

Diagnostic accuracy of Xpert MTB/RIF versus smear microscopy in the early diagnosis of tuberculosis in the real life of the “Umberto I” Hospital in Rome

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SUMMARY

Early diagnosis of tuberculosis (TB) is one of the primary challenges in curtailing the spread of TB. This study aimed to determine the diagnostic accuracy of Xpert MTB/RIF for the identification of *M. tuberculosis* in clinical specimens, and compare this to a microscopist’s diagnostic performance. Xpert MTB/RIF was positive in all specimens with culture-confirmed TB, giving a higher sensitivity than the smear microscopy (100% versus 63%). The use of the Xpert MTB/RIF, as part of routine assay, permits rapid diagnosis of TB and enables clinicians to start an effective treatment.

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Tuberculosis (TB) is an important and potentially fatal infection in humans, and it is estimated that one-third of the world’s population is infected by *Mycobacterium tuberculosis*, with the majority suffering from a latent form of infection (WHO, 2015). Despite lower TB mortality rates in high income countries, the early diagnosis and subsequent treatment of individuals with active TB remains essential to control the spread of the disease and is an important step for TB control programmes worldwide.

Smear microscopy is the most commonly used method with rapid results, identifies acid fast bacilli (AFB) not *M. tuberculosis*, which may affect its specificity in settings with a low burden of TB or places with a high prevalence of non-tuberculosis mycobacteria (NTM). Smear microscopy has limited sensitivity, which is further reduced in HIV-positive individuals. In addition it cannot distinguish between viable and non-viable organisms and cannot provide information on drug resistance, an understanding of which is critical for TB control (Siddiqi *et al.*, 2003; Steingart *et al.*, 2006).

Conventional mycobacterial culture (either solid or liquid), considered the gold standard for diagnosing of TB, is prone to contamination, the results are inevitably delayed due to the slow growth of mycobacteria, and in general require the use laboratories with appropriate biosafety infrastructure (Muyoyeta *et al.*, 2009).

The development of the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) has been considered an important breakthrough in the fight against TB. Xpert MTB/RIF de-

fects *M. tuberculosis* as well as mutation that confer rifampicin resistance using three specific primers and five unique molecular probes (Helbet *et al.*, 2010). It has been demonstrated that Xpert MTB/RIF is faster than conventional smear or culture methods, providing results in less than 2 hours and, importantly, it has excellent sensitivity and specificity. Results from controlled clinical validation trials involving 1730 individuals suspected of having TB or MDR-TB who were prospectively enrolled in 4 distinctly diverse settings showed that 92.2% of culture-positive patients were detected by a single direct Xpert MTB/RIF test. The sensitivity of a single Xpert MTB/RIF test in smear-negative culture-positive patients was 72.5% and this increased to 90.2% when three samples were tested. The specificity of Xpert MTB/RIF was 99% (Boehme *et al.*, 2010; Boehme *et al.*, 2011). In October 2013, WHO issued updated Policy Guidance, providing revised recommendations on using of Xpert MTB/RIF to diagnose pulmonary TB, paediatric TB, extrapulmonary TB and rifampicin resistance (WHO, 2013).

The aim of the present study was to evaluate if Xpert MTB/RIF, following smear microscopy in the routine diagnostic workup of TB, could improve diagnosis of TB and clinical outcomes in the real life of the “Umberto I” Hospital (Rome).

For this study, specimens of individuals with suspected TB were assessed between September 2013 and June 2015.

Direct and concentrated smears were prepared from clinical specimens after treating with N-acetyl-L-cysteine-NaOH for decontamination. Body fluids like bronchoalveolar lavage (BAL), urine, tracheal-bronchial aspirate (TBA), ascitic fluid, gastric aspirate, and synovial fluid, collected aseptically, were expected to have no contaminants, and assessed without decontamination. The smears were stained with Ziehl-Neelsen method according to WHO criteria (WHO, 2012). A 0.5 ml portion of the sed-

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iment was inoculated on solid media (Loewenstein-Jensen culture). Positive cultures were confirmed by use of microscopy and the species determination was performed using Genotype Mycobacterium (MTBC, CM, AS tests; Hain Lifescience, Nehren, Germany). A culture was considered positive if *M. tuberculosis* was detected; cultures with no growth or with growth of a mycobacterium other than *M. tuberculosis* were considered negative for analysis purposes. Xpert MTB/RIF tests were conducted and interpreted according to the manufacturer's recommendations, using a 4-module GeneXpert (Cepheid) instrument with automated readout. The protocol-specified tests (smear microscopy, culture and Xpert MTB/RIF) were performed for all samples in this sequence, and all tests were done by trained laboratory technical staff blinded to clinical information and radiological results.

All statistical analyses were done with GraphPad Prism Software, and considered significant in case of p values <0.05. Smear microscopy, and Xpert MTB/RIF sensitivity, specificity, and predictive values were calculated using culture results as the reference standard. Diagnostic yield was defined as the number of positive tests/total tests x100. The agreement between Xpert MTB/RIF and smear microscopy was performed using Cohen's kappa coefficient. Comparisons of proportions were performed using McNemar's chisquare and Fisher's exact tests for paired and unpaired data, respectively, and 95% confidence intervals (CI) were calculated.

Four hundred and thirty-three patients with suspected TB were assessed prospectively with three protocol-specified tests (smear microscopy, culture and Xpert MTB/RIF) and the results for overall are showed in Table 1.

Overall, by culture testing, 58 of the 433 samples (13.4%) were positive for *M. tuberculosis*, 15 (3.46%) were positive

for growth of non-tuberculosis mycobacteria, 354 (81.7%) were negative for any growth, and 6 (1.38%) were contaminated and excluded from the study (Table 1).

By smear microscopy testing, 46 of the 433 samples (10.6%) were AFB positive and 387 (89.3%) were AFB negative. In particular, smear microscopy was positive only in 37 of 58 specimens (63.7%) that were positive for *M. tuberculosis* growth, and in 9 of 15 specimens (60%) that had growth of a mycobacterium determined not to be *M. tuberculosis*. Of the 354 specimens with culture-negative for *M. tuberculosis*, all were sputum smear-negative (Table 1). The sensitivity of smear microscopy for detection of *M. tuberculosis*, calculated using culture-positive, was 63.8% (95% CI 50.1-76), the specificity was 100% (95% CI 98.9-100), the positive predictive value (PPV) was 100% (95% CI 91.5-96.5) and the negative predictive value (NPV) was 94.4% (95% CI 92.01-96.6).

By Xpert MTB/RIF testing, 58 of the 433 samples (13.4%) were positive for *M. tuberculosis* and 369 (85.2%) were negative (Table 1). All specimens with positive Xpert MTB/RIF results were culture-confirmed TB and 21 (36.2%) were sputum smear-negative. Among 354 specimens with culture-negative for *M. tuberculosis*, all had a negative Xpert MTB/RIF results (Table 1). The sensitivity of Xpert MTB/RIF was 100% (95% CI 93.8 - 100), specificity 100% (95% CI 98.9 - 100), PPV 100% (95% CI 93.8-100) and NPV 100% (95% CI 98.9 - 100).

We found that the overall TB diagnostic yield of Xpert MTB/RIF (13.4%) was higher than that for smear microscopy (8.54%), and there was an agreement between Xpert MTB/RIF and smear microscopy (95%, k=0.75).

Among the 58 specimens with *M. tuberculosis* detected by Xpert MTB/RIF, rifampicin resistance was detected for 2 (3.4%). The relatively low number of TB cases identified

Table 1 - Results of culture, Xpert MTB/RIF and smear microscopy for overall specimens.

	Overall specimen (n=433*)		
	Culture positive for MTB (n=58)	Culture negative (n=354)	Culture positive for NTM (n=15)
Smear - positive	37 (63.8%)	-	9 (60%)
Smear - negative	21 (36.2%)	354 (100%)	6 (40%)
Xpert - positive	58 (100%)	-	-
Xpert - negative	-	354 (100%)	15 (100%)

Definition of abbreviations: MTB = Mycobacterium tuberculosis; NTM = non tuberculous mycobacteria; smear = smear microscopy; Xpert = Xpert MTB/RIF assay.

*6 specimen were excluded from the analysis because they were contaminated.

Table 2 - Different types of pulmonary and extrapulmonary samples tested positive to smear microscopy and Xpert MTB/RIF assay.

Type of specimen	N° of specimen	Smear microscopy positive (n, %)	Xpert MTB/RIF positive (n, %)
Sputum	186	26 (13.9)	37 (19.8)
Bronchoalveolar lavage	74	5 (6.7)	10 (13.5)
Urine	85	1 (1.1)	5 (5.8)
Tracheal-bronchial aspirate	12	1 (8.3)	1 (8.3)
Ascitic fluid	8	1 (12.5)	2 (25)
Gastric aspirate	1	1 (100)	1 (100)
Synovial fluid	5	1 (20)	1 (20)
Tissue specimens	7	1 (14.2)	1 (14.2)

in our study prevented us from meaningfully assessing Xpert MTB/RIF performance for detection of rifampicin resistance.

The median time to detection TB by use of culture, defined as the period between enrolment and first *M. tuberculosis* confirmation, was 19 days (IQR 14-45), whereas Xpert MTB/RIF results were obtained within 2 h after the arrival of the samples.

In summary, we found that Xpert MTB/RIF provides higher sensitivity than smear microscopy, has a sensitivity close to solid culture, and is highly specific. Our data are similar to those reported by others which showed a sensitivity of Xpert MTB/RIF of approximately 98% for smear-positive/culture-positive TB and 72.5% for smear-negative/culture-positive disease, with specificity of approximately 99% (Boehme *et al.*, 2010; Boehme *et al.*, 2011). In addition, the Xpert MTB/RIF assay showed a good performance even in non-respiratory specimens from patients with suspected extra-pulmonary TB, as shown in *Table 2*. Instead, the low observed sensitivity of smear microscopy among our specimens highlights the inadequacy of this test, at least in a scenario in which relatively few individuals have TB disease. A limitation of our study is that the clinical diagnosis and clinical data of subjects were not available to the laboratory personnel.

We conclude that the adoption of Xpert MTB/RIF, as part of routine assay for detection of TB has the potential to improve the detection of pulmonary and extra-pulmonary TB. In addition, this assay provides results in about 2 hours, with information on rifampicin resistance, which is highly associated with multidrug-resistance and poor treatment outcomes using conventional TB treatment regimens.

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Conflicts of Interest

The Authors declare that they have no competing interests.

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