucibacillary forms of tuberculosis (smear-negative pulmonary tuberculosis and extrapulmonary tuberculosis).<sup>4,5</sup> The sensitivity of NAA tests has been maximum in smear-positive pulmonary tuberculosis. Another striking result is the widespread lack of consistency in research findings—studies have reported highly variable estimates of test accuracy.<sup>1-5</sup> Remarkably, in our meta-analysis on tuberculous meningitis, sensitivity estimates varied between 0% and 100%.<sup>4</sup> In general, the results of in-house PCR evaluations have been more inconsistent than those of commercial tests.<sup>4,5</sup>

The reliability of NAA tests for tuberculosis has been evaluated in several large, multicentric studies of interlaboratory reliability.<sup>6-10</sup> Typically, these studies have involved analyses of blinded clinical specimens with known amounts of *M. tuberculosis* bacilli. Variability in the PCR results obtained by various laboratories (for the same batch of specimens) is reflective of interlaboratory reliability. In general, these studies have shown that NAA tests tend to produce variable results across laboratories. Laboratories often tended to report false-positive results (i.e. specificity was a bigger

concern than sensitivity). Overall, these studies underscored the need for good laboratory practices (including quality assurance systems) to ensure the reliability of NAA tests.

What conclusions for clinical practice can be drawn from the current available best evidence on NAA tests for the diagnosis of tuberculosis? The following inferences appear to be well-supported by the evidence:

1. NAA tests cannot replace conventional tests such as microscopy and culture; if performed, they need to be used and interpreted in conjunction with conventional tests and clinical data.
2. In-house PCR tests are poorly standardized and they produce highly inconsistent and unreliable results—they have limited clinical applicability.
3. NAA tests, in general, have high specificity and positive predictive value. These test characteristics confer some value in terms of their ability to confirm (rule in) tuberculosis. A positive test in a patient with a reasonably high pre-test probability is fairly confirmatory of tuberculosis.
4. NAA tests, however, have a lower sensitivity and negative predictive value. This suggests that their ability to rule out disease is poor. A negative test, therefore, does not exclude the diagnosis of tuberculosis. A negative NAA test in a patient with a high index of clinical suspicion should prompt continued investigation.
5. Because NAA tests amplify dead bacilli and cannot distinguish viable from non-viable bacilli, they should not be used to monitor response to antituberculosis therapy. Further, NAA tests are not capable of quantifying *M. tuberculosis* in the sputum and clinical specimens; they are, therefore, not helpful in monitoring response to therapy.
6. NAA tests have the highest sensitivity in patients with smear-positive pulmonary tuberculosis. Their sensitivity tends to be poor in patients with smear-negative pulmonary tuberculosis and extrapulmonary forms of tuberculosis (e.g. tuberculous meningitis, pleuritis and lymphadenitis). Therefore, in patients with smear-negative pulmonary tuberculosis and those with suspected extrapulmonary disease, a negative NAA test does not rule out tuberculosis.
7. NAA tests should be performed only in established laboratories that have adequate quality assurance and monitoring systems in place. In the absence of such systems, these tests can produce false-positive results and lead to unnecessary intervention.

In summary, because of concerns regarding reproducibility, low sensitivity, potential for false-positive results under field conditions, high costs, and the requirement for sophisticated laboratory infrastructure, NAA tests may have a limited role in the diagnosis of tuberculosis in most developing, tuberculosis-endemic countries. In these countries, microscopy and culture continue to be the cornerstones for the diagnosis of tuberculosis.

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