EBioMedicine 71 (2021) 103552

Contents lists available at ScienceDirect

EBioMedicine

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

Commentary

# TB or NTM: Can a new multiplex PCR assay be the answer?

Appakkudal R Anand[a], Jyotirmay Biswas[b,*]

[a] L & T Microbiology Research Centre, Medical Research Foundation, 41, College Road, Chennai, India 600006
[b] Department of Uvea and Ophthalmic pathology, Medical Research Foundation, 41, College Road, Chennai, India 600006

ARTICLE INFO

*Article History:*
Received 11 August 2021
Accepted 11 August 2021
Available online xxx

Tuberculosis continues to be a major public health concern, being the leading cause of death due to an infectious agent. Simultaneously, the prevalence of nontuberculous mycobacterial (NTM) infections worldwide has been rising [1]. *Mycobacterium avium* complex (MAC) is the most frequent NTM associated with pulmonary disease [2]. MAC can often be misdiagnosed as *Mycobacterium* tuberculosis (Mtb), especially in countries with a high tuberculosis burden and treated with anti-tuberculosis drugs, to which MAC is usually not sensitive [3]. Conventional laboratory diagnosis relies on culture, to see whether Mtb or NTM is present, and is hampered by the slow growth of most mycobacteria and may take several weeks. Several molecular tests such as the WHO-recommended Xpert MTB/RIF (Cepheid) assay have provided a rapid alternative to culture for detection of Mtb [4]. However, most of these molecular Mtb assays do not detect NTM.

In this article of *EBioMedicine*, Sarro and colleagues [5] have developed a new highly sensitive multiplex MTB/NTM assay that can differentiate M. tuberculosis complex (MtbC) and the most common NTM, MAC. This multiplex assay has shown a high analytical sensitivity (5 CFUs/mL for TB and MAC and 20 CFUs/mL for other NTMs). The overall sensitivity, specificity, of the multiplex in cases without treatment failure were 83·3% and 96·6%, while the Xpert had value of 96·7% and 80·0%, respectively for the same patients compared to sputum culture. Thus, the method had a better specificity than Xpert for all TB-infection tested groups, although the Xpert had greater sensitivity. The assay also successfully detected all the MAC cases.

Over the past two decades, molecular methods have come to represent a reliable and rapid alternative for laboratory diagnostics of mycobacteria in clinical samples. The most significant advance in recent times has been the FDA approval and global distribution of the Xpert MTB/RIF, which detects the Mtb complex as well as key genetic determinants of rifampin resistance. However, the main drawback of Xpert is that it is less sensitive than sputum culture and does not detect NTM infections. The most accurate current method for the detection and identification of NTM species is PCR-DNA sequencing with hsp65, rpoB, and 16S rRNA being the target genes [6]. The advantage of PCR-sequencing is it is highly discriminatory, but the equipment and running costs are high. In contrast, real-time PCR is an alternative and cheaper technique. A few recent studies have evaluated real-time PCR for the detection of NTM [7–9]. Peixoto et al. [7] evaluated a multiplex real-time PCR with high-resolution melting for the identification and differentiation between Mtb and NTM, as well as among NTM species of clinical importance. However, this assay involved three different stages of PCR, one to differentiate Mtb and NTM and two more to differentiate the NTM species. Rocchetti et al. [8] used a multiplex real-time PCR on the BD Max open system to detect Mycobacterium spp. (pan-Mycobacterium), Mtb Complex group, and MAC group. Sevilla et al. [9] described the development and evaluation of a novel tetraplex real-time PCR for simultaneous detection of Mycobacterium genus, *M. avium* subspecies, and *M. tuberculosis* complex. Most recently, another duplex PCR targeting genes encoding catalase-peroxidase of MAC and Mtb to discriminate disseminated MAC and MtB infections in blood samples from HIV patients to diagnose disseminated tuberculosis was reported [10]. In this article, Sarro et al. have developed a novel one-step multiplex PCR platform that could be a convenient, cost-effective, and reliable method for simultaneous detection and differentiation between Mtb and MAC infections. This assay is extremely relevant to enhance discrimination of TB and NTM infections in pulmonary disease, with implications not only for selection of antimicrobial therapy diseases, but also for infection control.

Though molecular methods have had a huge impact in the laboratory diagnosis of mycobacterial related infections and therefore patient care during the last decade, we need to be aware of their limitations. These include technical problems, expensive reagents, high labor costs, limited multiplexing capacity, technical skills requirement, and the requirement for specialized infrastructure. Furthermore, owing to their ubiquitous presence in the environment, a specific drawback in NTM diagnosis by PCR is that its detection in non-sterile samples does not equate infection. Hence, PCR assay tends to be a double-edged sword due to high sensitivity, making it challenging to discern NTM biological sample contamination from a true NTM infection. This assay is an open-system, usable on many PCR platforms but not yet automated to guarantee consistency in performance. Further development of this assay is needed, exploring the potential options for automation.

Though culture presently continues to be the gold standard, being especially required for antibiotic susceptibility testing, the present

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2021.103527.
* Corresponding author.
*E-mail addresses:* dranand@snmail.org (A.R. Anand), drjb@snmail.org (J. Biswas).

https://doi.org/10.1016/j.ebiom.2021.103552

one-step multiplex assay is a welcome addition to the promising pipeline of molecular diagnostics for diagnosis of mycobacterial infections. The assay has the potential to be adapted to a scalable, quick and easy point of care automated system and warrants further larger trials, particularly in areas where TB and various types of NTM disease are both prevalent.

## Declaration of Competing Interest

Appakkudal R Anand and Jyotirmay Biswas have no competing interests.

## References

[1] Machado D, Couto I, Viveiros M. Advances in the molecular diagnosis of tuberculosis: from probes to genomes. Infect Genet Evol 2019;72:93–112.
[2] Somoskovi A, Mester J, Hale YM, Parsons LM, Salfinger M. Laboratory diagnosis of nontuberculous mycobacteria. Clin Chest Med 2002;23(3):585–97.
[3] Pennington KM, Vu A, Challener D, Rivera CG, Shweta FN, Zeuli JD, Temesgen Z. Approach to the diagnosis and treatment of non-tuberculous mycobacterial disease. J Clin Tuberc Other Mycobact Dis 2021;24:100244.
[4] World Health Organization. Global tuberculosis report 2013. World Health Organization; 2013.
[5] Sarro YDS, et al. Development and clinical evaluation of a new multiplex PCR assay for a simultaneous diagnosis of tuberculous and nontuberculous mycobacteria. EBioMedicine 2021. doi: 10.1016/j.ebiom.2021.103527.
[6] Kim SH, Shin JH. Identification of nontuberculous mycobacteria using multilocous sequence analysis of 16S rRNA, hsp65, and rpoB. J Clin Lab Anal 2018;32(1):e22184.
[7] Peixoto AD, Montenegro LM, Lima AS, Melo FL, Barbosa WL, Neves MM, et al. Identification of nontuberculous mycobacteria species by multiplex real-time PCR with high-resolution melting. Rev Soc Bras Med Trop 2020;53:e20200211.
[8] Rocchetti TT, Silbert S, Gostnell A, Kubasek C, Widen R. Validation of a multiplex real-time PCR assay for detection of mycobacterium spp., mycobacterium tuberculosis complex, and mycobacterium avium complex directly from clinical samples by use of the BD Max open system. J Clin Microbiol 2016;54(6):1644–7.
[9] Sevilla IA, Molina E, Elguezabal N, Pérez V, Garrido JM, Juste RA. Detection of mycobacteria, mycobacterium avium subspecies, and mycobacterium tuberculosis complex by a novel tetraplex real-time PCR assay. J Clin Microbiol 2015;53(3):930–40.
[10] Sharma S, Latawa R, Wanchu A, Verma I. Differential diagnosis of disseminated mycobacterium avium and mycobacterium tuberculosis infection in HIV patients using duplex PCR. Future Microbiol 2021;16(3):135–42.