Eksakta : Berkala Ilmiah Bidang MIPA
VOLUME 26 NO 04 2025, pp 516-529
ISSN : Print 1411-3724 — Online 2549-7464
DOI : https://doi.org/10.24036/eksakta/vol26-iss04/626

Eksakta
Berkala Ilmiah Bidang MIPA
http://www.eksakta.ppj.unp.ac.id/index.php/eksakta

Review

Serological and Non-Sputum Biomarkers for Tuberculosis Diagnosis: A Systematic Review of Host- and Pathogen-Derived Signatures

Wa Ode Diah Erwati[1], Andriansjah Rukmana[2*]

[1]Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Article Info

Article history :
Received September 25, 2025
Revised November 20, 2025
Accepted November 27, 2025
Published December 30, 2025

Keywords :
Tuberculosis,
serological biomarkers
antibody response
non-sputum diagnostic methods
mycobacterium tuberculosis

Abstract. Tuberculosis (TB) remains a major global health concern, with more than 10 million new cases reported annually. Conventional sputum-based diagnostics such as microscopy, culture, and GeneXpert MTB/RIF show limited sensitivity in children, individuals with HIV, and extrapulmonary TB, highlighting the need for accurate non-sputum alternatives. This systematic review evaluates the diagnostic performance of serological and other non-sputum biomarkers for active TB, focusing on both host and pathogen derived targets. Eleven eligible studies (2016–2024) involving 2,548 participants were analyzed. The reviewed studies employed multiplex immunoassays and protein microarrays to assess immune markers and *M. tuberculosis* antigens. Key findings indicate that several cytokines (e.g., IFN-γ, IP-10, IL-27) and antibodies to ESAT-6, CFP-10, and Ag85B differentiated active from latent infection. Multi-antigen panels achieved sensitivity and specificity above 85%, while saliva, serum, and skin-based assays showed potential for non-invasive and field-applicable diagnosis. The novelty of this review lies in its integrative approach analyzing both host and pathogen biomarkers, which are often studied separately. Such multi-marker, non-sputum serological strategies could complement current TB diagnostics and provide reliable, accessible tools for use in resource-limited and high HIV prevalence settings

Corresponding Author :
Wa Ode Diah Erwati
Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
Email : waodediaherwati2981@gmail.com

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of the world's leading infectious killers and a major global health concern. According to the Global Tuberculosis Report 2024, an estimated 10.8 million new TB cases and 1.25 million deaths occurred worldwide in 2023, with Indonesia ranking second after India in TB burden, recording more than one million cases annually [1-2]. Despite the availability of effective chemotherapy and diagnostic technologies, TB control continues to face challenges from ongoing transmission, drug resistance, and delayed or missed diagnosis [3].

Current diagnostic tools primarily rely on sputum-based methods, including smear microscopy, culture, and molecular assays such as GeneXpert MTB/RIF [4-5]. However, these approaches often have reduced sensitivity in children, people living with HIV, and patients with extrapulmonary disease, largely due to difficulties in obtaining adequate sputum samples [5]. These limitations highlight the need for alternative diagnostic strategies that use easily accessible, non-sputum specimens such as blood or urine [6-7].

In this context, serological testing has regained attention as a practical, non-sputum diagnostic option. Compared to molecular or culture-based assays, antibody or protein biomarker detection is faster, safer, and requires less technical expertise [8]. Although earlier commercial serological tests were not endorsed by the World Health Organization (WHO) due to poor accuracy [9], recent advances in immunoassay technology such as multiplex ELISA, protein microarrays, and proximity extension assays have significantly improved the reliability and sensitivity of antibody-based detection [10–12]. For instance, combinations of *M. tuberculosis* antigens such as ESAT-6, CFP-10, Ag85B, and Rv2031c have shown strong antibody responses in active TB patients, achieving diagnostic accuracies reaching 85–90% in several studies [10–13]. In addition, newer pathogen-derived proteins such as Resuscitation-Promoting Factors (Rpfs) have recently been recognized for their immunogenic potential and emerging diagnostic relevance [14].

Host-derived biomarkers, including cytokines (e.g., IFN-γ, IP-10, IL-27) and acute-phase proteins (e.g., CRP, SAA, NCAM-1), have also been investigated for distinguishing active from latent TB infection [15-16]. Some multi-marker biosignatures combining host immune responses and pathogen-derived antigens have demonstrated high sensitivity and specificity across diverse populations, suggesting a complementary diagnostic potential [17–19]. However, most studies have analyzed host or pathogen biomarkers separately, with limited efforts to integrate both dimensions within a single diagnostic framework [20-21]. This fragmentation has led to variability in reported results and challenges in translating biomarker findings into clinically useful tests.

Therefore, this systematic review aims to synthesize current evidence (2015–2025) on serological and other non-sputum-based biomarkers for TB diagnosis, with emphasis on both host- and pathogen-derived markers. The review addresses two major research gaps: (1) the limited availability of accurate, accessible diagnostic tools that do not rely on sputum, and (2) the lack of integrated evaluation of host–pathogen biomarker combinations in diagnostic research. By consolidating these findings, this review provides an updated perspective on biomarker discovery, diagnostic performance, and future directions for developing reliable serology-based TB diagnostics.

2. Methods

This systematic review was conducted in accordance with the PRISMA 2020 guidelines. Relevant studies were identified through comprehensive searches of PubMed, Google Scholar, and ProQuest databases for publications between January 2015 and January 2025. The following keywords and Boolean operators were used: ("tuberculosis" OR "TB") AND ("biomarker" OR "serology" OR "serological biomarker" OR "antibody response" OR "cytokine" OR "proteomics") AND ("latent TB" OR "LTBI") AND ("active TB" OR "ATB") AND ("microarray" OR "Mycobacterium tuberculosis antigen").

A total of 1,657 records were retrieved: PubMed (18), Google Scholar (1,327), and ProQuest (312). Duplicate records were identified through cross-database comparison and removed using built-in filtering functions and manual checking to ensure accuracy. The remaining 995 records were screened by title and abstract, and 880 records were excluded for irrelevance, review type, or lack of full text. One hundred fifteen full-text articles were further assessed for eligibility, of which 104 were excluded for reasons including unmet inclusion criteria (n = 37), non-serological antibody studies (n = 21), no ATB vs. LTBI comparison (n = 13), or unrelated research focus (n = 33). The study selection process is summarized in the PRISMA flow diagram (Figure 1).

The final synthesis included 11 eligible studies encompassing 2,548 participants. Inclusion criteria were: (1) evaluation of host- or pathogen-derived biomarkers for tuberculosis diagnosis; (2) inclusion of human participants with confirmed active TB, latent TB infection (LTBI), or healthy controls; and (3) reporting of diagnostic performance (e.g., sensitivity, specificity, or AUC). Exclusion criteria comprised non-English publications, reviews, editorials, conference abstracts, and studies lacking sufficient diagnostic data.

Only studies reporting validated diagnostic methods or performance metrics were included to ensure the reliability of extracted data. Data were synthesized descriptively based on reported diagnostic performance (sensitivity, specificity, and AUC), without additional statistical meta-analysis. Extracted information included study characteristics, population, diagnostic methods, biomarkers analyzed, and diagnostic accuracy outcomes. Because this study synthesized previously published data, no ethical approval was required.

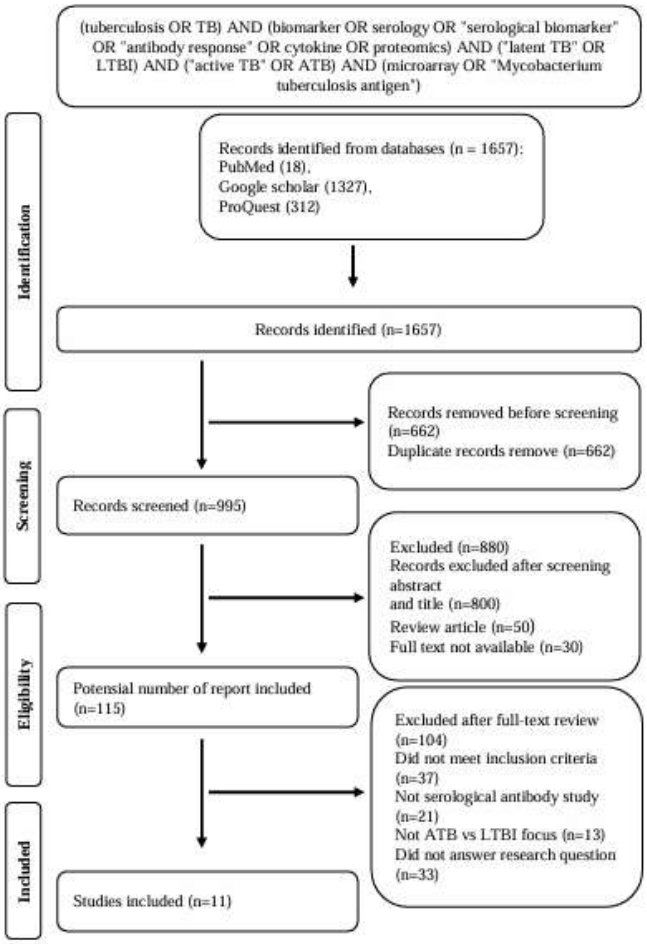

Figure 1. Schematic/Flowchart of research

3. Results and Discussion

Eleven primary studies published between 2016 and 2024 met the eligibility criteria. Together, these studies analyzed data from 2548 participants, including patients with active tuberculosis (ATB), latent tuberculosis infection (LTBI), healthy controls, and individuals with other respiratory diseases. The studies used various methods: four used protein microarray platforms to screen large sets of *Mycobacterium tuberculosis* (Mtb) antigens, three used a multiplex serological platform, four used ELISA, two used a serum proteomics approach, one used Western Blot, and one investigated host inflammatory markers using the Proximity Extension Assay.

Table 1. Characteristics of included studies (n = 11).

Author (Year)	Country	Design	Population (ATB/LTBI /HC)	Method	Key Antigens/Markers	Main Findings
Ruschca Jacobs et al. (2016) [22]	Cape Town, South Africa	Prospektif, observasional, case-control	ATB HIV(+)4/ ATB HIV (-) 14/ ORD HIV(+) 8/ ORD HIV (-) 25 Total: 51	Multiplex cytokine platform	IL-1β, IL-23, ECM-1, HCC1, fibrinogen, granzim A, GDF-15, SAA, IL-21, ENA-78, IL12(p40), IL-13, PAI-1, mioglobulin, TPA	A five-marker biosignature (IL-1β, IL-23, ECM-1, HCC1, fibrinogen) diagnosed TB with 88.9% sensitivity and 89.7% specificity irrespective of HIV status; excluding HIV-positive individuals, two eight-marker biosignatures achieved up to 100% accuracy.
Chang Liu et al. (2017) [30]	Houston, Texas	Case Control	HIV- (ATB+ culture 27/ LTBI 31/ NTM 32/ HC 21) HIV+ (ATB+ culture 23/ ATB - culture 17/ EPTB+ culture 23/ EPTB – culture 8/ Non TB 29) Total : 211	Antibody-conjugated nanodisc, Mass spectrometry	ESAT-6, CFP-10	NanoDisk-MS sensitively detected M. tuberculosis–specific peptides (CFP-10, ESAT-6) in serum, achieving >90% sensitivity in culture-positive TB and high specificity against healthy and LTBI controls. The method remained effective in HIV-positive patients and enabled

						monitoring of antigen decline during therapy
Cao Shu Hui et al. (2018) [23]	China	Cross-sectional	ATB 112/ LTBI 113/HC 94 Total : 319	Proteome microarrays + ELISA	Rv2031c, Rv1408, Rv2421c	M. tuberculosis antigens such as Rv2031c, Rv1408, and Rv2421c show potential as serological biomarkers for distinguishing LTBI from active TB, while antigen combinations or logistic regression–based predictive models provide greater accuracy than single-antigen assays.
Zhang Li Peng et al. (2020) [24]	China	Cross-Sectional	ATB 100/LTBI 60/HC 44 Total : 204	Proteome and mini-protein microarrays	MT1560.1-IgM, Rv0049-IgM, Rv0270-IgM, Rv0350-IgG, Rv0350-IgM, Rv0494-IgM, Rv1597-IgM, Rv1860-IgG, Rv1876-IgM, Rv2031c-IgG, Rv2352c-IgM, Rv2450c-IgM, Rv2511-IgG, Rv2688c-IgM, Rv3480c-IgM.	The multi-antigen panel demonstrated excellent diagnostic performance (sensitivity 85.4%; specificity 90.3%; AUC 94.4%), with predominant IgM responses indicating its role in distinguishing ATB from LTBI. This study also introduced several novel antigens, including MT1560.1, Rv0049, Rv0270, Rv1597, and Rv3480c, as potential ATB-specific biomarkers.

Zhihui Li et al. (2021) [21]	China	Cross-Sectional	Discovery : ATB 52/ LTBI 37/ HC 27 Validation : ATB 205/ LTBI 123/ HC 112 Total 556	Microarray protein + ELISA	Rv0934, Rv3881c, Rv1860, Rv1827	The 4-antigen panel (Rv0934, Rv3881c, Rv1860, Rv1827) showed the best performance, with 67.3% sensitivity and 91.2% specificity for ATB vs LTBI, and 71.2% sensitivity and 96.3% specificity for ATB vs HC. ELISA results were consistent with the microarray findings
Thomas C Morris et al. (2021) [29]	Karonga, Malawi dan Cape Town, Afrika Selatan.	Prospektif, case-control	Karonga, Malawi TB HIV–: 32 TB HIV+: 31 OD HIV–: 31 OD HIV+: 28 → Total = 122 Cape Town, South Africa TB HIV–: 30 TB HIV+: 29 OD HIV–: 31 OD HIV+: 37 → Total = 127 Grand total: 249	Multiplex Luminex assay	CRP, transthyretin, IFN-γ, complement factor H, ApoA-I, IP10, SAA	A seven-protein serum panel (CRP, transthyretin, IFN-γ, complement factor H, ApoA-I, IP-10, and SAA) achieved ~93% sensitivity and ~73% specificity in distinguishing ATB from OD across both HIV-positive and HIV-negative patients.
Nadege Nziza et al. (2022) [27]	Capetown, South Africa	Cross-sectional	ATB,HIV(+) 12/ ATB,HIV (-) 21/ LTBI,HIV (+) 22/ LTBI,HIV (-) 22	Luminex-based multiplex immunoassay	Rv2435c, Rv3583, Rv1528, Rv2034, Rv1508, LAM, Ag85A	A minimal panel of seven Mtb antigens combined with Fc antibody features provides robust discrimination

			Total : 77			between ATB and LTB in both HIV– and HIV+ populations
Jie Li et al. (2022) [13]	China	Cross-sectional	Discovery : ATB 60/ LTBI 60/ HC 60 Validation : ATB 100/ LTBI 100/ HC 100 Total : 480	Microarray protein + ELISA	Rv1860, Rv2031c, Rv3881c, Rv3803c, Rv0526	Microarray analysis identified five candidate proteins, refined to a four-protein panel (Rv1860, Rv2031c, Rv3881c, Rv3803c). The random forest model showed AUC >0.9, and ELISA validation (n=300) confirmed high accuracy, with 93.3% sensitivity/97.7% specificity for ATB vs LTBI and 86%/97.6% for ATB vs healthy controls
Yuan Yuan et al. (2023) [31]	China	Prospektif Cohort	ATB 257	ESAT6-CFP10 skin test (ECST).	ESAT-6, CFP10	The ESAT6-CFP10 skin test (ECST) showed a sensitivity of 72.7% and specificity of 90.5%, compared to 75.2% and 85.7% for IGRA, respectively. ECST achieved a higher discriminative performance with an AUC of 0.87 versus 0.83 for IGRA, with good agreement between tests (κ = 0.75). No serious adverse

						events were observed.
Andi C.Tran (2023) [26]	Maputo, Mozambik	Eksploratif Retros pektif	ATB 21/ LTBI 18/ HC 17 Total : 56	ELISA, Western Blot	IgA anti-MPT64, IgG anti-Ag85B, IgG anti-CFP (Mtb/BCG) , dan IgG anti-38 kDa & 19 kDa	IgA against MPT64 emerged as the strongest serological biomarker (AUC 0.96; Sensitivity 95%; Specificity 97%), while IgG against Ag85B and CFP provided only modest additional accuracy.
Sosina Ayalew et al. (2024) [28]	Addis Ababa, Ethiopia	Cross-sectional	ATB 30/ ORD,LTBI (+) 29/ ORD,LTBI (-) 29 Total : 88	Proximity Extension Assay (PEA)	IFN-γ, LIF, uPa, CSF-1, SCF, SIRT2, 4E-BP1, GDNF	Eight host serum proteins (IFN-γ, LIF, uPa, CSF-1, SCF, SIRT2, 4E-BP1, GDNF) discriminated PTB from ORD+LTBI (AUC 0.943; sensitivity 86%; specificity 97%) and PTB from ORD–LTBI (AUC 0.927; sensitivity 86%; specificity. 89%)

3.1 Overview of Biomarker Approaches

Researchers have explored a broad spectrum of tuberculosis (TB) biomarkers, encompassing both pathogen-based assays such as nucleic acid amplification and antigen detection and host-response measurements including antibody titers, cytokine profiles, and proteomic signatures. This review focuses on blood-based, non-sputum diagnostic approaches reflecting both immune and pathogen responses. Studies included antibody profiling against Mtb antigens, multi-antigen ELISA or microarray panels, and host cytokine or protein markers.

3.2 Key Study Findings

A total of 69 host biomarkers were examined in saliva from individuals with suspected tuberculosis, and a five-marker signature IL-1β, IL-23, ECM-1, HCC1, and fibrinogen was eventually singled out for its strong diagnostic performance. Sensitivity and specificity approached 89%, and these results held regardless of HIV status. The work suggests that saliva, despite its simplicity, can carry enough inflammatory signals to reliably distinguish active disease [22].

Proteome microarray platforms were then used in separate studies to map antibody profiles that differentiate active TB from latent infection. Several high-performing antigens emerged, including

Rv2031c, Rv1408, and Rv2421c, with AUC values ranging from 0.80 to 0.85. An expanded antigen panel later showed even higher overall accuracy. One antigen Rv2031c (HspX) appeared repeatedly across cohorts, pointing to its value as a stable diagnostic target. Overall, combining several antigens proved more effective than relying on single markers, and both IgG and IgM responses contributed meaningfully to diagnostic strength [23-24].

Further evaluation of multi-antigen combinations showed varying levels of accuracy depending on the study design. A broad initial screen identified four secretory antigens that yielded moderate sensitivity and high specificity, whereas a more focused selection of DosR-related antigens, refined with random forest modeling, produced notably stronger diagnostic performance. Despite differences in approach, several antigens particularly Rv1860 and Rv2031c appeared consistently important. Even so, both sets of findings share similar limitations, mainly the lack of multicenter validation and minimal testing in groups such as children or people living with HIV [13,25].

In another investigation, IgA responses to the antigen MPT64 stood out as a highly accurate marker, with sensitivity and specificity exceeding 95%. Active TB was also associated with elevated IgG and IgA levels to Ag85B and culture filtrate proteins, while individuals with latent TB showed higher IgG responses to HBHA. Although the sample size was small and limited to HIV-negative adults, the results suggest that IgA-based serology may have considerable promise in high-burden regions [26]

A different line of work expanded serological assessment by measuring how antibodies interact with Fc receptors. Features such as FcγR2A and FcγR3A engagement were shown to add discriminatory value beyond simple antibody titers. Building on this principle, an eight-protein serum panel was later identified and demonstrated very strong diagnostic performance (AUC 0.943), underscoring the usefulness of host proteomic signatures for clinical applications [27-28].

Serum biomarkers were also assessed in populations with high HIV prevalence using a 22-plex Luminex platform. From this, a seven-protein combination including CRP, transthyretin, complement factor H, ApoA-I, IFN-γ, IP-10, and SAA showed high sensitivity while maintaining reasonable specificity. Importantly, its performance remained consistent across both HIV-positive and HIV-negative participants, suggesting that the panel may be reliable in typical high-burden settings [29]

Pathogen-based diagnostic approaches also yielded encouraging findings. Using a NanoDisk-MS system, circulating CFP-10 and ESAT-6 antigens were detected in serum with sensitivities above 90%, even among individuals with HIV or extrapulmonary disease. This supports the idea that direct antigen detection can complement immune-based tests. A field-friendly ESAT6–CFP10 skin test was also evaluated and produced balanced sensitivity and specificity, along with better overall discrimination than IGRA. Because it can be administered without laboratory resources, the test may be especially useful in primary care, although broader validation—particularly in immunocompromised groups will still be needed [30-32].

3.3 Consistency of Findings

The updated WHO Target Product Profile (TPP) for rapid TB diagnostics introduces stricter performance expectations than the earlier version. In this revision, sputum-based low-complexity tests are required to reach a minimal sensitivity of 90%, while point-of-care formats may range slightly lower at 75–85% depending on the intended setting. The optimal target remains high at ≥95% sensitivity, and the specificity requirement continues to be set at ≥98% for both minimal and optimal levels [33].

When these newer benchmarks are used as a reference point, the findings from the reviewed studies show a gradual but noticeable improvement in diagnostic performance. Several assays now report sensitivities in the range of 85–90% with consistently high specificity, although only a few fully meet the higher minimal threshold set by the updated TPP. Even so, the collective evidence points toward meaningful progress in assay development, supported by advances in multi-marker strategies, better antigen selection, and validation in increasingly diverse patient populations. Variability across studies is still evident, reflecting differences in study design, analytical platforms, and cohort characteristics, but the overall trajectory remains positive.

In line with this trend, a systematic review of host blood protein biomarkers for tuberculosis screening was conducted, and several reproducible protein signatures with strong diagnostic potential across diverse settings were identified. The synthesis of available evidence shows that blood-based proteomic approaches are becoming increasingly robust, with recent studies gradually meeting or approaching WHO-recommended performance benchmarks. These developments suggest that protein signatures detectable in peripheral blood are no longer exploratory concepts but are maturing into viable tools for early case-finding, particularly in high-burden or resource-limited environments. Consistent with this progression, emerging data further indicate that multi-analyte combinations rather than single markers are most likely to deliver the level of diagnostic consistency and scalability required for real-world implementation [34].

3.4 Methodological Comparisons and Integration of Evidence

Most studies were carried out using a discovery–validation framework, in which proteome-wide screening was followed by ELISA-based confirmation. Through this two-step process, biomarker panels were refined and the likelihood of false positives was reduced. In contrast, functional antibody profiling and direct antigen detection through NanoDisk-MS represent alternative strategies that can circumvent variability in host immune responses. When viewed together, these complementary approaches show that combining host- and pathogen-derived biomarkers offers a stronger diagnostic foundation than relying on a single biomarker category [27-30].

More recent investigations have further reinforced this integrative model. When host-derived immune signatures such as cytokine patterns and antibody profiles were combined with pathogen-related readouts, including circulating antigens and cfDNA, diagnostic performance was markedly improved compared with approaches focused on only one axis. These findings strengthen the rationale for developing unified, blood-based biosignatures that simultaneously reflect immune activity and microbial burden [35]

Across these analyses, a clear convergence has emerged around several antigenic and protein targets, particularly Rv2031c, Rv1860, and CFP-10. These molecules repeatedly appeared as conserved diagnostic anchors across different populations and analytical platforms. Their consistent validation in independent cohorts supports their translational potential for serology-based tuberculosis diagnostics.

3.5 Clinical and Translational Implications

From a clinical perspective, multi-analyte serological panels demonstrate superior diagnostic accuracy compared to single-analyte assays, supporting their role as valuable adjuncts to conventional sputum-based tests. Host-response signatures serve as dynamic indicators of disease activity and treatment response, whereas pathogen-based antigen detection provides direct evidence of infection—particularly advantageous in extrapulmonary or HIV-associated tuberculosis.

Supporting this, elevated IgG responses to Rv1860 and Ag85B were observed among Indonesian patients with pulmonary tuberculosis when compared with healthy controls. This locally generated evidence strengthens the translational relevance of antigen-specific serology in endemic settings and highlights the feasibility of adapting such assays for field deployment within Indonesia. These findings

also indicate that antigen profiles identified in global studies can be recapitulated in local populations, suggesting good biological consistency and reinforcing the potential for standardized serological tools to be integrated into national TB diagnostic strategies [36].

Importantly, the combined assessment of immune and pathogen markers reflects a broader paradigm shift in TB diagnostics from isolated biomarker discovery toward integrated biosignatures that capture both infection status and host–pathogen interactions. This integrative framework offers a promising direction for developing serology-based, point-of-care diagnostic tools suitable for real-world application.

3.6 Study Limitations and Future Direction

Despite notable progress in biomarker discovery, translating these findings into practical diagnostics remains challenging. Many studies relied on small, single-center cohorts, limiting external validity and generalizability across diverse epidemiological settings [3]. Key populations such as children, individuals with extrapulmonary tuberculosis, and people living with HIV continue to be underrepresented, even though they represent groups in which diagnostic accuracy is most needed [8]. Methodological variability, including differences in antigen selection, assay platforms, and analytical models, contributes to inconsistent results and limits comparability across studies [15]. Moreover, while multi-analyte panels generally outperform single-marker assays, their performance often remains below the optimal sensitivity and specificity targets set by the World Health Organization [16].

Only a few studies have undergone multicenter validation, underscoring the need for broader and more representative assessments. Without evaluations across diverse settings, the generalizability and real-world applicability of many promising biomarkers remain uncertain [29].

Future research should prioritize three critical directions: (1) validating high-performing biosignatures across large, geographically diverse cohorts to ensure reproducibility; (2) simplifying complex biomarker combinations into affordable, field-deployable assays; and (3) integrating host- and pathogen-derived markers into unified diagnostic platforms that reflect both immune response and pathogen presence. Such efforts could bridge the gap between discovery and implementation, advancing the development of next-generation serological tools that align with WHO performance standards and are practical for use in high-burden settings such as Indonesia.

4. Conclusion

This systematic review demonstrates that both host- and pathogen-based biomarkers offer significant potential to enhance tuberculosis diagnosis, particularly through serological and other non-sputum-based approaches. Multi-analyte biosignatures consistently outperform individual biomarkers in diagnostic accuracy, yet most studies still fall short of the minimal and optimal performance levels outlined in the updated WHO Target Product Profiles (TPPs). Serological assays measuring antibody responses to *M.tuberculosi*s antigens combined with host-derived proteins, cytokines, and immune mediatorsrepresent practical, non-sputum alternatives that could complement or surpass the limitations of conventional diagnostics. Similarly, pathogen-targeted methods, including direct antigen detection and ESAT6–CFP10-based skin tests, provide direct evidence of infection and hold promise for use in difficult-to-diagnose populations such as individuals with extrapulmonary TB or HIV co-infection.

Despite encouraging progress, translation into clinical practice remains limited. Most studies involve small, single-center cohorts and rely on resource-intensive technologies, constraining scalability and access in high-burden settings. Consistent with previous reports, future research should prioritize large, multicenter validation studies across diverse populations and the development of simplified, affordable serological assays suitable for routine use in primary healthcare systems.

In conclusion, while TB biomarkers are not yet ready to replace traditional diagnostic methods, integrating host- and pathogen-derived biosignatures into unified, multi-analyte platforms presents a promising direction for achieving accurate, accessible, and scalable diagnostics. Such advancements could play a pivotal role in strengthening global TB control efforts, particularly in resource-limited countries like Indonesia.

References

[1] World Health Organization. (2024). *WHO consolidated guidelines on tuberculosis. Module 6: tuberculosis and comorbidities*. World Health Organization.

[2] Ministry of Health of the Republic of Indonesia. (2023).*Tuberculosis Control Annual Report.* Ministry of Health.

[3] Walzl, G., McNerney, R., du Plessis, N., Bates, M., McHugh, T. D., Chegou, N. N., & Zumla, A. (2018). Tuberculosis: advances and challenges in development of new diagnostics and biomarkers. *The Lancet Infectious Diseases*, *18*(7), e199-e210.

[4] Li Y, et al. (2022). Evaluation of the GeneXpert MTB/RIF Ultra assay for the diagnosis of pulmonary and extrapulmonary tuberculosis in China. *Front Microbiol.*13:1012212.

[5] Graham, S. M., Marais, B. J., & Amanullah, F. (2022). Tuberculosis in children and adolescents: progress and perseverance. *Pathogens*, *11*(4), 392.

[6] MacLean, E., Broger, T., Yerlikaya, S., Fernandez-Carballo, B. L., Pai, M., & Denkinger, C. M. (2019). A systematic review of biomarkers to detect active tuberculosis. *Nature microbiology*, *4*(5), 748-758.

[7] Du Preez K, et al. (2024). Advances in TB diagnostics: Integration of host and pathogen biomarkers. *Eur J Med Res.*29(1):92.

[8] Nogueira, B. M., Krishnan, S., Barreto-Duarte, B., Araújo-Pereira, M., Queiroz, A. T., Ellner, J. J., ... & Andrade, B. B. (2022). Diagnostic biomarkers for active tuberculosis: progress and challenges. *EMBO Molecular Medicine*, *14*(12), e14088.

[9] World Health Organization. (2011). *Commercial serodiagnostic tests for diagnosis of tuberculosis: policy statement* (No. WHO/HTM/TB/2011.5). World Health Organization.

[10] Jaganath, D., Rajan, J., Yoon, C., Ravindran, R., Andama, A., Asege, L., ... & Cattamanchi, A. (2020). Evaluation of multi-antigen serological screening for active tuberculosis among people living with HIV. *PloS one*, *15*(6), e0234130.

[11] Demissie, A., Leyten, E. M., Abebe, M., Wassie, L., Aseffa, A., Abate, G., ... & VACSEL Study Group. (2006). Recognition of stage-specific mycobacterial antigens differentiates between acute and latent infections with Mycobacterium tuberculosis. *Clinical and vaccine immunology*, *13*(2), 179-186.

[12] Huang W, et al. (2023). Proteomic profiling of TB patient serum reveals immune biomarkers with diagnostic potential. *Front Immunol.* 14:1172456.

[13] Li, J., Wang, Y., Yan, L., Zhang, C., He, Y., Zou, J., ... & Zhang, X. (2022). Novel serological biomarker panel using protein microarray can distinguish active TB from latent TB infection. *Microbes and Infection*, *24*(8), 105002.

[14] Tanriver, G., Khan, S. A., Góra, A., Chegou, N. N., & Mahmoudi, S. (2025). Exploring the multifaceted roles of resuscitation-promoting factors in tuberculosis: Implications for diagnosis, vaccine development, and drug targeting. *Biotechnology Reports*, e00886.

[15] Shaukat S, et al. (2023). Cytokine biomarkers for differentiating active from latent tuberculosis infection. *BMC Infect Dis.*23:456.

[16] Lu, Y., Chillarige, Y., Izurieta, H. S., Wei, Y., Xu, W., Lu, M., ... & Forshee, R. A. (2019). Effect of age on relative effectiveness of high-dose versus standard-dose influenza vaccines

among US Medicare beneficiaries aged≥ 65 years. *The Journal of infectious diseases*, *220*(9), 1511-1520.
[17] Chen Y, et al. (2024). Combined host and pathogen-derived biomarkers for tuberculosis diagnosis. *Sci Rep.*14:5218.
[18] Chatterjee A, et al. (2024). Serological and molecular biomarkers for TB detection: Current progress and perspectives. *Lancet Microbe.* 5(4):e251–e260.
[19] Kumar V, et al. (2022). Integration of multi-omics data for tuberculosis biomarker discovery. *Nat Commun.* 13:7341.
[20] Broger T, et al. (2024). Host biomarker combinations for tuberculosis diagnosis and treatment monitoring. *Front Immunol.* 15:1382081.
[21] Wang H, et al. (2025). Advances in non-sputum-based tuberculosis diagnostics: Current developments and challenges. *Biomedicines.* 13(9):2076.
[22] Jacobs, R., Maasdorp, E., Malherbe, S., Loxton, A. G., Stanley, K., Van Der Spuy, G., ... & Chegou, N. N. (2016). Diagnostic potential of novel salivary host biomarkers as candidates for the immunological diagnosis of tuberculosis disease and monitoring of tuberculosis treatment response. *PloS one*, *11*(8), e0160546.
[23] Cao, S. H., Chen, Y. Q., ZHENG, S. H., ZHANG, Z. G., & LI, C. Y. (2018). Screening of serum biomarkers for distinguishing between latent and active tuberculosis using proteome microarray. *Biomedical and Environmental Sciences*, *31*(7), 515-526.
[24] Peng, Z., Chen, L., & Zhang, H. (2020). Serum proteomic analysis of Mycobacterium tuberculosis antigens for discriminating active tuberculosis from latent infection. *Journal of International Medical Research*, *48*(3), 0300060520910042.
[25] Li, Z., Hu, J., Liu, P., Cui, D., Di, H., & Wu, S. (2021). Microarray-based selection of a serum biomarker panel that can discriminate between latent and active pulmonary TB. *Scientific Reports*, *11*(1), 14516.
[26] Tran, A. C., Boariu, E., García-Bengoa, M., Kim, M. Y., Vergara, E. J., Mussá, T., & Reljic, R. (2023). Serological analysis reveals differential antibody responses between TB patients and latently infected individuals from the TB endemic country of Mozambique. *Frontiers in medicine*, *10*, 1286785.
[27] Nziza, N., Cizmeci, D., Davies, L., Irvine, E. B., Jung, W., Fenderson, B. A., ... & Alter, G. (2022). Defining discriminatory antibody fingerprints in active and latent tuberculosis. *Frontiers in immunology*, *13*, 856906.
[28] Ayalew, S., Wegayehu, T., Wondale, B., Tarekegn, A., Tessema, B., Admasu, F., ... & Mihret, A. (2024). Candidate serum protein biomarkers for active pulmonary tuberculosis diagnosis in tuberculosis endemic settings. *BMC infectious diseases*, *24*(1), 1329.
[29] Morris, T. C., Hoggart, C. J., Chegou, N. N., Kidd, M., Oni, T., Goliath, R., ... & Hamilton, M. S. (2021). Evaluation of host serum protein biomarkers of tuberculosis in sub-Saharan Africa. *Frontiers in immunology*, *12*, 639174.
[30] Liu, C., Zhao, Z., Fan, J., Lyon, C. J., Wu, H. J., Nedelkov, D., ... & Hu, Y. (2017). Quantification of circulating Mycobacterium tuberculosis antigen peptides allows rapid diagnosis of active disease and treatment monitoring. *Proceedings of the National Academy of Sciences*, *114*(15), 3969-3974.
[31] Yuan, Y., Xia, L., Wu, Q., Liu, X., & Lu, S. (2023). Utility of recombinant fusion protein ESAT6-CFP10 skin test for differential diagnosis of active tuberculosis: A prospective study. *Frontiers in Immunology*, *14*, 1162177.
[32] World Health Organization. (2024). *WHO consolidated guidelines on tuberculosis. Module 3: diagnosis–rapid diagnostics for tuberculosis detection*. World Health Organization.
[33] World Health Organization. (2025). *Target product profile for tuberculosis screening tests*. World Health Organization.

[34] Gaeddert, M., Glaser, K., Chendi, B. H., Sultanli, A., Koeppel, L., MacLean, E. L., ... & Denkinger, C. M. (2024). Host blood protein biomarkers to screen for tuberculosis disease: a systematic review and meta-analysis. *Journal of Clinical Microbiology*, *62*(11), e00786-24.

[35] Li, Z., Hu, Y., Wang, W., Zou, F., Yang, J., Gao, W., ... & Chen, X. (2024). Integrating pathogen-and host-derived blood biomarkers for enhanced tuberculosis diagnosis: a comprehensive review. *Frontiers in Immunology*, *15*, 1438989.

[36] Dewi, D. N. S. S., Mertaniasih, N. M., Soedarsono, Hagino, K., Yamazaki, T., Ozeki, Y., ... & Matsumoto, S. (2023). Antibodies against native proteins of Mycobacterium tuberculosis can detect pulmonary tuberculosis patients. *Scientific Reports*, *13*(1), 12685.