J Clin Tuberc Other Mycobact Dis 21 (2020) 100181

Contents lists available at ScienceDirect

J Clin Tuberc Other Mycobact Dis

journal homepage: www.elsevier.com/locate/jctube

Utility of *Mycobacterium tuberculosis* PCR in ruling out active disease and impact on isolation requirements in a low prevalence setting

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ARTICLE INFO

Keywords:
Mycobacterium tuberculosis
Molecular testing
Infection prevention

ABSTRACT

Objective: To analyze and interpret clinical microbiology data for specimens tested with the fluorochrome stain (AFB stain), mycobacterial culture and a laboratory-developed *Mycobacterium tuberculosis* (MTB) PCR in order to understand the performance of each test and to demonstrate the utility of MTB PCR to assist with decisions regarding discontinuation of airborne isolation.

Methods: Retrospective cohort analysis of 2798 respiratory specimens from 2006 patients in the period between November 1st, 2011 and January 1st, 2018.

Results: 53.7% were males, median age was 61 years, and 43 patients were HIV positive. Results demonstrated positive mycobacterial cultures for MTB in 52 specimens (1.9%) and for nontuberculous mycobacteria (NTM) or aerobic actinomycetes (eg., *Nocardia* spp.) in 435 specimens (16%). Using mycobacterial culture as the gold standard, AFB smear had a sensitivity of 48.1% while MTB PCR had a sensitivity of 96.0% in AFB smear positive specimens and an overall sensitivity of 57.7% with PPV of 94% and a NPV of 99%.

Conclusions: The combination of a positive AFB smear with a negative MTB PCR offers a rapid result to rule out active pulmonary MTB in a low prevalence setting. In this study, that combination reliably excluded active tuberculosis (NPV of 99.2%). The combination of a positive AFB smear with a negative MTB PCR indicated pulmonary NTM infection with the results available within 1 day. There was little benefit to pursuing collection and testing of more than 2 respiratory specimens in a low prevalence setting for both long term diagnostic or rapid isolation discontinuation purposes.

1. Introduction

Mycobacterium tuberculosis (MTB) infections continue to be a major challenge to human health worldwide and are the leading cause of death amongst single infectious causes [1].

The most recent CDC update in 2018 reported more than 9000 new MTB cases (a rate of 2.8 cases per 100,000 persons) in the United States. Worldwide, reports estimate 10 million cases of tuberculosis (TB) in 2017 with approximately 1.3 million MTB related deaths in HIV-negative patients [2].

Pulmonary MTB is of specific concern due to the potential for high infectivity compared to extrapulmonary MTB. Definitive diagnosis however remains challenging due to slow growth of MTB in the culture media which can take up to 8 weeks. A rapid definitive diagnosis is vital to infection prevention strategies in order to limit transmission in the healthcare setting and in the community.

Suspected MTB patients are usually kept in a single bed, negative-pressure ventilation room and healthcare personnel are required to use respiratory protection until an MTB diagnosis is excluded. Exclusion of MTB is both labor and resource intensive as it involves collection, examination, and resulting of 3 negative acid-fast bacilli (AFB) smears prepared from respiratory specimens, obtained 8–24 h apart, with at least 1 early morning specimen [3].

The sensitivity of acid fast bacilli (AFB) smear for diagnosis of pulmonary MTB is low at 40–60% and it only slightly improves with the subsequent 2nd and 3rd smears [4,5]. AFB smear is not specific for MTB and is positive in cases of NTM as well. Molecular testing with MTB-specific nucleic acid amplification tests (NAAT) demonstrated high sensitivity of up to 95–100% in AFB smear positive specimens and approximately 50% to 80% in AFB smear negative specimens which led to the endorsement of rapid molecular testing by the WHO and the Food and Drug administration in 2010 and 2013 respectively [6–10].

Subsequently in April 2016, a panel of MTB experts published a consensus that recommended discontinuation of airborne isolation in

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https://doi.org/10.1016/j.jctube.2020.100181

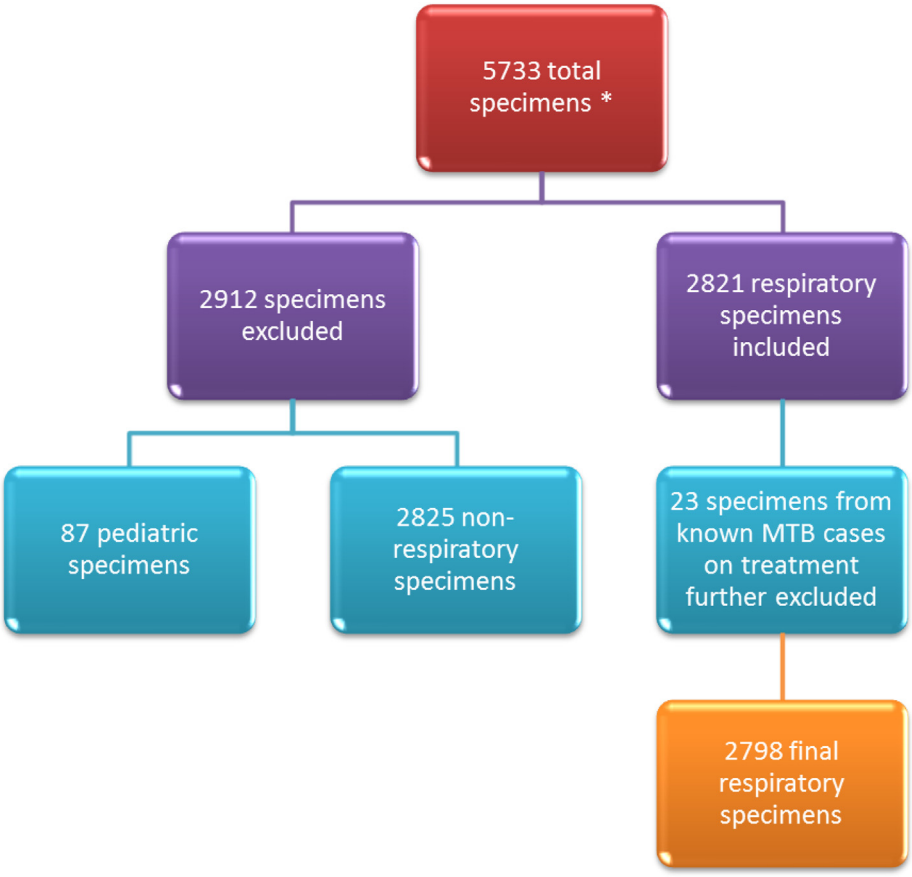

Fig. 1. Specimen Inclusion and Exclusion Criteria MTB = *Mycobacterium tuberculosis.*

patients with 2 Xpert NAAT negative sputum specimens that are obtained at least 8 h apart [7]. However not all institutions across the United States use the Xpert NAAT test and different infection prevention protocols may be utilized. At our institution, a laboratory-developed MTB complex PCR assay is used and historically, we have required 3 negative AFB smears to discontinue airborne isolation.

The aim of this study is to evaluate our laboratory-developed PCR test performance in patients being evaluated for MTB by multiple testing modalities in a low prevalence setting and to explore the significance of specimens with concomitant positive AFB smear and negative MTB PCR in regards to safety in ruling out pulmonary tuberculosis and discontinuing airborne isolation.

2. Patients and methods

The study was approved by an Institutional Review Board of the Mayo Clinic. Data was obtained through the review of the microbiologic database. Data was retrospectively collected and analyzed specimens from respiratory sources that were tested in the microbiology lab by AFB smear, the laboratory-developed MTB PCR and mycobacterial culture simultaneously. MTB cultures were considered the gold standard for MTB diagnosis in this project. Slightly more than half of the specimens were from residents of Minnesota where the TB incidence rate is 3.1 cases per 100,000 population [11].

Using Advanced Cohort Explorer software, data was extracted for the period between March 2011 and January 2018.

Specimen sources included were sputum, induced sputum, bronchoalveolar lavage fluid (BAL), bronchial washings, tracheal secretions and *trans*-bronchial biopsies.

The MTB complex PCR is a laboratory developed assay that uses primers and fluorescence resonance energy transfer (FRET) probes to target and detect a unique 203 base pair sequence within the *katG* gene [12]. The PCR assay is performed on the LightCycler 2.0 instrument (Roche Applied Sciences, Indianapolis, IN). Primers and probes were synthesized by TIB MOLBIOL (Primer-Probe Set [535], Adelphia, NJ). The PCR assay was performed using the LC FastStart DNA Master hybridization probe kit (Roche Applied Sciences, Indianapolis, IN). PCR amplification parameters were denaturation at 95 °C for 10 min, followed by 45 amplification cycles at 95 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s. Following amplification, melting curve analysis was performed by measuring the fluorescent signal during the following cycling parameters: 95 °C for 0 s, 59 °C for 20 s, 45 °C for 20 s with a 0.2 °C/s transition, and 85 °C for 0 s with a 0.2 °C/s transition. The performance of the MTB PCR assay was compared to the GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA) and the correlation of the two assays was 99.2% (n = 499 specimens total, 29 Xpert positive specimens) with a kappa coefficient of 0.93 indicating near perfect agreement.

A detailed chart review of the patients with a final diagnosis of pulmonary tuberculosis was performed to analyze the number and source of specimens tested for each of these patients and their results.

2.1. Statistical analysis

Data was analyzed using the Fisher exact test or χ^2 analysis for nominal data and the median test and interquartile ranges for non-parametric continuous data. P values < 0.05 were considered significant. All statistical analysis was performed using JMP software, version 14.1.0 (SAS Institute Inc.).

Table 1
Characteristics of 2798 respiratory isolates from 2011 to 2018

Characteristics	Number (%)
Specimen source	
Sputum	2009 (71.8)
Bronchoalveolar lavage fluid	384 (13.7)
Bronchial washing	179 (6.4)
Tracheal secretions	166 (5.9)
Trans-bronchial lung biopsies	60 (2.1)
Patient place of residence	
USA residents	2617 (93.5)
Minnesota	1527 (54.6)
Iowa	255 (9.1)
Wisconsin	139 (5.0)
Others	696 (24.9)
International	181 (6.5)
Saudi Arabia	40 (1.4)
Kuwait	39 (1.4)
UAE	30 (1.1)
Others	72 (2.6)

3. Results

A total of 5733 specimens collected in the period between March 2011 and January 2018 had an AFB smear, MTB PCR and mycobacterial culture performed simultaneously. Of the 5733 specimens, 2912 specimens were excluded including 87 specimens from patients < 18 years of age and 2825 non-respiratory specimens. 23 specimens were excluded for patients with known active MTB who were on antituberculous treatment at the time of testing. The final analysis included 2798 respiratory specimens from 2006 patients with AFB smear, MTB PCR and mycobacterial culture performed on each specimen at the reporting laboratory (Fig. 1).

The most common respiratory specimen source was sputum (71.8%), followed by BAL fluid (13.7%), bronchial washing (6.4%), tracheal secretions (5.9%) and *trans*-bronchial lung biopsies (2.1%). The majority of the specimens were from patients residing in the United States (93.5%) whereas 6.5% of specimens were from patients residing in other countries. Specimen sources and patient epidemiology are summarized in Table 1.

The majority of the patients were male (53.7%) and the median age was 61 years old. The majority of the patients were white (75.5%) with other ethnicities including African American (7.5%), Asian (including Asian Indian, Chinese, Filipino, Pakistani, 5.9%) and 11.1% had either an unknown race or who chose not to disclose their race. HIV status was documented for 1112 of the 2006 total patients with 2.1% HIV positive, 53.3% HIV negative, and 44.6% unknown status. (Table 2).

Mycobacterial cultures were positive for NTM and other closely related organisms such as *Nocardia* spp., *Gordonia* spp., *Streptomyces* spp. and *Rhodococcus equi* in 435 specimens (15.5%). Cultures were positive for MTB in 52 specimens (1.9%) and cultures were negative in 2311 specimens (82.6%). Of the positive MTB cultures, 40 (77%) were from sputum specimens, 7 (13%) from BAL fluid, 4 (8%) from bronchial washings, and 1 (2%) from a lung biopsy.

Compared to mycobacterial cultures positive for MTB, the AFB smear had a sensitivity of 48.1% (25/52). MTB PCR had a sensitivity of 96.0% (24/25) for smear positive specimens and overall sensitivity of 57.7% (30/52) regardless of smear status.

When stratified by HIV status, the incidence of positive MTB cultures in HIV positive patients (2.3%) was essentially equivalent to that of HIV negative patients (1.7%) but the overall numbers are small with only 43 specimens from HIV positive patients. In HIV positive, MTB culture positive specimens, the sensitivity of AFB smear was 100% and MTB PCR was 100% while in HIV negative, MTB culture positive specimens, AFB smear sensitivity was 61.6% and MTB PCR sensitivity was 66.7%.

Table 2
Characteristics of 2006 patients from 2011 to 2018.

Characteristics	Number (%)
Patient demographics	
Age (median, range)	61 (18–99
Male	1077 (53.7)
Female	929 (46.3)
Race	
White	1514 (75.5)
African American	150 (7.5)
Asian	119 (5.9)
Unknown/Declined to answer	223 (11.1)
HIV status	
Unknown	894 (44.6%)
HIV positive	43 (2.1%)
HIV negative	1069 (53.3%)
Patients with pulmonary TB	33
Age (median, range)	51 (23–89)
HIV status	
Unknown	10 (30.3%)
Positive	2 (6.1%)
Negative	21 (63.6%)
Race	
White	9 (27.3%)
African American	4 (12.1%)
Asian	13 (39.4%)
Unknown/Declined to answer	7 (21.2%)

Of 171 total AFB smear positive specimens, 25 grew MTB in culture and 24/25 (96.0%) were MTB PCR positive from the first specimen tested. Of the remaining AFB smear positive specimens, 134/146 (91.7%) grew nontuberculous mycobacteria or *Nocardia* spp. while 12/146 (8.2%) did not show any microorganism grow in culture. All of the 146 smear positive specimens that grew NTMs or had no growth were also MTB PCR negative. The combination of a positive AFB smear with a negative MTB PCR was able to rule out MTB in all but one instance. A second specimen from the single patient with a positive AFB smear/negative MTB PCR was positive by the MTB PCR so two negative MTB PCR results from smear positive specimens ruled out MTB in 100% of cases ($p < 0.0001$).

Of 33 patients with culture-confirmed pulmonary MTB, 14 patients (42.4%) had a positive AFB smear and a positive MTB PCR from their first sample. Of 12 patients with two specimens collected and culture-confirmed pulmonary MTB, 9/12 (75%) had a positive AFB smear and a positive MTB PCR from the first two specimens collected. Of 5 patients with three specimens collected and culture-confirmed pulmonary MTB, 4/5 (80%) had a positive AFB smear and a positive MTB PCR from the first three specimens collected (Fig. 2).

4. Discussion

Mycobacterial culture remains the current gold standard for diagnosing MTB but the time required for results can take up to 8 weeks. This extended time to diagnosis poses a big challenge from infection prevention standpoint in cases of active pulmonary tuberculosis because airborne isolation is required for at least a portion of this time.

Although the initiation and maintenance of airborne isolation is essential to protect healthcare professionals and other patients from the risk of tuberculosis transmission, it is time and resource exhausting and it negatively affects both the patient and healthcare providers' experience. The performance of multiple AFB smears and MTB PCR has been evaluated to enhance timing of effective anti-mycobacterial therapy initiation and infection prevention protocol initiation and discontinuation.

Our study confirms the low sensitivity of AFB smears for the detection of MTB. MTB PCR had a sensitivity of 96% when used for AFB

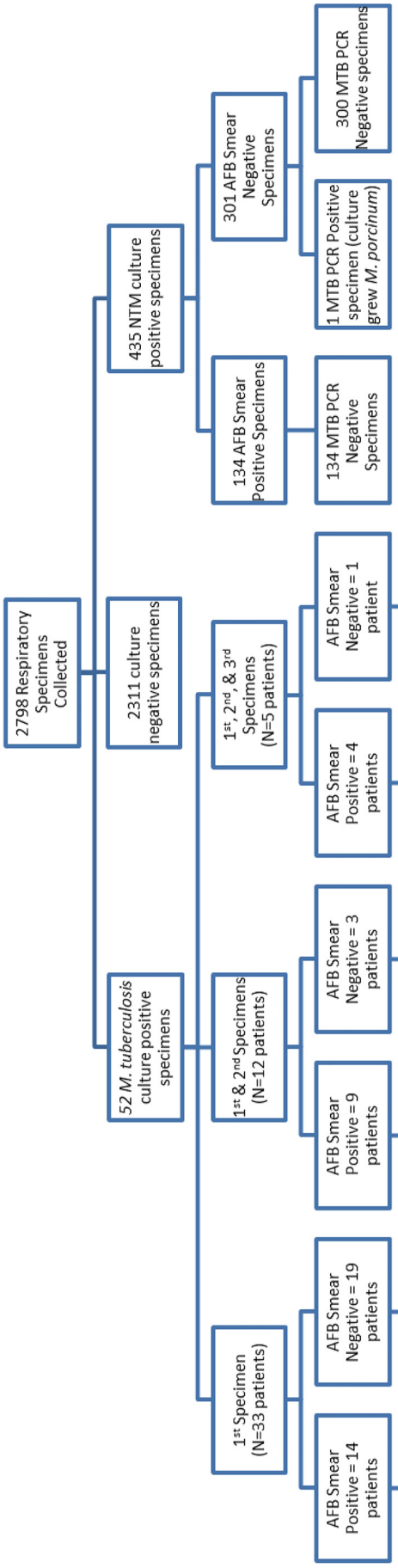

Fig. 2. Respiratory Specimens stratified by Mycobacterial Culture, AFB Smear and MTB PCR Results.

smear positive specimens but its overall sensitivity drops to 58% when smear negative specimens were included so MTB cannot be excluded in these cases. One could argue that peforming an MTB PCR on a smear negative specimen would not be cost effective given the low sensitivity but the benefit for patients with positive PCR results may outweigh the cost.

The combination of a positive AFB smear and a negative MTB PCR offers a rapid, effective way to rule out active pulmonary MTB in a low prevalence setting and indicated pulmonary NTM infection in 96% of the cases ($p < 0.0001$). A single patient was MTB culture positive with a positive AFB smear and a negative MTB PCR after one specimen was collected. The MTB PCR was positive after collection of a second specimen from this patient demonstrating that two negative MTB PCR results would effectively rule out MTB.

In addition, a positive MTB culture was successfully obtained by the collection of 2 specimens in all cases. The collection of more than 2 specimens did not result in any additional positive rapid diagnostics (AFB smear or MTB PCR). However, in high risk patients with high pretest probability for *Mycobacterium tuberculosis*, further testing should be considered.

5. Conclusion

A laboratory-developed MTB PCR provided high specificity and a PPV of 96%.

In a low prevalence setting, a combination of 2 negative AFB smears and 2 negative MTB PCR from respiratory specimens supports safely removing a patient from airborne isolation. This finding is in agreement with the April 2016 consensus statement by the NTCA and the APHL.

The combination of a positive AFB smear and a negative MTB PCR from a respiratory specimen offers the unique opportunity to reliably exclude MTB and provides data supporting the early discontinuation of airborne isolation.

Our study is limited by the relatively low prevalence of *Mycobacterium tuberculosis* complex and HIV positive patients in our institution.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Financial support. None reported.

Potential conflicts of interest. All authors report no conflicts of interest relevant to this article.

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