



# Accuracy of polimerase chain reaction for the diagnosis of pleural tuberculosis



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## Summary

**Introduction:** Polymerase chain reaction (PCR)-based techniques to detect *Mycobacterium tuberculosis* DNA in respiratory specimens have been increasingly used to diagnose pulmonary tuberculosis. Their use in non-respiratory specimens to diagnose extrapulmonary tuberculosis is, however, controversial. In this study, we estimated the accuracy of three in-country commercialized PCR-based diagnostic techniques in pleural fluid samples for the diagnosis of pleural tuberculosis.

**Methods:** Patients underwent thoracentesis for diagnosis purposes; pleural fluid aliquots were frozen and subsequently submitted to two real time PCR tests (COBAS<sup>®</sup>TAQMAN<sup>®</sup>MTB and Xpert<sup>®</sup>MTB/Rif) and one conventional PCR test (Detect-TB<sup>®</sup>). Two different reference standards were considered: probable tuberculosis (based on clinical grounds) and confirmed tuberculosis (bacteriologically or histologically).

**Results:** Ninety-three patients were included, of whom 65 with pleural tuberculosis, 35 of them confirmed. Sensitivities were 29% for COBAS<sup>®</sup>TAQMAN<sup>®</sup>MTB, 3% for Xpert<sup>®</sup>MTB/Rif and 3% for Detect-TB<sup>®</sup>; specificities were 86%, 100% and 97% respectively, considering confirmed

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tuberculosis. Considering all cases, sensitivities were 16%, 3% and 2%, and specificities, 86%, 100%, and 97%.

**Discussion:** Compared to the 95% sensitivity of adenosine deaminase, the most sensitive test for pleural tuberculosis, the sensitivities of the three PCR-based tests were very low. We conclude that at present, there is no major place for such tests in routine clinical use.

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## Introduction

Tuberculosis is still a leading cause of death worldwide, and Brazil is one of the 22 countries with the highest burden of the disease [1]. Pleural tuberculosis is the second most common form of the disease [2,3], and its diagnosis remains a challenge [4]. Sensitivity of smears for acid-fast bacilli (AFB) is extremely low (<5%) [4–6], cultures have a long delay and also low sensitivity (<60%) [7–10]. Histopathological examination of the pleural tissue is the most sensitive diagnostic test (80–85%) [4,6,10,11]; however, it requires a pleural biopsy, a procedure that increases risks and costs [11]. The adenosine deaminase (ADA) enzyme is another pleural fluid marker of tuberculosis. Despite its high sensitivity (56–100%) [12–14], ADA activity reflects only a non-specific immunologic response [11]. Previous studies showed a poor positive predictive value of ADA for tuberculosis diagnosis in low-tuberculosis incidence settings [12,14]. Despite these limitations, in practice, ADA, AFB and cultures of pleural fluid as well as pleural biopsies have been recommended as the reference tests for diagnosing pleural tuberculosis [4,11,12,14,15].

After a century of stagnation regarding new technologies for the diagnosis of tuberculosis, new molecular-based technologies were approved for the detection of *Mycobacterium tuberculosis* DNA in respiratory specimens in the past two decades, and automated systems, such as the Xpert<sup>®</sup>MTB/Rif, commercially available in the last couple of years, are being rapidly incorporated for the diagnosis of pulmonary tuberculosis in high-burden countries [16–18]. However, their use in extrapulmonary samples is still controversial [19]. In-house PCR-based tests in pleural fluid have a high specificity (98%), but low and heterogenous sensitivity (43–77%), but automated systems were less studied [20]. In order to study the usefulness of PCR technique in the diagnosis of pleural tuberculosis in routine practice, in the present study, we aimed to evaluate the accuracy of three commercially available tests: two real time PCR tests (COBAS<sup>®</sup>TAQMAN<sup>®</sup>MTB and Xpert<sup>®</sup>MTB/Rif) and one conventional PCR test (Detect-TB<sup>®</sup>).

## Methods

From September 2007 to March 2011, all patients with a pleural effusion needing a thoracentesis for diagnostic purposes hospitalized in the 7th ward (an Internal Medicine Unit) of Hospital Geral da Santa Casa da Misericórdia do Rio de Janeiro were eligible. Adults (>18 years old)

were invited to participate and those who signed an informed consent were prospectively included. Patients were excluded if they had bleeding disorders contraindicating thoracentesis, if the fluid volume was insufficient for storage or if a final diagnosis could not be ascertained.

In this pragmatic study, diagnosis and management were carried out according to the clinicians' practice and Brazilian Guidelines [21]. Consent was obtained for the experimental (PCR) techniques only. Pleural fluid was forwarded for biochemical (protein, glucose and ADA level), for cytometric (total white cells, mononuclear, neutrophils), for bacteriological (AFB smears and *M. tuberculosis* culture in liquid media – BACTEC mycobacterial growth indicator tube [MGIT] 960 System, BD) and sporadically, Gram stain and culture for pyogenic bacteria evaluation. Pleural tissue, obtained with a Cope needle, was forwarded for histopathological analysis and for *M. tuberculosis* culture (MGIT). Aliquots were frozen at –80 °C. Spontaneous or induced sputum specimens were also forwarded for AFB and culture, when available (data not shown). According to the Brazilian Guidelines [21], the diagnosis of confirmed tuberculosis was made if any specimen was positive for AFB or culture, or if granuloma with or without caseous necrosis was present on a biopsy. A clinical diagnosis of probable tuberculosis was made if patients had symptoms compatible with tuberculosis (fever, night sweats and weight loss), an exudative pleural fluid or an ADA level > 40 IU/L and if they had clinical improvement after antituberculous therapy. Patients were clinically managed according to these results.

For the three PCR techniques, samples were simultaneously unfrozen in 2012 and processed according to the manufacturer's recommendations (except for the freezing step) by two experienced lab technicians (EFSSKO and SEM), blinded to the clinical results. In brief, COBAS<sup>®</sup>TAQMAN<sup>®</sup>MTB amplifies and detects the *rRNA 16S* gene sequence [22–24], Xpert MTB<sup>®</sup>/Rif automatically amplifies and detects the *rpo β* gene [25], and the conventional Detect-TB<sup>®</sup> amplifies and detects the *IS 6110* insertion [26].

To assess the similarity between groups of participants Wilcoxon non-parametric test was used for medians. Proportions were compared using the Fischer's exact test.

Sensitivities, specificities and their 95% confidence interval (CI) were calculated for each test using (i) the confirmed cases as the reference standard and (ii) all cases (confirmed and probable) as the second reference standard, according to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) initiative recommendations [27].

Sensitivities were compared among those with the final diagnosis of tuberculosis using the  $\chi^2$  McNemar test. Specificities could not be compared since, by definition, there are no false-positive for tuberculosis tests [28].

## Ethics

The study was in accordance with the Brazilian CNS 196/96 (at present replaced by 466/12) and 441/11 resolutions, which follow the Helsinki declaration regarding human being research rights. It was authorized by the institutional review board of the *Hospital Geral da Santa Casa da Misericórdia do Rio de Janeiro*, the latest version was also approved by the National Ethical Committee (#771/2009). Only patients accepting to sign the informed consent, which foresaw freezing and further processing of samples, were included.

## Results

Out of 203 eligible patients, 110 were excluded: 21 did not have a final diagnosis and 89 did not have sufficient fluid to store (Fig. 1). Their sex, age, HIV-status and proportion of tuberculosis were similar to the included patients (Table 1), although they tended to be younger. Out of the 93 included patients, 63 (68%) had a final diagnosis of pleural tuberculosis, of whom 35 (56%) were confirmed. Other diagnoses are summarized in Fig. 1.

Participants' characteristics according to final diagnosis are compared in Table 2. Patients with tuberculosis were younger. In Tables 3A and 3B, test results based on which diagnosis was performed as well as experimental (PCR) test

**Table 1** Characteristics of eligible participants submitted to investigation of a pleural effusion.

	Included N = 93	Not included N = 98 <sup>a</sup>	P value
Median age (IQR)	50 (40; 57)	43 (27; 61)	0.08
Sex			0.75
Female	19 (20%)	19 (19%)	
Male	74 (80%)	79 (81%)	
HIV			0.37
Positive	5 (5%)	10 (10%)	
Negative	61 (66%)	65 (66%)	
Unknown	27 (29%)	23 (24%)	
Tuberculosis <sup>b</sup>			0.18
Yes	63 (68%)	69 (77%)	
No	30 (32%)	20 (23%)	

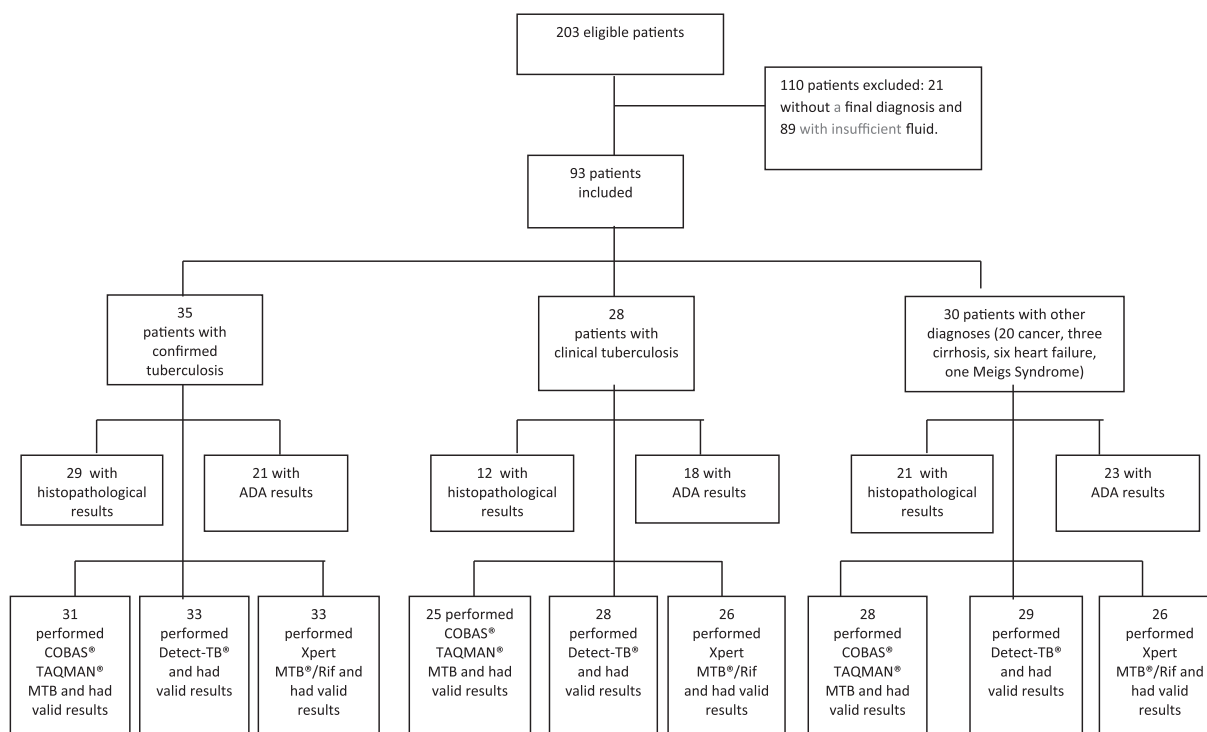
IQR – interquartile range.

<sup>a</sup> Among 21 participants without a final diagnosis, 12 did not have available information on clinical characteristics.

<sup>b</sup> 9 patients without a final diagnosis were excluded from this analysis.

results are displayed. Missing results are due to temporary unavailability of consumables in the hospital or to absence of pleural tissue in biopsy specimens, since this was a pragmatic study. Gram staining and culture for pyogenic bacteria were performed in 54% of participants. Despite two positive gram stains (one in a patient with a final clinical diagnosis of tuberculosis and the other in a patient with a final diagnosis of Meigs' Syndrome), all cultures for non-specific bacteria were negative.

As shown in Tables 3A and 3B, the three PCR-based tests had a very low sensitivity, although COBAS<sup>®</sup>TAQMAN<sup>®</sup>MTB



**Figure 1** Flowchart of study participants.

**Table 2** Characteristics of pleural TB suspects included in the study, according to their final diagnosis.

	Confirmed TB N (%)	Clinical TB N (%)	Other diagnosis N (%)	P value
Median age (IQR)	45 (35; 53)	46,5 (32; 57)	56 (49; 62)	<0.01
Sex				<0.01
Female	1 (3%)	10 (36%)	8 (27%)	
Male	34 (97%)	18 (64%)	22 (73%)	
HIV				0.05
Positive	4 (12%)	1 (4%)	0 (0%)	
Negative	20 (57%)	23 (82%)	18 (60%)	
Unknown	11 (31%)	4 (14%)	12 (40%)	

IQR – interquartile range, TB – tuberculosis.

had a significantly higher sensitivity than Xpert<sup>®</sup>MTB/Rif ( $p = 0.02$ ) and Detect-TB<sup>®</sup> ( $p = 0.02$ ). Conversely, ADA had the highest sensitivity, followed by the histopathological examination. The three PCR tests had a significantly lower sensitivity when compared to ADA ( $p < 0.01$ ) and to the histopathological examination ( $p < 0.01$ ).

Specificities of Xpert<sup>®</sup>MTB/RIF and Detect-TB<sup>®</sup> were the highest, 100% (89–100%) and 97% (81–100%, respectively).

Among 17 hemorrhagic samples, nine had false-negative results and four had indeterminate results in at least one of the three PCR-based tests.

## Discussion

In this pragmatic, routine study, sensitivities of three PCR-based commercially available tests for diagnosing pleural tuberculosis were very low. Despite their excellent

specificities, the low sensitivities make them unsuitable for routine use in clinical practice, unless if used as a confirmatory test after triage with more sensitive tests, such as ADA. Cost-effectiveness studies for this approach would, however, be necessary, since it would be unlikely that this strategy would be cost-effective, due to the high costs of the tests and the relatively low cost of tuberculosis treatment.

The very low sensitivities found in our study contrast with previous results in the literature, as reviewed by Pai et al., and with our own results in a previous series, using an in house PCR technique. Most series reviewed in Pai's meta-analysis [20] used in house tests and thus did not include automated tests, sensitivities varied from 25% to up to 87%. A more recent meta-analysis that evaluated the accuracy of Xpert<sup>®</sup>MTB/RIF in 1385 pleural fluid samples confirmed a low pooled sensitivity (43.7%; 95% CI 24.8%; 64.7%) and a high pooled specificity (98.1%; 95% CI 95.3%; 99.2%) if culture for MTB was used as the reference standard. Using a composite standard reference (histopathological examination, clinical signs and AFB smear), sensitivity was reduced to levels similar to those found in the present study (17%; 95% CI 7.5%; 34.2%) [29].

One of the possible reasons for the low sensitivity of PCR-based tests is that pleural effusions are thought to be due to a local inflammatory reaction. However, even among those with bacteriologically confirmed tuberculosis, PCR tests were mostly negative in our study.

Another possible explanation for the very low sensitivities in our study was the presence of inhibitory substances. We did not submit clinical specimens to any processing prior to the technique itself. Blood and other inhibitors, such as heparin or pus may interfere with cell lysis, inactivating the DNA polymerase or interfering with nucleic acids [30]. This can cause false-negative or invalid results, as seen in our sample [11,30]. Eliminating inhibitory substances in clinical specimens has been reported to increase the sensitivity of

**Table 3A** Sensitivity and specificity of tests: 3A – Only patients with confirmed diagnosis (confirmed TB and other diagnosis).<sup>a</sup>

Tests	N	True positive results among TB patients	Sensitivity (95% CI)	True negative results among other diagnosis	Specificity (95% CI)
PF AFB smear <sup>b</sup>	65	1	3% (0%; 16%)	–	–
PF culture <sup>c</sup>	63	16	47% (31%; 64%)	–	–
Pleural histopathological examination <sup>d</sup>	50	26	90% (73%; 97%)	–	–
PF ADA <sup>e</sup>	44	20	95% (76%; 100%)	20	87% (67%; 96%)
PF PCR					
Detect-TB <sup>®f</sup>	62	1	3% (0%; 19%)	28	97% (81%; 100%)
COBAS <sup>®</sup> TAQMAN <sup>®</sup> MTB <sup>g</sup>	59	9	29% (16%; 47%)	24	86% (68%; 95%)
Xpert MTB <sup>®</sup> /Rif <sup>h</sup>	59	1	3% (0%; 17%)	26	100% (89%; 100%)

ADA – Adenosine deaminase, PCR – polymerase chain reaction, PF – pleural fluid, 95% – CI confidence interval, TB – tuberculosis.

<sup>a</sup> Analysis of 35 patients with confirmed TB and 30 patients with other confirmed diagnosis (see Fig. 1).

<sup>b</sup> Acid-fast bacilli smear in pleural fluid.

<sup>c</sup> Pleural fluid culture was not performed in two patients.

<sup>d</sup> Histopathological examination in pleural tissue was not done in 15 patients.

<sup>e</sup> Adenosine deaminase (ADA) measurement in pleural fluid was not performed in 21 patients. ADA positive was considered when results were higher than 40 IU/L.

<sup>f</sup> Detect-TB<sup>®</sup>-One patient did not have enough fluid to perform the test and two other patients had invalid results.

<sup>g</sup> COBAS<sup>®</sup> TAQMAN<sup>®</sup> MTB-Six patients had invalid results.

<sup>h</sup> Xpert MTB<sup>®</sup>/Rif-One patient did not have enough material and five other patients had invalid results.

**Table 3B** Sensitivity and specificity of tests: 3B – All patients included in the study (confirmed or probable pleural tuberculosis and other diagnosis).<sup>a</sup>

Tests	N	True positive results for TB patients	Sensitivity (95% CI)	True negative results for other diagnosis	Specificity (95% CI)
PF AFB smear <sup>b</sup>	93	1	2% (0%; 9%)	—	—
PF culture <sup>c</sup>	91	16	26% (16%; 38%)	—	—
Pleural histopathological examination <sup>d</sup>	62	26	63% (48%; 76%)	—	—
PF ADA <sup>e</sup>	62	35	90% (76%; 97%)	20	87% (67%; 96%)
PF PCR					
Detect-TB <sup>f</sup>	90	1	2% (0%; 10%)	28	97% (81%; 100%)
COBAS <sup>®</sup> TAQMAN <sup>®</sup> MTB <sup>g</sup>	84	9	16% (8%; 28%)	24	86% (68%; 95%)
Xpert MTB <sup>®</sup> /Rif <sup>h</sup>	85	2	3% (0%; 12%)	26	100% (89%; 100%)

ADA – Adenosine deaminase, PCR – polymerase chain reaction, PF – pleural fluid, 95% – CI confidence interval, TB – tuberculosis.

<sup>a</sup> Analysis of 63 patients with clinical TB and 30 patients with other confirmed diagnosis (see Fig. 1).

<sup>b</sup> Acid-fast bacilli smear in pleural fluid.

<sup>c</sup> Pleural fluid culture was not performed in two patients.

<sup>d</sup> Histopathological examination in pleural tissue was not done in 31 patients.

<sup>e</sup> Adenosine deaminase (ADA) measurement in pleural fluid was not performed in 31 patients. ADA positive was considered when results were higher than 40 IU/L.

<sup>f</sup> Detect-TB<sup>®</sup>-One patient did not have enough fluid to perform the test and two other patients had invalid results.

<sup>g</sup> COBAS<sup>®</sup> TAQMAN<sup>®</sup> MTB-Nine patients had invalid results.

<sup>h</sup> Xpert MTB<sup>®</sup>/Rif-One patient did not have enough material and seven other patients had invalid results.

PCRs. Likewise, concentrating the clinical specimen might yield better results [6,31–33].

Finally, the low sensitivities found in our study could also be explained by the long storage time in freezers, despite the optimal temperature (–80 °C). Indeed, fresh samples have a better sensitivity 50% (95% CI 36%; 64%) than frozen specimens (26%; 95% CI 14%; 40%) [17]. Manufacturers recommend the use of tests in specimens frozen for up to 6 months or refrigerated for up to 4–10 days. Some of our specimens were frozen for up to 4 years. However, no relationship was seen between false-negatives and date of specimen collection (data not shown).

One of the main limitations of our study was the high number of presumptive (non-confirmed) cases. This was due to the routine, pragmatic nature of the study, conducted in a public secondary health facility. Culture of pleural tissue, which could significantly improve accuracy of diagnosis, were not performed. In addition, many patients did not have one of the routine tests. However, even among confirmed cases, sensitivity was unacceptably low, confirming the findings of the systematic reviews. Another important limitation was the use of frozen specimens. Conversely, the main strength of the study is that every sample, in routine conditions, was processed, comparing three different commercially available kits.

In summary, despite the high specificity, because of their very low sensitivities, it is unlikely that PCR-based tests will have a major clinical usefulness for the diagnosis of pleural tuberculosis.

### Author's contribution

Conception and hypothesis delineation: AT, EFSSKO, AK, MO. Data collection: EFSSKO, MLB, EBN, EMS. PCR tests:

EFSSKO, EMS. Data analysis and interpretation: AT, EFSSKO, MLB, AK, MO. Manuscript drafting: AT, EFSSKO, MLB, AK, MO. Final review: all co-authors.

### Conflict of interests

The authors declare no conflicts of interests.

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