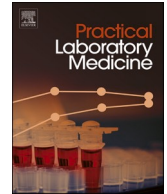




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Research Article

Direct identification of *Mycobacterium* species from liquid medium (MGIT) using FastPrep-2 bead beating system and MALDI-TOF mass spectrometry technology: a comparison with solid media and PCR-based method

Ali M. Bazzi^{a,*}, Abdulaziz A. Sunki^a, Mohab J. Hamdi^a, Abdullah O. Khaldi^a, Jaffar A. Al-Tawfiq^{b,c,d,**}

^a Microbiology Laboratory, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia

^b Infectious Disease Unit, Specialty Internal Medicine, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia

^c Division of Infectious Diseases, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

^d Division of Infectious Diseases, Department of Medicine Johns Hopkins University, Baltimore, MD, USA

A B S T R A C T

Background: Mycobacterial infections present significant global health challenges. Traditional diagnostic methods are inadequate for the identification of *Mycobacterium* species, highlighting the need for more efficient techniques. Matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) technology offers potential advantages in rapid and accurate pathogen identification.

Objective: This study evaluates the accuracy and precision of the MALDI-TOF using the FastPrep-2 bead beating system and VITEK MS version 3.2 for identifying *Mycobacterium* species directly from *Mycobacteria* Growth Indicator Tube (MGIT), liquid medium, compared to MALDI-TOF (VITEK MS) based on the traditional solid medium (Lowenstein–Jensen). We also compared the result of the MALDI-TOF from MGIT to *M. tuberculosis* results by PCR-based method.

Methods: The study included 16 *mycobacterium tuberculosis* (MTB) and 37 *nontuberculous mycobacteria* (NTM). Isolates were grown in LJ solid medium and MGIT liquid medium, and lysed using the FastPrep-2 bead beating system. Identification was performed using VITEK MS version 3.2 from liquid.

Results: NTM comprised 70 % (37/53) of the total isolates evaluated. **Of these, 92 % (34/37) were successfully identified using VITEK MS from MGIT liquid medium.** Overall, the method achieved 88.6 % accuracy for identifying *Mycobacterium* species from liquid medium, compared to 96.2 % from solid medium. The agreement between both methods was moderate (Kappa = 0.470, $p < 0.001$). MTB isolates were identified with 100 % accuracy, and the approach demonstrated excellent reproducibility with 100 % intra-assay and inter-day consistency.

Conclusion: Using VITEK MS version 3.2 to directly identify MTB and NTM from MGIT liquid medium provides a rapid, cost-effective, and reliable method for identifying *Mycobacterium* species. Further optimization and database expansion are recommended to enhance accuracy for rare and less common mycobacterial species.

1. Introduction

Mycobacterial infections caused by *Mycobacterium tuberculosis* (MTB) present significant global health challenges owing to their complex nature [1]. Despite advancements in diagnostic tools, tuberculosis (TB) remains a leading cause of mortality worldwide [2].

* Corresponding author.

** Corresponding author. Infectious Disease Unit, Specialty Internal Medicine, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia

E-mail addresses: ali.bazzi@JHAH.com, Bazziamh@gmail.com (A.M. Bazzi).

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The problem is further compounded by the rise in multidrug-resistant strains, particularly in immunocompromised populations suffering from HIV/AIDs [3]. Consequently, there has been an increasing prevalence of non-tuberculous mycobacteria (NTM) infections both in developing and developed regions. In North America and Australia, the annual prevalence of non-tuberculous mycobacterial (NTM) disease ranges from 3.2 to 9.8 cases per 100,000 people [4]. In the USA, the prevalence of NTM-positive cultures is between 1.4 and 6.6 per 100,000 individuals [5]. Meanwhile, data from the UK show an increase in the incidence of NTM-positive cultures from 4 to 6.1 per 100,000 people between 2007 and 2012 [6]. Additionally, a study in Canada reported a significant rise in the prevalence of NTM pulmonary disease (NTM-PD), from 29.3 cases per 100,000 individuals between 1998 and 2002 to 41.3 cases per 100,000 individuals from 2006 to 2010 [7].

Several factors contribute to the rising prevalence of non-tuberculous mycobacterial (NTM) disease. Genetic mutations in NTM have led to strains with enhanced virulence, while environmental and climate changes driven by expanding human-made infrastructure also play a role [8]. Additionally, alterations in host immunity due to longer life expectancy and a higher number of immunocompromised individuals contribute to increased susceptibility [8,9]. The rising incidence of chronic lung diseases further exacerbates the situation. In high-income countries, the decline in tuberculosis burden has resulted in reduced herd immunity, making populations more vulnerable to NTM infections [10]. The widespread availability of CT scanning and advanced laboratory facilities has improved the diagnosis of NTM, and increased awareness among healthcare professionals about the disease has led to more frequent identification [8,9]. This growing burden highlights the need for developing an accurate procedure for the identification of mycobacterial species to inform targeted treatment regimens and prevent inappropriate use of broad-spectrum antimicrobials.

Traditionally used diagnostic methods are labour-intensive and slow, requiring weeks for definitive results [3]. The most effective diagnostic test is gene sequencing, but it has a high cost and technical challenges, which require highly trained personnel [11]. These issues have created a gap in the timely diagnosis of NTM, which has a diverse resistance pattern. Therefore, there is a need to develop reliable, rapid and accessible diagnostic methods that can be implemented in clinical laboratories for accurate diagnosis. One such method is Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This method offers unique advantages such as cost-effectiveness, speed, and reduction in labour, enabling it for pathogen identification at the species level [12]. Despite growing interest, the application of MALDI-TOF MS in mycobacteriology remains less explored compared to its use for other bacterial groups. This could be associated with the complex cell wall structure of mycobacteria and the biosafety risks. Contrarily, with the recent advancements in the form of Bruker MALDI-TOF MS method, it has become possible to disrupt the mycobacterial cell wall and apply MALDI-TOF MS to diagnose these pathogens [3].

The potential of MALDI-TOF MS in the direct identification of mycobacteria from liquid media offers an opportunity to overcome the limitations of current diagnostic techniques [13]. Liquid media-based identification has been shown to improve turnaround times and potentially reduce contamination risks, particularly in high-burden clinical settings [13]. The main challenge lies not only in integrating MALDI-TOF MS to identify mycobacteria species but also in ensuring clinically reliable results.

The mechanical disruption of mycobacterial cells is increased using the FastPrep-2 bead beating system [14]. In this system, there are small glass beads agitated at high speeds to lyse bacterial cells. This facilitates efficient protein extraction, which is required for MALDI-TOF MS identification. The bead beating method shortens lysis time, reduce cross-contamination risk and enhance reproducibility [15]. The FastPrep-2 system is reliable to prepare challenging mycobacterial samples for downstream mass spectrometry analysis. To our knowledge, this is one of the few studies evaluating the FastPrep-2 bead beating system with VITEK MS version 3.2 directly from MGIT broth [16]. This approach may support TB elimination strategies by differentiating MTB from NTM more efficiently and at lower cost. Therefore, the present study aimed to address this gap in the literature by examining the accuracy and precision of the Vitek MS version 3.2 in the accurate identification of Mycobacterium species directly from liquid media. In this research, MALDI-TOF MS is used for rapid and accurate identification of both MTB and NTM species. It is hypothesized that this method would significantly improve patient outcomes by improving earlier identification and more targeted interventions. Furthermore, this study will contribute to the growing body of literature supporting the integration of advanced techniques in microbiological diagnosis, in the context of rising antimicrobial resistance.

2. Materials and methods

2.1. Study design

This prospective observational study analyzed a total of 53 mycobacterial isolates collected over a five-year period (2017–2022). The isolates included 16 *Mycobacterium tuberculosis* (MTB) samples confirmed by the Xpert MTB/RIF PCR test, and 37 nontuberculous mycobacteria (NTM) samples representing 13 different species. Among the NTM, 20 were proficiency testing (PT) samples obtained from the College of American Pathologists (CAP), which are pre-characterized isolates with undisclosed identities used for external quality assessment. These survey samples mimic clinical isolates and are used to assess laboratory performance. The remaining 17 NTM isolates were derived from patient clinical samples collected across various hospital wards (e.g., infectious diseases, pulmonology, and ICU). NTM isolates were previously identified at Mayo Clinic using either 16S rRNA gene sequencing or MALDI-TOF MS following standard protocol [3,11]. 16S rRNA sequencing was conducted using universal bacterial primers targeting the conserved 16S rDNA region and aligned with database reference sequences for species-level identification [11]. Proficiency testing isolates from CAP were pre-characterized but blinded to the testing laboratory. All the isolates were cultured under standardized conditions using both Lowenstein-Jensen (LJ) solid medium and Mycobacteria Growth Indicator Tube (MGIT) liquid medium. The study aimed to evaluate the performance of VITEK MS version 3.2 in the direct identification of these mycobacterial species from liquid media, specifically MGIT, using FastPrep-2 Bead Beating System. Additionally, the study compared the identification accuracy across different methods,

highlighting the robustness of MALDI-TOF MS in reducing the time required for identification by bypassing the sub-culturing step.

2.2. Sample preparation

A formal sample size calculation was not performed. The study included all available and eligible clinical and proficiency testing isolates collected during the study period. The samples from MGIT broth were centrifuged at a high speed. This centrifugation step was optimized at 13,000 g for 2 min to ensure the effective concentration of bacterial cells, which is essential for accurate identification in mass spectrometry. The supernatant was carefully discarded to remove any residual media components in the post-centrifuge phase to avoid its interference with the mass spectrometric analysis [17]. The remaining pellet was washed thoroughly with 500 mL phosphate buffer (PBS), ensuring the removal of non-cellular debris. The pellet was centrifuged again. For each sample, 1 mL of MGIT broth was used as input volume for MALDI-TOF MS processing. For inactivation, each pellet was incubated at 95 °C for 60 min, cooled to room temperature, and then 700 µL of absolute ethanol was added, and transferred to 1.5 mL Eppendorf tube with 200 µL of 0.5 mm glass beads from BIOMERIEUX were used during the lysis step. The samples were lysed in approximately 5 s using the FastPrep-2 bead beating system at 4000 cycles/min, the bacterial suspensions were transferred to another 2 mL Eppendorf tube, centrifuged for 1 min at 13,000 rpm to remove the supernatant. Finally, the pellets were treated with 10 µL 70 % formic acid, vortexed for 5 s, then treated with 10 µL 100 % acetonitrile, vortexed for 5 s, and centrifuged at 13,000 rpm for 1 min an aliquot of 1 µL from each sample was applied directly onto the VITEK MS target plate, air dried, treated with 1 µL VITEK MS-CHCA matrix solution, and analyzed using VITEK MS version 3.2. All procedures involving the handling of live mycobacteria prior to heat inactivation were conducted in a Biosafety Level 2 (BSL-2) laboratory equipped with negative air pressure and appropriate biosafety cabinets, ensuring safe containment and preventing exposure.

The FastPrep-2 system (MP Biomedicals) is a mechanical homogenizer optimized for rapid cell lysis using bead beating. Its advantages include reduced lysis time (5 s), minimal cross-contamination, and improved protein yield compared to vortexing or sonication. The bead beating technology employed in this study offers a significant advantage of enabling the direct application of the bacterial pellet from liquid medium for identification by saving 15–30 min of lysing work compared to traditional homogenization methods, such as vortexing, and sonication, and maintaining high yields of intact proteins, thereby eliminating the risk of cross-contamination and time-consuming clean-up associated with manual lysis methods. [18]. The omission of conventional subculturing step onto solid media reduces the overall turnaround time for identification and mitigates the risks of contamination and overgrowth by fast-growing bacteria.

2.3. Identification methods

Three different identification methods were used to evaluate the accuracy and efficiency of the VITEK MS version 3.2 system. In the first method, the mycobacterial species were identified from LJ solid media with the help of the VITEK MS version 3.2 system. In this method, the standard method for mass spectrometry was compared with an innovative liquid media approach. In the second method, mycobacterial species were identified directly from the MGIT liquid medium using the same VITEK MS version 3.2 system. This method aimed to validate the efficacy of liquid media as a reliable source for direct identification, bypassing the need for solid media subculturing. The third method employed a PCR-based identification using the Xpert MTB/RIF assay (GeneXpert system) which serves as the molecular gold standard for the detection of *Mycobacterium tuberculosis* complex (MTBC) [19]. The Xpert MTB/RIF assay is highly specific and sensitive nucleic acid amplification technology allowed for an independent comparison of MALDI-TOF MS performance, particularly for MTB identification. For comparison, the results of NTM identification by MALDI-TOF MS were evaluated against reference identifications provided by CAP or Mayo Clinic using sequencing. The comparative analysis across these methods provided crucial insights into the strengths and limitations of MALDI-TOF MS in clinical settings and its potential integration into routine diagnostic workflows.

2.4. Validation procedures

The precision and reproducibility of VITEK MS version 3.2 system in identifying mycobacterial species were rigorously evaluated through a series of validation procedures. Intra-assay variability was assessed by performing three consecutive identifications of the same species within a single day. This allowed for the examination of consistency in identification under controlled conditions and within the same experimental run. Furthermore, inter-day variability was tested by repeating the identification procedures on three different days, using the same five species of mycobacteria to assess the robustness of the VITEK MS version 3.2 system over time. These five species, selected for their clinical significance and diverse profiles, provided a comprehensive representation of the variability inherent in mycobacterial identification. The analysis of variability across both intra-assay and inter-day runs ensured that the system's precision was maintained, further validating the reliability of the VITEK MS version 3.2 system for routine clinical diagnostics. The inclusion of multiple species and repeated measurements reflects the stringent criteria necessary for establishing the validity of this identification method.

3. Results

3.1. Identification accuracy

The study included a total of 53 mycobacterial isolates, including 16 *Mycobacterium tuberculosis* (MTB) and 37 Nontuberculous mycobacteria (NTM) representing 13 different species. All MTB samples tested negative for rpoB gene mutations using the Xpert MTB/RIF assay, indicating rifampicin sensitivity. Table 1 shows the number of MTB that were correctly identified by each of the two methods and the accuracy compared to the gold standard PCR method.

For the 37 NTM, 2 (5.4 %) were not identified by MALDI-TOF MS (Vitek MS) from both liquid which is similar to the standard method of solid media. The remaining 35 samples, 31 (83.7 %) were identified by MALDI-TOF MS (Vitek MS) from liquid media similar to that from the solid media. The 4 samples that were not identified from the liquid media are 2 *Mycobacterium avium* (MAC), 1 *Mycobacterium chelonae*, and 1 *Mycobacterium szulgai*. Table 2 shows the number of NTM that were correctly identified by each of the two methods and the accuracy of each method compared to CAP survey samples results and MAYO clinic referral lab results.

Mycobacterium chelonae showed a slight discrepancy, with only 2 of 3 samples correctly identified from liquid medium, whereas the standard method of solid medium identification was 100 % accurate. *Mycobacterium szulgai* could not be identified from a liquid medium but was successfully identified from a solid medium, indicating potential limitations in the sample preparation or detection sensitivity for this species in a liquid medium. *Mycobacterium riyadhense* and *Mycobacterium conceptionense* could not be identified by the VITEK MS system from either solid or liquid medium, likely due to the absence of these species in the system's database. This underscores the need for ongoing database updates to include rarer NTM species.

The overall accuracy of identifying *Mycobacterium* species (MTB and NTM) using the VITEK MS version 3.2 system directly from a liquid medium was 88.6 % (47/53). The standard method is identification through solid medium and as per this standard the accuracy recorded was 96.2 %. The current accuracy identified through liquid medium was found to be closely aligning with the standard, demonstrating that MALDI-TOF MS is an effective method for direct identification from liquid medium. All samples of *Mycobacterium tuberculosis* complex were correctly identified by the VITEK MS version 3.2 system from liquid mediums, which is consistent the standard methods of solid medium and the Xpert MTB/RIF PCR assay. The identification of NTM species from liquid medium identification demonstrated an accuracy of 92 %, which is lower than the accuracy of standard method of solid medium reported to be 100 %.

3.2. Reproducibility and precision

The precision of the method was evaluated for intra-assay and inter-day variability across five species: *Mycobacterium tuberculosis* complex, *Mycobacterium avium* complex (MAC), *Mycobacterium fortuitum*, *Mycobacterium abscessus*, and *Mycobacterium chelonae*. Both intra-assay and inter-day reproducibility were 100 %, demonstrating that the VITEK MS version 3.2 system, when applied to a liquid medium yields consistent and reliable results.

Cohen's Kappa statistic was calculated to assess the agreement between identification results from solid and liquid media using SPSS version 26. A p-value < 0.05 was considered statistically significant. The Kappa value was 0.470 (p < 0.001), indicating moderate agreement between the two methods (Table 3). This result reflects a reasonable level of consistency, though some variability exists, particularly in the identification of non-tuberculous *Mycobacterium* (NTM) species from liquid medium.

4. Discussion

Our findings confirm that VITEK MS version 3.2 IVD system is a reliable method for identifying *Mycobacterium* species directly from the liquid medium. With an overall accuracy of 88.6 %, this approach demonstrates a significant advancement in diagnostic pathway compared to solid culture methods. These findings align with previous studies, Balada-Llasat et al. (2013), and Rodriguez-Temporal et al. (2014), reported similar identification accuracies (85–92 %) from liquid media using MALDI-TOF MS [13,20]. More recently, other studies have reinforced the feasibility of MALDI-TOF MS in liquid media for *mycobacteria*. Fernández-Esgueva et al. (2021) further demonstrated accurate identification directly from MGIT cultures, although they emphasized that results varied by species and the quality of protein extraction [21]. Topić Popović et al. (2023) improved sensitivity through enhanced lysis and extraction protocols, underscoring the importance of sample preparation methods [22].

Our study revealed 100 % identification of *Mycobacterium tuberculosis tuberculosis* (MTB), consistent with previous literature emphasizing the high sensitivity and specificity of MALDI-TOF MS for MTB [19]. However, the comparison of solid and liquid media revealed a moderate agreement (Cohen's Kappa = 0.470, p < 0.001), due to lower performance for some non-tuberculous mycobacteria (NTM). For instance, *M. chelonae* and *M. szulgai* had reduced identification rates in liquid media, which may relate to protein yield variability or extraction efficiency from broth cultures. The inability to identify the latter two species reflects the limitations of the VITEK MS

Table 1
Comparative accuracy for *Mycobacterium tuberculosis* (MTB) identification.

MTB	VITEK MS Solid Medium	VITEK MS Liquid Medium	Xpert MTB/RIF PCR assay Reference method
Samples correctly identified	16/16	16/16	16
Accuracy of test methods %	100	100	–

Table 2

Comparative Accuracy for NTM Identification using VITEK MS version 3.2 for identifying *Non-Tuberculous Mycobacterium* (NTM) directly from liquid medium compared to the traditional solid medium method.

NTM Species	Samples Tested	Number of isolates Correctly Identified by VITEK MS from Solid Medium	Number of isolates Correctly Identified by VITEK MS from Liquid Medium
<i>Mycobacterium avium</i> complex (MAC)	11	11	9
<i>Mycobacterium fortuitum</i>	5	5	5
<i>Mycobacterium abscessus</i>	4	4	4
<i>Mycobacterium kansasii</i>	4	4	4
<i>Mycobacterium chelonae</i>	3	3	2
<i>Mycobacterium mucogenicum</i>	2	2	2
<i>Mycobacterium goodii</i>	1	1	1
<i>Mycobacterium xenopi</i>	1	1	1
<i>Mycobacterium scrofulaceum</i>	1	1	1
<i>Mycobacterium marinum</i>	2	2	2
<i>Mycobacterium szulgai</i>	1	1	0
<i>Mycobacterium riyadhense</i>	1	0	0
<i>Mycobacterium conceptionense</i>	1	0	0
Total identified		35	31
Overall Accuracy of test method %^a		94.5	83.7
Accuracy of test method based on VITEK MS database %^b		100	88.5

^a Accuracy to total NTM samples.

^b Accuracy after excluding *M. riyadhense* and *M. conceptionense*.

Table 3

Cohen's Kappa agreement between VITEK MS identification from solid medium and liquid medium.

	Value	Asymp. Std. Error	Approx. T	Approx. Sig.
Kappa	0.47	0.216	4.035	<0.001
N. of Valid Cases	53			

database, a concern also noted by El Khechine et al. (2011), who observed frequent misidentifications when rare species were absent from spectral libraries [23].

5. Strengths and clinical significance

A key strength of our study is the method's reproducibility, with 100 % intra-assay and inter-day consistency, which affirms its potential as a standardized tool in clinical laboratories. Griffith et al. (2020) also reported high reproducibility, emphasizing that MALDI-TOF MS can produce reliable and repeatable results even across different laboratory settings [24]. This reproducibility is particularly important given the increasing incidence of nontuberculous mycobacteria (NTM) infections globally. As NTM species are becoming more prevalent, particularly in immunocompromised patients, rapid and accurate identification of NTM can significantly improve clinical outcomes through earlier initiation of targeted therapy [25]. Moreover, our study contributes to the literature by demonstrating the ability of MALDI-TOF MS to identify NTM species directly from a liquid medium, with a slightly reduced accuracy (92 %) compared to solid media (100 %). This mirrors the findings by Saleeb et al. (2011), who observed similar trends, attributing the reduced accuracy to the challenges associated with protein extraction from a liquid medium, which may impact the mass spectra quality [26]. Our study contributes by validating a high-throughput protocol that bypasses subculturing, shortening turnaround times and supporting targeted therapy decisions.

6. Challenges and limitations

Identification of MAC by MALDI-TOF MS remains challenging due to protein profile overlap among subspecies. Rare species like *M. riyadhense* and *M. conceptionense* could not be identified due to database gaps in VITEK MS. A formal sample size calculation was not performed, which may limit the generalisability of our findings. However, all available clinical and proficiency testing isolates collected over the five-year period were included to maximise data robustness. It is consistent with previous findings by Fernandez-Esgueva (2021), who noted that NTM species with complex lipid compositions or fewer protein biomarkers often exhibit weaker MALDI-TOF signals [21]. Body et al. (2018) found that database incompleteness led to misidentifications or failures in species differentiation [27]. Expanding spectral libraries to include rare and emerging NTM species is essential for the broader clinical adoption of this technique [28].

7. Clinical implications and future directions

The clinical implications of our findings are significant, particularly in the global health context of rising NTM infections [29]. The ability of MALDI-TOF MS to provide accurate identification directly from a liquid medium can expedite clinical decision-making, reducing the time from sample collection to diagnosis. This is particularly relevant in settings where tuberculosis (TB) and NTM coinfections are prevalent, and distinguishing between these pathogens is critical for initiating appropriate treatment. However, as demonstrated by our results and others [24], database refinement and method optimization are needed to enhance the identification of rare or less common species.

Future studies should focus on addressing the limitations observed in NTM species identification from liquid media. Techniques to improve protein extraction and spectra quality from liquid cultures should be explored, as suggested by Saleeb et al. (2011) [26]. Moreover, advancements in machine learning algorithms for spectra interpretation could help circumvent database limitations by improving the discriminatory power of MALDI-TOF MS for rare species. Integrating MALDI-TOF MS with molecular techniques, such as PCR, could also provide a complementary approach, allowing for more comprehensive and accurate mycobacterial identification.

8. Conclusion

The present study has revealed that VITEK MS version 3.2 has been a highly accurate and precise method for the identification of Mycobacterium species directly from a liquid medium utilizing MALDI-TOF MS. The results demonstrate that the VITEK MS version 3.2 system achieved an overall accuracy of 88 % in identifying mycobacterial species from the liquid medium. The main advantage of this approach is the reduced time and cost of mycobacterial identification as this approach bypassed the need for subculturing on solid media. Furthermore, the VITEK MS version 3.2 system has achieved an exceptional accuracy of 100 % for identifying *Mycobacterium tuberculosis* as compared to the PCR method. Additionally, the precision of results also demonstrates the reliability of the VITEK MS version 3.2 system in both intra-assay and inter-day assessments. Moreover, direct identification of mycobacterial species from a liquid medium using MALDI-TOF MS can streamline workflows, enhance turnaround times, and potentially improve patient outcomes by facilitating quicker and more accurate pathogen identification. This study supports the adoption of high-speed preparation methods and MALDI-TOF MS technology as an effective tool in the diagnostic arsenal against tuberculosis and non-tuberculous mycobacterial infections, ultimately contributing to more efficient and cost-effective patient care.

CRedit authorship contribution statement

Ali M. Bazzi: Writing – original draft, Methodology, Formal analysis, Data curation. **Abdulaziz A. Sunki:** Methodology, Data curation. **Mohab J. Hamdi:** Methodology, Data curation. **Abdullah O. Khaldi:** Methodology, Data curation. **Jaffar A. Al-Tawfiq:** Writing – review & editing, Validation, Supervision.

Ethical

The study was approved by the institution IRB.

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.

Data availability

The data that has been used is confidential.

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