

REVIEW

Antigens of *Mycobacterium tuberculosis* Used in the Diagnosis of Tuberculosis: A Literature Review

Antígenos de *Mycobacterium tuberculosis* utilizados en el diagnóstico de tuberculosis. Revisión bibliográfica

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ABSTRACT

Introduction: tuberculosis is a chronic infectious disease primarily caused by the bacterium *Mycobacterium tuberculosis*. It most commonly affects the lungs (pulmonary tuberculosis), although it can also involve other organs (extrapulmonary tuberculosis).

Objective: to identify specific *M. tuberculosis* antigens used in tuberculosis diagnosis, as well as the various detection methods, evaluating their effectiveness and applicability in different clinical contexts.

Method: a comprehensive search of studies published between January 2014 and May 2024 was conducted in biomedical databases, using Boolean operators to locate relevant articles. Inclusion and exclusion criteria were applied to select original studies on antigens and diagnostic methods.

Results: the findings of this review highlight the relevance of various *M. tuberculosis* antigens, such as ESAT6, CFP10, MPT64, LAM, and Ag85B, in tuberculosis diagnosis. Detection methods include immunoenzymatic techniques and nucleic acid amplification, each with variations in sensitivity and specificity. The selection of the most appropriate diagnostic approach depends on the clinical and epidemiological context, emphasizing the need for tailored strategies.

Conclusions: the use of specific *M. tuberculosis* antigens is essential for early detection and management of tuberculosis. Emerging technologies offer more precise and accessible alternatives, with the potential to enhance diagnosis in primary care settings and high-risk populations, contributing to global disease control and improved public health outcomes.

Keywords: *Mycobacterium Tuberculosis*; Antigens; Diagnosis; Tuberculosis.

RESUMEN

Introducción: la tuberculosis es una enfermedad infecciosa crónica causada principalmente por la bacteria *Mycobacterium tuberculosis*. Afecta con mayor frecuencia a los pulmones (tuberculosis pulmonar), aunque también puede comprometer otros órganos (tuberculosis extrapulmonar).

Objetivo: identificar los antígenos específicos de *M. tuberculosis* utilizados en el diagnóstico de tuberculosis, así como los diversos métodos de detección, evaluando su efectividad y aplicabilidad en diferentes contextos clínicos.

Método: se realizó una búsqueda exhaustiva de estudios publicados entre enero de 2014 y mayo de 2024 en bases de datos biomédicas, utilizando operadores booleanos para localizar artículos relevantes. Se aplicaron criterios de inclusión y exclusión para seleccionar estudios originales sobre antígenos y métodos diagnósticos.

Resultados: los hallazgos de esta revisión destacan la relevancia de diversos antígenos de *M. tuberculosis*,

como ESAT6, CFP10, MPT64, LAM y Ag85B, en el diagnóstico de la tuberculosis. Los métodos empleados para su detección incluyen técnicas inmunoenzimáticas y amplificación de ácidos nucleicos, cada uno con variaciones en sensibilidad y especificidad. La selección del enfoque diagnóstico más adecuado depende del contexto clínico y epidemiológico, evidenciando la necesidad de estrategias adaptadas a cada escenario.

Conclusiones: el uso de antígenos específicos de *M. tuberculosis* es clave para la detección temprana y el manejo de la tuberculosis. Las tecnologías emergentes ofrecen alternativas más precisas y accesibles, con el potencial de mejorar el diagnóstico en atención primaria y poblaciones de alto riesgo, contribuyendo al control global de la enfermedad y a mejores resultados en salud pública.

Palabras clave: *Mycobacterium Tuberculosis*; Antígenos; Diagnóstico; Tuberculosis.

INTRODUCTION

Mycobacterium tuberculosis, the microorganism responsible for tuberculosis, remains a global health challenge.⁽¹⁾ In 2022, tuberculosis caused 1,3 million deaths, making it the second deadliest infectious disease after COVID-19, surpassing HIV/AIDS. The prevalence of pulmonary tuberculosis is high in Latin America, with Peru standing out for having a significantly high prevalence in 2023. Two studies conducted in Peru reported prevalences of 5,60 % (with 611 cases) and 0,97 % (with 3734 cases), followed by Ecuador with 918 patients and a prevalence of 1,3 %, and Brazil with a prevalence of 43 % per 100 000 patients.⁽²⁾

Early and accurate diagnosis of tuberculosis is essential for controlling the disease, as it facilitates rapid treatment implementation and helps prevent its spread. However, current diagnostic methods face significant limitations in sensitivity, specificity, and accessibility, complicating both early detection and proper case management of tuberculosis.⁽³⁾

A promising strategy to improve tuberculosis diagnosis is the identification and use of specific *Mycobacterium tuberculosis* antigens. These new testing methods can enhance accuracy, optimize operational characteristics, and improve end-user access to tuberculosis infection detection tests.⁽⁴⁾ *M. tuberculosis* antigens are proteins or protein fragments produced by the bacterium that can be recognized by the host's immune system. Recently, several biomarkers have been of interest in developing rapid and reliable methods for tuberculosis detection. Some of the most studied antigens include CFP-10, ESAT-6, Ag85A, Ag85B, CFP-7, and PPE18.⁽⁵⁾

In this context, these antigens have become key components in developing new tuberculosis (TB) diagnostic strategies. These antigens play an essential role in various diagnostic tests, such as the Tuberculin Skin Test (PPD), the QuantiFERON-TB Gold (QFT-G) assay, and other interferon-gamma (IFN- γ) detection-based tests.⁽⁶⁾

The host response to *M. tuberculosis* involves both cellular and humoral immunity. Cellular immunity, mediated by specific T cells, is crucial for tuberculosis defense, and the evaluation of these cells' response through tests like QFT-G reflects this immunological interaction. On the other hand, humoral immunity, which involves the production of specific antibodies against *M. tuberculosis* antigens, has also been studied as a potential biomarker for tuberculosis diagnosis, although its clinical application is still under investigation.⁽⁷⁾

The objective of this review is to critically analyze the role of *M. tuberculosis* antigens in tuberculosis detection. To achieve this, current scientific evidence will be examined, evaluating its clinical utility, limitations, and challenges in diagnostic practice. Additionally, the latest advances in antigen research and their potential to optimize diagnostic strategies, especially in complex epidemiological contexts, will be discussed.

METHOD

Design: literature review.

Definition of Search Terms

An exhaustive search of terms and keywords reflecting the research topics of interest was conducted.

Data Collection

An information search was carried out between January 2014 and May 2024. The most relevant biomedical databases were consulted, including PubMed, Elsevier, Scielo, Redalyc, and Google Scholar.

Searches were conducted in both Spanish and English. The Spanish keywords were: antígenos, diagnóstico, tuberculosis, *M. tuberculosis*. In English, the keywords used were: antigen, diagnosis, tuberculosis, *M. tuberculosis*. Boolean operators such as "AND," "OR," and "NOT" in English, and "Y," "O," and "NO" in Spanish were applied. Keywords were combined with these operators to locate scientific articles relevant to the study's objective.

Inclusion Criteria

Research studies on specific *M. tuberculosis* antigens within the framework of tuberculosis diagnosis.

Studies published between January 2014 and May 2024.
Original articles on *M. tuberculosis* antigens used in tuberculosis diagnosis.

Exclusion Criteria

- Literature reviews, systematic reviews, and other types of reviews.
- Articles without publication date information or author names.
- Articles presenting duplicate data or results previously published in other documents.

Analysis Procedures and Data Processing Techniques

To carry out this narrative literature review, bibliographic sources were selected and initially examined based on title, objective, and results. Subsequently, an analytical reading of the selected articles was performed. Data were then observed, compared, and interpreted. Finally, the research was drafted based on the analyzed studies.

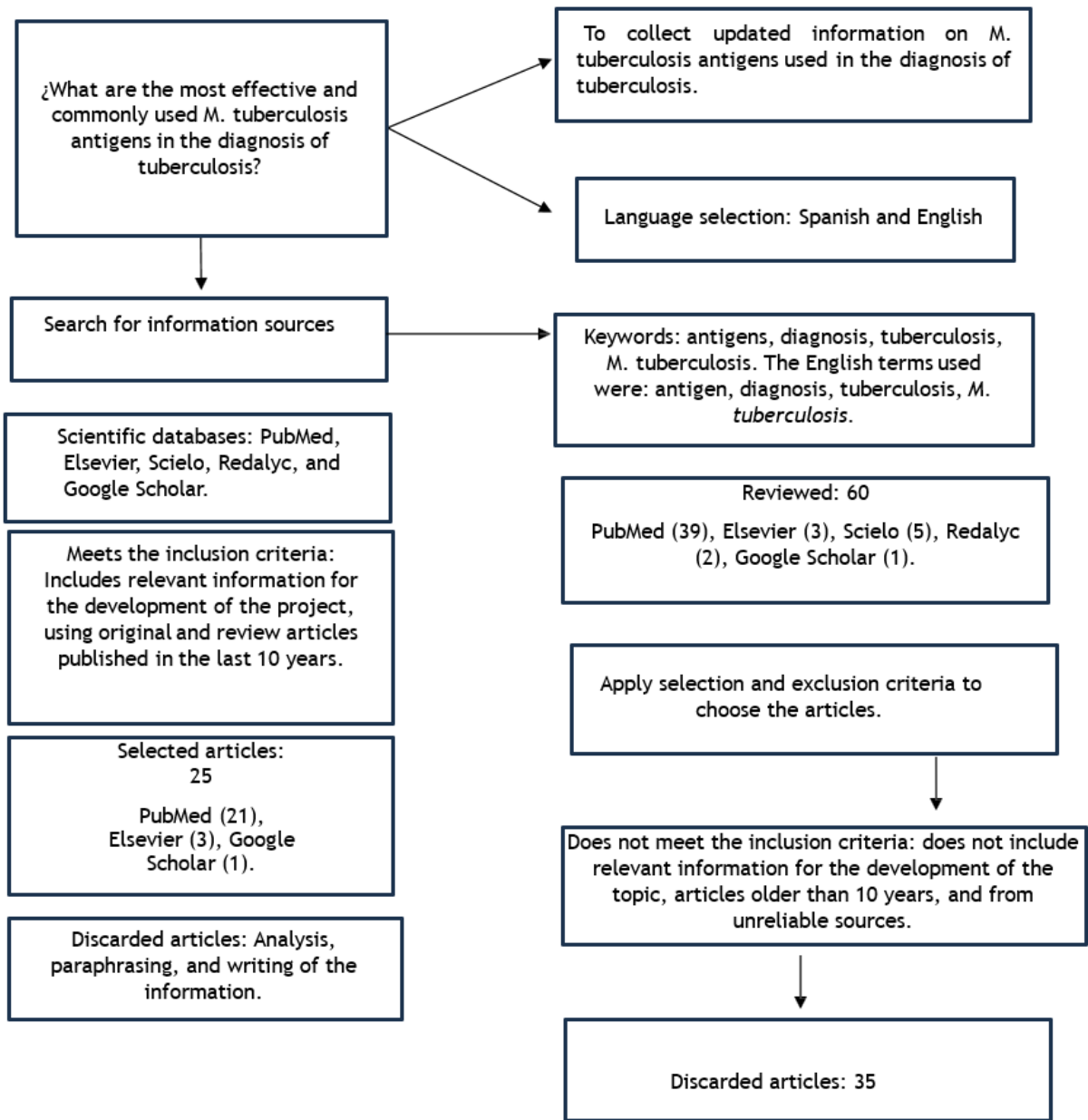


Figure 1. Flowchart of Scientific Article Selection for *M. tuberculosis* Antigens in Tuberculosis Diagnosis

RESULTS

In table 1, the antigens of *M. tuberculosis* used in the 25 studies analyzed for the diagnosis of tuberculosis are shown:

Table 1. Antigens of *M. tuberculosis* used in the diagnosis of tuberculosis

No.	Author	Year	Antigens Used
1	Xu et al. ⁽¹⁰⁾	2022	ESAT6, CFP10
2	Arora et al. ⁽¹¹⁾	2015	MPT64
3	Ashraf et al. ⁽¹²⁾	2014	Rv3803c, Rv2626c
4	Tan et al. ⁽¹³⁾	2017	ESAT-6 ,CFP-10, Rv3615c
5	Mao et al. ⁽¹⁴⁾	2021	CFP-10
6	Wang et al. ⁽¹⁵⁾	2023	ESAT-6,CFP-10
7	Broger et al. ⁽¹⁶⁾	2019	LAM, ESAT-6
8	Pope et al. ⁽¹⁷⁾	2018	MPT64
9	Sharma et al. ⁽¹⁸⁾	2019	MPT64, PstS1
10	Dahiya et al. ⁽¹⁹⁾	2020	CFP-10
11	Dahiya et al. ⁽²⁰⁾	2020	MPT64, CFP-10
12	Dirix et al. ⁽²¹⁾	2022	ESAT-6
13	Yan et al. ⁽²²⁾	2022	38KD, MPT32, MPT64, CFP10, Mtb81- EspC, LAM
14	Petrone et al. ⁽²³⁾	2018	IP-10
15	Luo et al. ⁽²⁴⁾	2017	Rv0310c, Rv1255c
16	Brock et al. ⁽²⁵⁾	2019	LAM
17	Xu et al. ⁽²⁶⁾	2021	Ag85B
18	Tang et al. ⁽²⁷⁾	2014	ESAT6, CFP10
19	Bethu et al. ⁽²⁸⁾	2023	HspX, MPT 64
20	Mahmood et al. ⁽²⁹⁾	2022	Rv3874, Rv3875
21	Dass et al. ⁽³⁰⁾	2023	MPT51, MPT64
22	Bjørgaas Helle et al. ⁽³¹⁾	2024	MPT64
23	You et al. ⁽³²⁾	2017	Rv0220, Rv2958c, Rv2994 , Rv3347c
24	Phunpae et al. ⁽³³⁾	2014	Ag85
25	Singh et al. ⁽³⁴⁾	2015	Ag85B

Table 2 summarizes studies on the sensitivity and specificity of immunoenzymatic tests for diagnosing tuberculosis. It highlights that ELISA and ELONA show variable performance. The antigens Rv0220, MPT64, Ag85, and Aptamer CE24 demonstrate high efficacy in active TB.

Table 2. Sensitivity and Specificity Results of Tests Based on Immunoenzymatic Techniques in the Diagnosis of Tuberculosis

Antigen(s)	Detection Method	Sensitivity	Specificity	Clinical Context
Rv3803c	ELISA	69,3 %	76,4 %	Acute TB / Latent TB
Rv2626c		77,1 %	85,1 %	
ESAT-6/CFP-10/ Rv3615c	ELISPOT	81,9 %	91,7 %	Active TB
38KD-MPT32- MPT64+CFP10- Mtb81-EspC+ LAM	ELISA	74 %	88,2 %	TBp
Rv0310c/Rv1255c	ELISA	82,5 %	71 %	Smear-positive/ TBp Smear-negative/TBp
HspX		76,9 %	71 %	
	ALISA	70 %	75 %	TBp
		50 %	73 %	TBe
MPT 64		70 %	75 %	TBp
		50 %	73 %	TBe
Rv3874/Rv3875	ELISA	53,3 %	98 %	TBp

MPT51	ELISA	70 %	86,3 %	TBe
MPT64		90 %	92 %	
Rv0220	ELISA	91,3 %	98,3 %	TBa
Rv2958c		85,9 %	91,7 %	
Rv2994		89,1 %	93,3 %	
Rv3347c		80,4 %	93,3 %	
Ag85	ELISA	89,6 %	94 %	TBa
ESAT6/CFP10	ELONA	Aptamer CE24: 100 %	Aptamer CE24: 94,1 %	TBp/TBe
		Aptamer CE15: 89,6 %	Aptamer CE15: 94,1 %	

Table 3 presents the sensitivity and specificity results of tests based on Nucleic Acid Amplification Techniques for the diagnosis of tuberculosis (TB). These tests allow the direct detection of the genetic material of *M. tuberculosis*, improving diagnostic accuracy compared to conventional methods.

Table 3. Sensitivity and specificity results of tests based on Nucleic Acid Amplification Techniques in the diagnosis of Tuberculosis				
Antigen(s)	Detection Method	Sensitivity	Specificity	Clinical Context
CFP-10	GNP-RT- I-PCR	83,7 %	93,5 %	TBp
		76,20 %	93,80 %	TBe
MPT64/ CFP-10	MB-AuNP-I-PCR	89,30 %	97,90 %	TBp
		78,10 %	98,30 %	TBe
MPT64/PstS1	RT- I-PCR	93,2 %	92,8 %	TBp
		77,9 %	91,3 %	TBe
Ag85B	I-PCR	83 %	90 %	TBp
		68,6 %	92 %	TBe
Ag85B	RPA	90 %	98 %	TBp

RT-I-PCR: Real-Time Immuno-Polymerase Chain Reaction, GNP-RT-I-PCR: RT-I-PCR based on gold nanoparticles
MB-AuNP-I-PCR: Immuno-PCR with functionalized gold particles coupled to detection antibodies, oligonucleotides, and magnetic beads, RPA: Recombinase Polymerase Amplification.

DISCUSSION

This review analyzes the various *Mycobacterium tuberculosis* antigens used in the diagnosis of tuberculosis, assessing their effectiveness in terms of sensitivity and specificity. To this end, 25 studies were examined, which utilized different antigens and detection methods, providing a comprehensive overview of the current state of diagnostic tests for this disease.

ESAT-6 and CFP-10 are antigens that have been extensively used in diagnostic tests such as QuantiFERON-TB Gold (QFT) and T-SPOT.TB. These antigens are known for their high specificity, as they are not present in BCG strains or in most non-tuberculous mycobacteria (NTM). This provides a significant advantage in settings where BCG vaccination is common or where NTM infections are prevalent. Despite their high specificity, the sensitivity of ESAT-6 and CFP-10 can be compromised in immunocompromised populations, such as HIV patients, due to a high frequency of disease with negative smear and high rates of extrapulmonary TB.⁽⁸⁾ The variability in immune response in these groups can reduce the effectiveness of these tests, leading to false-negative results. Furthermore, the ability of these antigens to differentiate between active tuberculosis and latent tuberculosis infection (LTBI) is limited, which has driven the search for other markers or antigen combinations that may improve this distinction.

A study by Peña *et al.*⁽⁹⁾ quantified the production of IFN- γ in response to the antigens CFP-10, ESAT-6, Rv2624c, Rv2626c, and Rv2628. The results showed that subjects with LTBI secreted significantly higher levels of IFN- γ against Rv2626c than healthy donors, allowing differentiation between LTBI and active TB.⁽¹⁰⁾ Latency antigens, such as HspX, Rv2623, and Rv2031c, have been of interest due to their potential to identify LTBI, a condition in which the bacillus remains in a non-replicative state but has the potential to reactivate. HspX, in particular, has been studied for its ability to detect infections in people exposed to the bacillus but without symptoms of active disease.⁽¹¹⁾

One of the main challenges with the use of latency antigens is their low sensitivity in diagnosing active TB. This is because these antigens are more expressed during the latent phase of infection, limiting their utility in diagnosing active cases. Additionally, the variability in immune response to these antigens can depend on the host's immune status, further complicating their application. Recently, it has been proposed that combining latency antigens with active-phase antigens may improve diagnostic accuracy for both LTBI and active TB. The combination of HspX with Ag85 showed promising results in initial studies, suggesting a viable path for the development of more robust tests.⁽¹²⁾

Rv3615c, along with ESAT-6 and CFP-10, has been explored as part of the ELISPOT test, which measures the response of Mtb-specific T cells. This antigen has proven particularly useful in detecting infections at early stages, before the clinical manifestation of disease. However, the addition of Rv3615c to ESAT-6 and CFP-10 does not always significantly improve the sensitivity of the test, suggesting that its use may not be necessary in all contexts. Moreover, the production and standardization of this antigen present technical challenges that may limit its applicability in low-resource settings.⁽¹³⁾

A study by Tan et al.⁽¹⁴⁾ evaluated the combination of Rv3615c with other latency and active-phase antigens, finding that this combination improved sensitivity in patients with extrapulmonary TB but not so much in pulmonary TB. This suggests that Rv3615c may have a more specific role in certain subgroups of patients.⁽¹⁵⁾ The Ag85 complex, consisting of the proteins Ag85A, Ag85B, and Ag85C, is one of the most studied antigens for the diagnosis of active TB. This antigen plays a crucial role in the synthesis of the Mtb cell wall and is highly immunogenic, making it a good candidate for diagnostic tests.^(16,17,18)

However, Ag85's ability to distinguish between active TB and LTBI is limited, reducing its utility in certain clinical contexts. Additionally, variability in the immune response to the Ag85 complex in different patient groups can influence diagnostic results, suggesting the need for further standardization of tests that use it. It is worth noting that although Ag85 shows high specificity, its sensitivity varies widely depending on the sample type (sputum, blood, etc.) and the patient's immune status. This reinforces the idea that Ag85 should be used in combination with other antigens to improve diagnostic accuracy.⁽¹⁹⁾

Despite the advancements, current techniques have limitations. The sensitivity of some antigens, such as Rv3803c (69,3 %) and Rv1255c (68,2 %), although useful, may not be sufficient in clinical settings where earlier and more accurate detection is required.⁽²⁰⁾

The Immuno-PCR (I-PCR) technique has proven to be a promising tool in tuberculosis diagnosis, especially in cases of pulmonary TB with negative smear and paucibacillary extrapulmonary TB, where other diagnostic methods fail. Compared to ELISA, I-PCR offers greater accuracy and speed, enabling early diagnosis and better monitoring of disease progression and response to anti-tuberculosis treatment. However, high background noise and the complexity of the protocol present significant challenges that need to be addressed. The implementation of liquid formats with nanoparticles could optimize this technique, reducing assay duration and improving its accuracy.⁽²¹⁾

The use of urinary biomarkers for diagnosing pulmonary tuberculosis has gained interest, especially with the extraction of transrenal DNA. However, the sensitivity and specificity of these biomarkers vary widely, depending on both the extraction method and the patient's immune status. Despite their potential, biomarkers like IP-10, though promising, lack specificity and might be more useful for treatment monitoring than for initial diagnosis. Mass spectrometry has shown potential in detecting metabolomic and proteomic biomarkers, although reliable markers that consistently predict treatment outcomes are still lacking.⁽²²⁾

In the future, tuberculosis diagnosis will benefit significantly from several emerging innovations. Biomarkers under development promise better identification of the progression from latent infection to clinical disease, as well as the prediction of reactivations and providing accurate endpoints for clinical trials. Advanced molecular technology is improving with new tests for detecting drug resistance, which will allow more effective identification of resistant strains. Additionally, advances in bioinformatics and systems biology are facilitating the validation of biomarkers and a deeper understanding of the immune response.⁽²³⁾

In summary, the review of M. tuberculosis antigens and their detection methods reveals a varied landscape with significant advances in immunoenzymatic and combined techniques. The integration of advanced methods and the combination of antigens offers considerable potential for improving diagnostic accuracy and tuberculosis management, though further studies are needed to validate these methods in diverse clinical settings and broader populations.

CONCLUSIONS

The antigens ESAT6, CFP10, MPT64, LAM, and Ag85B have proven to be essential in the diagnosis of tuberculosis, with variations in their sensitivity and specificity depending on the method used. Immunoenzymatic techniques and nucleic acid amplification methods have shown differences in performance, with ELISA for Rv0220 and Ag85B, as well as MB-AuNP-I-PCR in pulmonary tuberculosis, standing out. The variability in the results highlights the importance of selecting the most appropriate antigen and method according to the clinical context, suggesting that the combination of multiple approaches could improve diagnostic accuracy.

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The authors declare that there is no conflict of interest.

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