BIOCELL

Tech Science Press

http://doi.org/10.32604/biocell.2026.077540

REVIEW

Emerging Roles of Exosomes and Exosomal Antigens as Potential Diagnostic and Prognostic Agents for *Mycobacterium tuberculosis* Infection

Mohammad Muzaffar Mir[1,*], Rashid Mir[2,3], Badr A. Alsayed[4], Mohammed M. Jalal[3], Malik A. Altayar[3], Hanan M. Aljammaz[5], Zinab Alatawi[6], Khalid A. Alfifi[2], Basmah M. Alenzi[7], Mohammad Tanveer Khaji[8], Umair Ismail[9], Ghada Mohamed[10,11], Nada Zai Sageer[12], Abdullatif Taha Babakr[13], Saba M. Mir[14] and Ulfat Jan[15]

[1]Department of Clinical Biochemistry, College of Medicine, University of Bisha, Bisha, Saudi Arabia
[2]Prince Fahd Bin Sultan Chair for Biomedical Research, University of Tabuk, Tabuk, Saudi Arabia
[3]Department of Medical Lab Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk, Saudi Arabia
[4]Department of Internal Medicine, Faculty of Medicine, University of Tabuk, Tabuk, Saudi Arabia
[5]Family Medicine Resident, Tabuk Health Cluster, Tabuk, Saudi Arabia
[6]Department of Family Medicine, University of Tabuk, Tabuk, Saudi Arabia
[7]Ministry of Health, Tabuk Region, Tabuk, Saudi Arabia
[8]Microbiology Department, King Salman Armed Forces Hospital—Northwestern Region, Tabuk, Saudi Arabia
[9]Molecular Biology Department, King Salman Armed Forces Hospital—Northwestern Region, Tabuk, Saudi Arabia
[10]Pathology Department, National Cancer Institute, Cairo University, Cairo, Egypt
[11]Histopathology Lab, King Fahad Specialist Hospital, Tabuk, Saudi Arabia
[12]King Faisal Hospital, Ministry of Health, Makkah, Saudi Arabia
[13]Department of Medical Biochemistry, Faculty of Medicine, Umm al-Qura University, Makkah, Saudi Arabia
[14]Department of Microbiology, Government Medical College, Baramulla, India
[15]Genome Diversity Unit, University of Tabuk, Tabuk, Saudi Arabia
*Corresponding Author: Mohammad Muzaffar Mir. Email: mmmir@ub.edu.sa

Received: 11 December 2025; Accepted: 13 March 2026; Published: 27 July 2026

ABSTRACT: The tuberculosis (TB) epidemic continues to be one of the largest public health challenges affecting people globally, especially due to late diagnosis, disease monitoring, and prognosis. Currently used diagnostic tools have variable sensitivity and accessibility, and many have limited ability to differentiate between latent and active TB. In case of TB, exosomes from cells infected with *Mycobacterium tuberculosis* (*M. tuberculosis*) have disease-specific antigens, microRNAs, and other molecular components, which make them potential diagnostic and prognostic biomarkers. This review focuses on previously published literature regarding the role of exosomes and exosomal antigens in TB screening and prognostication. It highlights their potential as non-conventional biomarkers due to their stability in biological fluids, specificity, and the availability of less invasive sampling techniques. Moreover, it covers recent developments in techniques for isolating and characterizing exosomes and also ways in which the gaps between exosome-based biomarkers have been challenged in real-world clinical settings. In the future, research needs to prioritize large validation studies, achievement-in-exosome-based assays, and incorporation of multi-omics techniques to improve their clinical relevance. The use of exosome-based biomarkers shows promise for improving diagnostic processes for tuberculosis and may support a worldwide campaign for tuberculosis control.

KEYWORDS: Tuberculosis (TB); *Mycobacterium tuberculosis*; biomarkers; exosomes; exosomal antigens

1 Introduction

Tuberculosis is among the leading causes of death worldwide and remains a major infectious killer, causing approximately 1.3 million deaths globally in recent years [1,2] *M. tuberculosis* was estimated to infect approximately one-quarter of the world's population, which corresponds to nearly 2 billion people. The condition has remained one of the top 10 international health issues for many years [3]. Attributed to its complex nature, high incidence rate, and fatal impact, tuberculosis (TB) has been labeled an epidemic by the WHO. It has been reported that TB claimed 1.4 million lives in 2019 [1]. *Mycobacterium bovis* and *M. tuberculosis* cause an ancient disease which believed to have started over 40,000 years ago. TB can spread among infected people with pulmonary illness through airborne droplets, and, hence, those who inhale these infections. Thus, TB is associated with immunologic suppression disorder [4]. Most of the TB cases, approximately 90 percent, forego an exaggerated immune system response to *M. tuberculosis* infection, more commonly referred to as latent TB infection (LTBI) [5]. Active TB, especially with further immune suppression, occurs in 10% of the population [6]. It can move to regions such as bones or the brain, and these conditions are termed extrapulmonary TB. The macrophages in the lungs ingest the bacilli, thus the latter settle. Granulomas formed encircle the infection and serve the purpose of containing it [7]. TB primarily affects the lungs through infection with *Mycobacterium tuberculosis*, which spreads through airborne droplets generated by coughing or sneezing, and causes chronic pulmonary disease rather than typical pneumonia [8]. TB is associated with increased risk in individuals with immunosuppression, rather than being an immunologic suppression disorder itself. (Fig. 1). Recent research has highlighted the role of host–pathogen interactions at the cellular level, particularly through extracellular vesicles such as exosomes, which carry molecular signatures reflective of *Mycobacterium tuberculosis* infection [9].

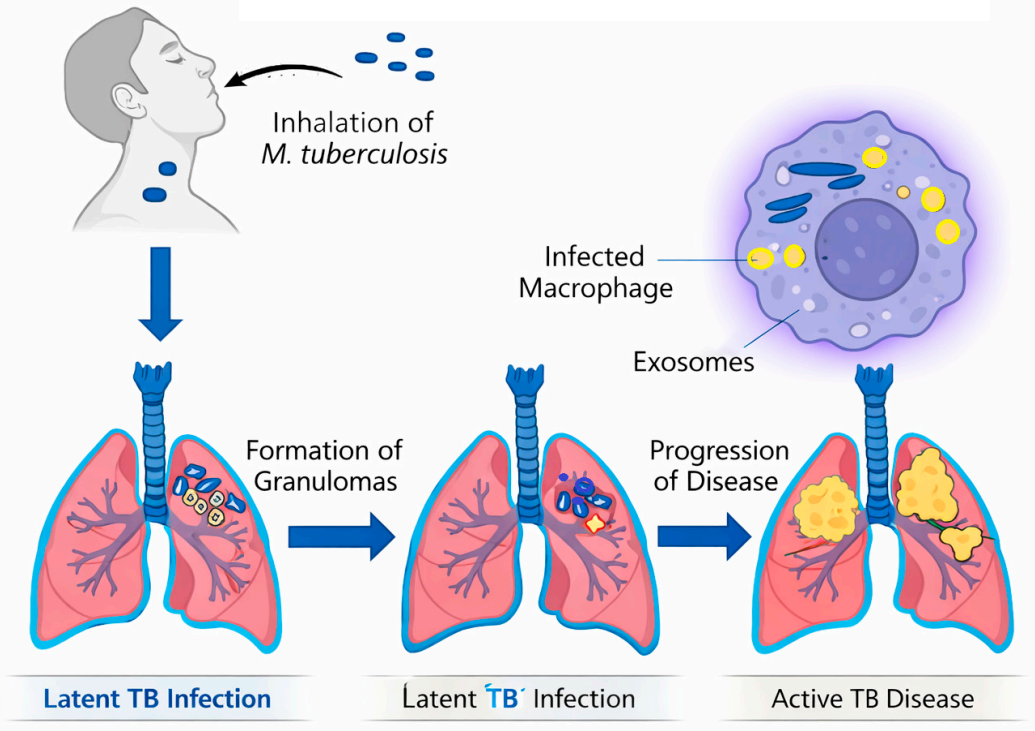

Figure 1: Shows inhalation of *M. tuberculosis* and granuloma formation. BioRender (BioRender.com, version 2024.1, BioRender, Toronto, ON, Canada).

Exosomes came to the attention of biologists in the 1980s during reticulocyte studies and are 30 to 150 nm in size [10]. These extracellular vesicles are secreted by all cells in the body and were initially regarded as mere cellular debris. Today, exosomes are understood to be sophisticated messengers containing proteins, lipids, and nucleic acids, and are enclosed within a lipid bilayer [11]. Exosomes provide diagnostic potential through their lipid layers that carry proteins, lipids, and mRNA, miRNA, and even DNA. They are formed in multivesicular bodies (MVB), which are part of the endosomal pathway. The intercellular signaling functions of exosomes enable them to influence repair, immunity, and development [12]. Exosomes can mediate signaling by fusing with target cells, binding with receptors, or getting internalized. Dendritic cell exosomes, such as those responsible for activating T-cells, are classified as immune exosomes, while stem cell exosomes are growth factor exosomes responsible for aiding healing. These exosomes can cross barriers such as the blood-brain barrier, allowing them to affect distant tissues [13].

The role of exosomes in developing disease is evident in cancer, where exosomes promote metastasis, and in neurodegenerative cases spread toxic proteins [14]. During TB, the exosomes of *M. tuberculosis*-infected cells modulate immunity. Although exosomes do have biocompatibility issues, their cargo, when placed in blood or saliva, turns them into liquid biopsies for diagnosis. The main challenge that seems to stand in their way is related to their isolation and unclear origins. Nevertheless, their role in both health and disease issues makes these exosomes transformative. In this review, we attempt to explain the complex role of exosomes in tuberculosis, starting from their contribution towards disease progression and the immune response, where these vesicles orchestrate or affect host defenses by transporting modulatory substances that may either aid in the survival of *M. tuberculosis* or strengthen the immune response. The review formulates approaches for implementing exosome-derived innovations into clinical practice and equity advocacy by proposing their incorporation into international tuberculosis control frameworks and low-cost models in economically disadvantaged environments, where exosomes can be harnessed to shed light on dormant and resistant forms of TB, improve patient management, and contribute to the vision of eliminating TB by the year 2030.

Objectives, Goals, and Scope of the Review

The main aim of this review is to critically assess the new role of exosomes and exosome-associated antigens as diagnostic and prognostic biomarkers of *M. tuberculosis* infection. Considering the ongoing high incidence of tuberculosis in the world and the shortcomings of current diagnostic methods—especially to differentiate between active disease and latent infection and to determine disease progression and response to treatment—this review aims to evaluate the possibility of exosome-based biomarkers to add clinical value.

This article aims to review the existing data on the biological basis, molecular composition, and functional significance of exosomes released in the course of *M. tuberculosis* infection, and their diagnostic and prognostic value in particular. These involve the assessment of the evidence of reported presence of exosomal antigens, microRNAs, and other molecular cargo that have been suggested as predictors of disease presence, activity, and outcome. Moreover, this review will analyze the reported exosome-based biomarker performance in preclinical and clinical trials, and pinpoint major methodological and translational weaknesses and barriers to their validation and daily clinical use.

This review is in the form of a narrative literature review and has a narrow scope that limits itself to host-derived exosomes and their molecular constituents in relation to the diagnosis and prognosis of tuberculosis. Other subjects (e.g., exosome-based therapeutic uses, artificial intelligence-based diagnostic tools, ethical implications, and the development of point-of-care devices) are touched upon only to the extent that they directly relate to biomarker discovery or evaluation or clinical relevance. The guiding

question in the review is to answer how exosomal components are modified during *M. tuberculosis* infection, whether these changes can be consistently used to distinguish between active tuberculosis and latent infection, and to what extent exosome-based biomarkers could help in disease surveillance and prognostic evaluation in the real-world clinical setting.

2 Biology of Exosomes

2.1 Structural and Molecular Characteristics

The exosome is a type of extracellular vesicle that is characterized by a spherical shape and a lipid bilayer membrane that encloses molecular cargo specific to the cell of origin [15]. This membrane, which is a product of the endosomal compartment, is lipid-rich in cholesterol, sphingomyelin, and phosphatidylserine, which increase stability and rigidity so that their contents are protected during movement outside the cell. This lipid bilayer contains transmembrane proteins, of which tetraspanins, cluster of differentiation 9 (CD9), CD63, and CD81 are notable since they act as exosome markers and have a role in interaction with target cells [16]. Exosome targeting and uptake are facilitated by other surface proteins, including integrins and adhesion molecules [17,18].

Exosome internal cargo is exceptionally heterogeneous, containing biologically active proteins, nucleic acids, and lipids. These include cytoplasmic structural proteins such as actin and tubulin, and heat shock proteins like heat shock protein 70 (HSP70) and HSP90, as well as some exosome membrane-associated proteins, like Alix and TSG101 [19]. Genetic information that can be transferred to alter cellular behaviors includes mRNAs, microRNAs (miRNAs), and small quantities of DNA [20]. For instance, miRNAs are capable of post-uptake gene silencing of specific targets. Also, lipid signaling molecules like prostaglandins are contained within the lumen. This composition is not arbitrary; it is curated according to the condition of the parent cell. In tuberculosis, exosomes from macrophages infected with *M. tuberculosis* and exosomes harboring bacterial lipids such as lipoarabinomannan and host inflammatory proteins showcase the intricate dynamics of infection [21].

2.2 Exosome Biogenesis

The endosomal pathway marks the beginning of exosome formation, differentiating them from other vesicles like microvesicles, which bud off directly from the plasma membrane. The process begins with the early endosomes that result from the inward budding of the plasma membrane (Fig. 2). These endosomes mature into late endosomes, which further generate intraluminal vesicles (ILVs) through the inward budding of the endosomal membrane. This step is facilitated by the endosomal sorting complex required for transport (ESCRT). It involves the action of Alix and TSG101, who aid in the sorting of cargo into ILVs. There is also an ESCRT-independent, ceramide-rich lipid raft pathway that accentuates the complexity of exosome biogenesis. These ILVs represent capsules loaded with bioactive materials. Their accumulation takes place within the large endosomal compartments known as multivesicular bodies (MVBs). The MVBs face two eventualities: fusion with lysosomes for degradation or secretion as exosomes. The fusion of MVBs with plasma membranes is a calcium-dependent process regulated by Rab GTPases (e.g., Rab27a, Rab27b) and SNARE proteins [22,23]. Exosomes emerge from the cleavage of MVBs. They release bioactive materials to extracellular space, where they can be targeted either locally or distally. During tuberculosis, cells infected by *M. tuberculosis* consider enhancing exosome generation, which may affect how MVBs are shaped [24]. Signals related to stress, such as hypoxia and infection, can change the fate of MVBs to boost secretion and heightened exosome production [25]. Still, the precise stimulus and the mechanism for cargo selection have not been studied and therefore remain a challenge for therapy-oriented strategies.

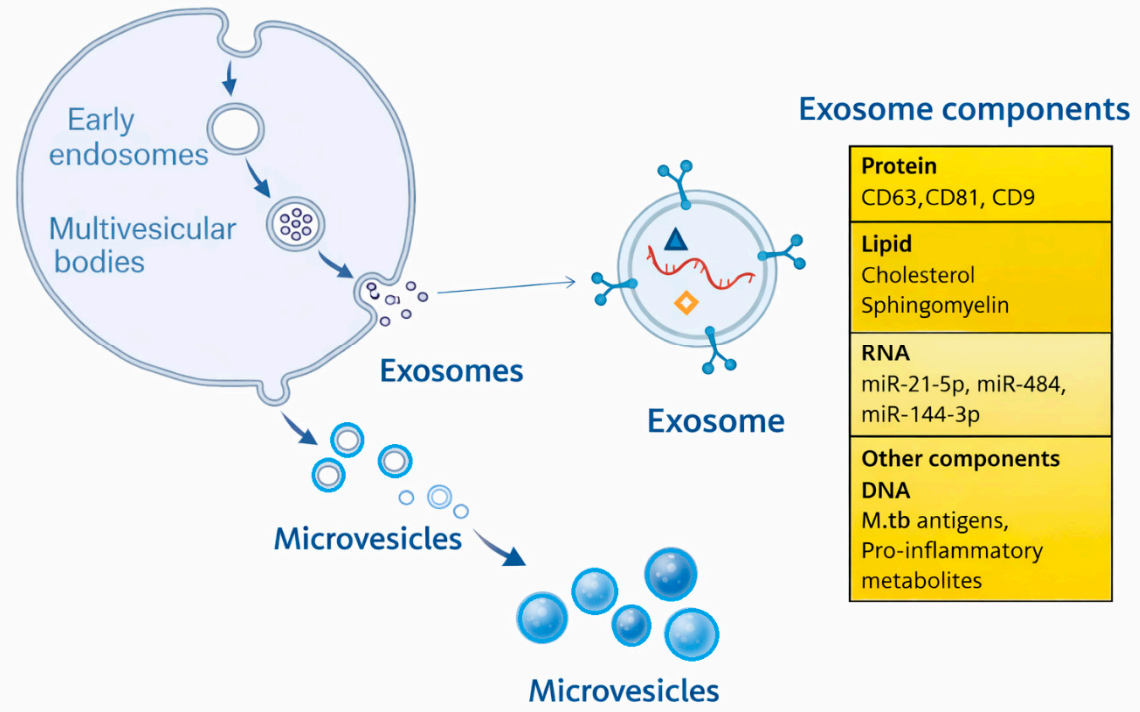

Figure 2: Exosome biogenesis via the endosomal pathway and representative exosomal components relevant to *M. tuberculosis* infection, shown in distinction from plasma membrane–derived microvesicles. BioRender (BioRender.com, version 2024.1, BioRender, Toronto, ON, Canada).

3 Exosomes in Tuberculosis Pathogenesis

Exosomes serve important functions in the context of *M. tuberculosis* infection, both to promote the survival of the bacterial pathogen and to mediate the mechanisms of host defense [26]. Consider the case of infection, whereby *M. tuberculosis* is known to invade alveolar macrophages or other cells. In the case of alveolar macrophages, they undergo a process of escape and are not destroyed [27]. There is a feedback mechanism with respect to exosomes released, which can be further augmented by factors of bacterial infection and host body stress signals, which include hypoxic conditions or inflammation [28]. These vesicles give out *M. tuberculosis*-produced substances to cells that have not been infected, thereby priming the host environment in anticipation of further infection and modifying immune system responses to be more aggressive [29].

3.1 Host–Pathogen Interactions Mediated by Exosomes

M. tuberculosis makes use of exosomes for the purpose of increasing its pathogenicity [30]. Exosomes derived from an infected cell can block phagosome-lysosome fusion in the recipient macrophage, and this is an important strategy employed by *M. tuberculosis* for enduring in the harsh environment of the macrophage [31,32]. This could also happen because such exosomes carry bacterial effectors that disrupt the host trafficking pathways at already advanced stages of infectious blockade. Granuloma formation, which is the characteristic signature of TB, could be facilitated by exosomes through recruiting immune components and sustaining a persistent inflammatory response [33]. In LTBI, exosomes could be of help in maintaining the bacterial form dormant by altering cytokine balance by elevating the levels of anti-inflammatory IL-10 despite the fact that stress-induced changes could set off the dormant particles [34]. On the other hand, host-derived exosomes try to combat *M. tuberculosis* infection. Pro-inflammatory signals from activated

macrophages or dendritic cells in the form of exosomes may serve to enhance the innate immune responses of surrounding cells. However, *M. tuberculosis*'s capacity to usurp these system mechanisms tends to favor the survival of the pathogen, especially in immunocompromised conditions such as co-infection with human immunodeficiency virus (HIV), where the outcome is further complicated by exosome-mediated immune suppression [35]. This highlights the importance of exosomes in the complex pathogenesis of TB.

3.2 Exosomal Cargo in TB

The exosomal cargo in TB is made up of proteins, lipids, and nucleic acids. Many constituents are derived from the host, including heat shock proteins (HSP70), inflammatory cytokines like TNF-α and IL-1β, as well as *M. tuberculosis* proteins ESAT-6 and CFP-10, which are virulence factors exported through the bacterial type VII secretion system [36]. Depending on the situation, these proteins are able to either inhibit macrophage killing pathways or activate T cell responses. Table 1 provides a summary of the exosomal proteins and lipids found in people who were infected with *M. tuberculosis* infections.

Nucleic acids, and more specifically miRNAs, are strong regulators within TB exosomes (Table 2). Some host miRNAs, like miR-155, are inflammatory, while others, like miR-146a, are inhibitory, showing how *M. tuberculosis* has programmed host genes to be used [37]. Small non-coding RNAs, as well as other *M. tuberculosis*-derived RNAs, have been found in exosomes, possibly silencing defense genes or aiding in bacterial survival [38]. The fact that this cargo is exosome-bound and correlates with the severity of the infection enhances the potential of exosomes as biomarkers. For instance, increased levels of ESAT-6 or LAM in the exosomes could indicate active TB, and different profiles of miRNAs could be used to distinguish LTBI from reactivation [39].

Table 1: Summary of Exosomal Proteins and Lipids in Subjects Infected with *M. tuberculosis*.

No.	Exosomal Biomarker (s)	Biomarker Type	Biofluid	Source of Exosomes	n	Cohort Type	Control Group	Analytical Method	Expression Pattern	Diagnostic Performance	External Validation
1	HSP90, Vimentin, Coronin-1C, Moesin	Protein	Cell culture supernatant	*M. tuberculosis*–infected macrophages	NR	*In vitro*	Uninfected macrophages	Tandem MS	Increased	Not reported	No
2	AcpM, Ag85A, Ald, DnaK, GroES, Mpt51, Mpt53, Mpt63, MrsA	Protein	Serum	Human serum exosomes	21 ATB	Adult	Healthy controls	MRM–MS	Increased	Not reported	No
3	LBP	Protein	Serum	Human serum exosomes	60 ATB	Adult	Healthy controls	ELISA	Increased	AUC 0.78	No
4	CD36, MHC-I	Protein	Serum	Human serum exosomes	60 ATB	Adult	Healthy controls	ELISA	Decreased	AUC 0.72	No
5	Rv1827, Rv2220, Rv0350	Protein	Serum	Human serum exosomes	48 LTBI	Adult	Healthy controls	MRM–MS	Increased	Not reported	No
6	Hsp16.3	Protein	Plasma	Plasma exosomes	52 ATB	Adult	Healthy controls	Western blot	Increased	Sensitivity 76%; Specificity 82%	No
7	HP, PRG4, STOM, CD151, ICAM2, ORM1, SAA1, SLC2A3	Protein	Plasma	Plasma exosomes	80 TB	Adult	Healthy controls	TMT-based proteomics	Differential	Not reported	No
8	C1R, GRIP1	Protein	Plasma	Plasma exosomes	54 TB	Adult	Healthy controls	TMT proteomics	Increased	AUC 0.84	No
9	Phosphatidylserine (PS)	Lipid	Cell culture supernatant	*M. tuberculosis*–infected macrophages	NR	*In vitro*	Uninfected macrophages	Western blot	Increased	Not reported	No

Table 1: *Cont.*

No.	Exosomal Biomarker (s)	Biomarker Type	Biofluid	Source of Exosomes	n	Cohort Type	Control Group	Analytical Method	Expression Pattern	Diagnostic Performance	External Validation
10	Lipoarabinomannan (LAM)	Lipid/Antigen	Urine	Urinary exosomes	PTB n = 74; EPTB n = 53	Adult	Healthy + non-TB diseases	Immuno-PCR	Increased	Sensitivity 68–74%; Specificity 91–93%	No
11	TAGs, Cholesteryl esters	Lipid	Plasma	Plasma exosomes	80 TB	Adult	Healthy controls	ESI-MS	Increased	Not reported	No

Note: Heat shock protein 90 (HSP90), heat shock protein 70 (HSP70), heat shock protein 16.3 (HSP16.3), coronin actin-binding protein 1C (Coronin 1C), acyl carrier protein, mycobacterial (AcpM), antigen 85 complex A (Ag85a), aldolase (Ald), chaperone protein DnaK (DnaK), chaperonin GroES (GroES), mycobacterial protein 51 (Mpt51), mycobacterial protein 53 (Mpt53), mycobacterial protein 63 (Mpt63), mycothiol reductase A (MrsA), lipopolysaccharide-binding protein (LBP), cluster of differentiation 36 (CD36), major histocompatibility complex class I (MHC-I), haptoglobin (HP), proteoglycan 4 (PRG4), stomatin (STOM), cluster of differentiation 151 (CD151), intercellular adhesion molecule 2 (ICAM2), orosomucoid 1 (ORM1), serum amyloid A1 (SAA1), solute carrier family 2 member 3 (SLC2A3), complement C1r subcomponent (C1R), glutamate receptor-interacting protein 1 (GRIP1), phosphatidylserine (PS), lipoarabinomannan (LAM), culture filtrate protein 10 (CFP-10), triacylglycerols (TAG), cholesteryl esters (CEs), active tuberculosis (ATB), latent tuberculosis infection (LTBI), multiple reaction monitoring–mass spectrometry (MRM-MS), enzyme-linked immunosorbent assay (ELISA), tandem mass tag (TMT), electrospray ionization mass spectrometry (ESI-MS), immuno-polymerase chain reaction (I-PCR).

Table 2: Summary of Exosomal miRNAs found in subjects infected with *M. tuberculosis*.

No.	Exosomal miRNAs	Source of Exosomes	Screening Method	Expression Pattern	References	Biological/Functional Context	Disease Context	Model/Species Note
1	miR-20b-5p	Macrophage supernatant (*M. tuberculosis*-infected)	RT-PCR	Decreased	[40]	Reported to regulate hypoxia- and immune-related pathways; decreased levels may favor pro-inflammatory responses.	*In vitro* infection model	Murine macrophage model; findings may not fully translate to human TB.
2	miR-27-3p, let-7a-5p, let-7c-5p, miR-25-3p, miR-98-5p, miR-30a-3p, etc.	Macrophage supernatant (*M. tuberculosis*-infected)	RNA sequencing	Increased	[41]	let-7 family and miR-27/30 families implicated in macrophage activation, cytokine signaling, and autophagy regulation.	*In vitro* infection model	Macrophage-derived exosomes; experimental model, not patient samples.
3	miR-194-5p, miR-5110	Macrophage supernatant (*M. bovis*-infected)	RNA sequencing	Decreased	[41]	miR-194 is associated with immune modulation and epithelial–immune crosstalk.	*In vitro* infection model	*M. bovis* infection; results may differ from *M. tuberculosis* infection in humans.

Table 2: *Cont.*

No.	Exosomal miRNAs	Source of Exosomes	Screening Method	Expression Pattern	References	Biological/Functional Context	Disease Context	Model/Species Note
4	miR-185-5p	Plasma (TB patients)	RNA sequencing	Increased	[42]	miR-185 is linked to the regulation of TLR signaling and inflammatory pathways.	Active TB (ATB)	Human clinical samples.
5	miR-423-5p, miR-17-5p, miR-20b-5p	Serum (TB patients)	RNA sequencing	Increased	[43]	miR-17/20b cluster is involved in cell proliferation and immune regulation; miR-423-5p is proposed as a diagnostic biomarker.	Active TB (ATB)	Human serum-derived exosomes.
6	let-7e-5p, let-7d-5p, miR-450a-5p, miR-140-5p	Serum (LTBI patients)	RNA sequencing	Increased	[44]	let-7 family miRNAs are associated with immune homeostasis and controlled inflammation.	Latent TB infection (LTBI)	Human clinical samples.
7	miR-1246, miR-2110, miR-370-3p, miR-28-3p, miR-193b-5p, etc.	Serum (TB patients)	RNA sequencing	Increased	[44]	Several miRNAs are linked to apoptosis, macrophage activation, and host–pathogen interactions.	Active TB (ATB)	Human serum-derived exosomes.
8	miR-26a-5p	Serum (ATB patients)	RNA sequencing	Decreased	[44]	miR-26a-5p targets genes involved in autophagy and inflammatory signaling; downregulation may enhance bacterial survival.	Active TB (ATB)	Human clinical samples.
9	miR-484, miR-425, miR-96, etc.	Serum (TB patients)	qPCR	Increased	[45]	Associated with mitochondrial function, immune regulation, and oxidative stress responses.	Active TB (ATB)	Human serum-derived exosomes.
10	miR-205-5p, miR-200c-3p, miR-141-3p, etc.	Pleural effusion (TB patients)	RNA sequencing	Increased	[46]	miR-200 family implicated in epithelial integrity and inflammatory signaling.	Pleural TB	Human pleural effusion samples.
11	miR-483-5p, miR-375	Pleural effusion (TB patients)	RNA sequencing	Decreased	[46]	miR-375 is involved in immune cell differentiation and cytokine regulation.	Pleural TB	Human pleural effusion samples.

Table 2: *Cont.*

No.	Exosomal miRNAs	Source of Exosomes	Screening Method	Expression Pattern	References	Biological/Functional Context	Disease Context	Model/Species Note
12	miR-33a-3p, miR-153-3p, miR-373-5p, etc.	Pleural effusion (TB patients)	RNA sequencing	Increased	[47]	Linked to lipid metabolism and immune signaling, relevant to mycobacterial persistence.	Pleural TB	Human clinical samples.
13	miR-3120-5p, miR-489-3p, miR-4669-5p, etc.	Pleural effusion (LTBI patients)	sRNA sequencing	Decreased	[47]	Reduced expression may reflect immune quiescence during latent infection.	Latent TB infection (LTBI)	Human clinical samples.
14	miR-143-3p, miR-210-3p, miR-20a-5p, etc.	Serum (LTBI patients)	sRNA sequencing	Increased	[48]	miR-210 associated with hypoxia responses; miR-143 linked to immune regulation.	Latent TB infection (LTBI)	Human serum-derived exosomes.
15	miR-23b, miR-17, miR-584, etc.	Serum (ATB patients)	sRNA sequencing	Increased	[48]	miR-23b and miR-17 are implicated in inflammatory signaling and macrophage function.	Active TB (ATB)	Human serum-derived exosomes.

Note: MicroRNA (miR), lethal-7 (let-7), reverse transcription PCR (RT-PCR), quantitative real-time polymerase chain reaction (qPCR), RNA sequencing (RNA-seq), small RNA sequencing (sRNA-seq), active tuberculosis (ATB), latent tuberculosis infection (LTBI).

4 Exosome Isolation, Detection, and Analytical Technologies

4.1 Exosome Isolation and Characterization Methods

The process of exosomal antigen detection begins with the isolation of exosomes from biofluids, followed by analysis of the exosomal cargo. Basic procedures for exosome isolation are represented in Fig. 3.

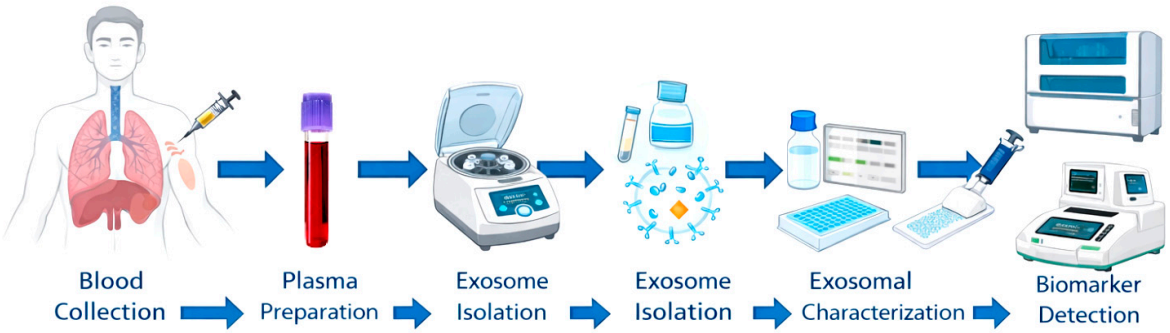

Figure 3: Schematic illustration of stepwise exosomal isolation for biomarker diagnosis. BioRender (BioRender.com, version 2024.1, BioRender, Toronto, ON, Canada).

Ultracentrifugation remains the most commonly used method, characterized by high-speed centrifugation (100,000–120,000× *g*) for pelleting exosomes, making it the gold standard. It is highly effective but time-consuming and demands sophisticated equipment. Moreover, it produces impure samples because of the concomitant proteins that are co-isolated. Despite these limitations, ultracentrifugation is still widely adopted as a reference method against which newer techniques are evaluated. In comparison, Size-Exclusion Chromatography (SEC) uses porous columns to separate exosomes by size, enabling higher purity and improved scalability.

Use porous columns to separate exosomes by size and gain high purity and scalability. Though less harsh on the vesicles, these methods require optimization for TB samples [49]. Another approach, immunoaffinity capture, is a highly selective but expensive method of isolating vesicles that employs antibodies against exosome markers such as CD63 and CD9 or TB antigens such as ESAT-6 [50]; however, its applicability is restricted by the availability and cost of suitable antibodies. Precipitation-based methods, which use aggregating reagents (such as ExoQuick) to precipitate exosomes and facilitate centrifugation, offer a rapid and simple alternative [51]. Nevertheless, these approaches carry a significant risk of non-specific contamination, which can compromise downstream analyses. Taken together, each isolation strategy presents distinct advantages and limitations in terms of purity, yield, cost, and scalability, underscoring the need for careful method selection and, in some cases, the combination of techniques depending on the study objectives.

During the initial stages of exosome analysis, effective separation from complex biofluids such as blood, sputum, and urine is essential, as these matrices contain numerous contaminating proteins and particles that can interfere with downstream analyses [52]. Following isolation, exosomes are further evaluated to determine their size, concentration, and molecular cargo, which collectively inform their biological relevance and diagnostic utility. Traditional isolation techniques, particularly ultracentrifugation at forces of 100,000–120,000× *g*, remain widely regarded as the gold standard because of their ability to yield large quantities of exosomes. However, this approach is associated with notable limitations, including the need for expensive instrumentation, prolonged processing times of 4–6 h, and reduced sample purity due to the co-isolation of soluble proteins and other vesicular components.

In response to these challenges, recent innovations have focused on improving isolation speed, accessibility, and cost-effectiveness while maintaining acceptable levels of purity. Microfluidic-based platforms have emerged as a particularly promising alternative, employing miniaturized channels and filtration systems to isolate exosomes based on size, morphology, or surface markers such as CD63 or ESAT-6 [53]. These devices can process microliter-scale samples within minutes and achieve purity levels comparable to ultracentrifugation. Notably, TB-specific microfluidic chips designed to target *Mycobacterium tuberculosis* (Mtb) antigens are under active development and show considerable potential for rapid analysis of sputum and blood samples, supporting their future application in point-of-care diagnostics.

Immunoaffinity-based isolation represents another targeted strategy, relying on magnetic beads or functionalized chips coated with antibodies against exosomal markers such as CD9 or TB-associated antigens, including lipoarabinomannan (LAM) [54]. This approach enables highly selective enrichment of TB-relevant exosomes from complex biofluids, particularly urine. Despite its specificity, immunoaffinity capture remains constrained by the high cost of antibodies and limited scalability, which currently restricts its widespread implementation in large-scale or low-resource screening programs. Acoustic trapping techniques offer a centrifugation-free alternative by exploiting sound waves to separate exosomes based on size, achieving processing times of less than one hour [55]. While the relatively low equipment requirements make this method attractive for use in resource-limited settings, further optimization is necessary to ensure consistent performance across diverse TB-related biofluids. In contrast, precipitation-based methods using commercial reagents such as ExoQuick provide a rapid and user-friendly means of exosome recovery via low-speed centrifugation [56]. However, their susceptibility to non-specific contamination limits their suitability for sensitive TB-focused analyses.

Following isolation, rigorous characterization of exosomes is critical for quality control and validation. Techniques such as nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS) are routinely employed to quantify vesicle size distribution and concentration, ensuring consistency and reliability of isolated samples [57]. Morphological confirmation is typically achieved through transmission electron microscopy, while flow cytometry using fluorescently labelled antibodies enables profiling of exosome surface markers [58]. Together, these complementary methods confirm the identity of TB-associated exosomes, including those carrying markers such as LAM. Nevertheless, the dependence of many characterization techniques on centralized laboratory infrastructure poses challenges for field-based applications. The development of portable and miniaturized analytical platforms, including emerging portable NTA devices, may help overcome these barriers and advance the feasibility of exosome-based tuberculosis diagnostics in resource-limited settings.

4.2 Antigen Detection and High-Throughput Screening

This method employs antibodies aimed at specific antigens (like LAM & ESAT-6) for detection [59]. It is sensitive and readily available but requires known targets. Enzyme-linked immunosorbent assay (ELISA), therefore, remains a practical choice for targeted antigen detection when prior knowledge of TB-specific markers is available. In contrast, mass spectrometry uses powerful techniques to comprehensively profile exosomal proteins and identify novel TB antigens [60]. While potent, it is expensive and dependent on the laboratory facilities. Western blotting is commonly used to confirm the presence of specific antigens, such as Ag85B, with a high degree of specificity; however, it is low-throughput and only semi-quantitative [61]. Polymerase chain reaction (PCR) offers a high level of sensitivity for detecting exosomal *M. tuberculosis* DNA or host miRNAs and can complement protein-based analyses, although it is sensitive but not widely used on the sought-after antigens [62]. In addition to these detection approaches, nanoparticle tracking

analysis (NTA) is employed to quantify the size and concentration of exosomes, supporting quality control before antigen detection rather than direct antigen identification [57]. Collectively, these techniques vary in sensitivity, specificity, throughput, and resource requirements, and are often applied in combination to enhance the robustness and interpretability of exosome-based TB antigen detection.

There is a need to further screen for TB-specific exosomal antigens such as ESAT-6 and Ag85B [63]. These antigens require high-throughput screening due to the complex nature of the cargo [64]. Traditional methods like ELISA or Western blotting are not suitable for high-throughput screening as they focus on known antigens one at a time. As a result, discovery-oriented approaches that enable simultaneous interrogation of multiple targets are increasingly required to advance exosome-based TB biomarker research.

Mass Spectrometry Based Proteomics: The use of liquid chromatography (LC-MS/MS) allows for the examination of thousands of exosomal proteins at once [65]. This includes the examination of *M. tuberculosis* markers like CFP-10 and host responses such as TNF-α. Although sensitive and comprehensive, these tests are expensive and are confined to laboratories, which limits their use in low and middle-income countries. Nevertheless, mass spectrometry remains a powerful discovery tool and is often used as a reference platform for validating candidate exosomal biomarkers.

Antibody Microarrays: These chips, containing hundreds of antibodies specific to TB antigens and grasping multiple targets in a single exosome sample, are able to implement multiplexing alongside speed and efficiency [66]. They have noted increased levels of LAM and ESAT-6 in exosomes from patients with active TB; however, tailored diagnostic arrays remain uncommon. The limited availability of standardized and clinically validated antibody panels currently restricts the broader implementation of this technology.

Aptamer-Based Screening: Specific antigens are bound by high-affinity synthetic DNA/RNA molecules called aptamers. Aptamer high-throughput libraries examine exosomal cargo in large quantities, uncovering new TB biomarkers [67]. Although these settings with low stability and costs are advantageous, the development of *M. tuberculosis* antigens lags. Further optimization and validation of TB-specific aptamers are therefore required before widespread diagnostic adoption.

Surface-Enhanced Raman Spectroscopy (SERS): This focuses on the amplification of molecular signals from exosomal antigens using nanoparticles, which enables rapid, label-free screening [68]. Preliminary research was able to detect *M. tuberculosis* lipids in exosomes, but sensitivity still needs improvement. Advances in nanoparticle design and signal enhancement may help address these current technical limitations.

These methods demonstrate the ability to uncover TB-specific signatures—for example, increased ESAT-6 levels in active vs. latent TB, but all face difficulties in reproducibility, validation in cross-diverse cohorts, and reproducibility. To overcome these shared challenges, future efforts should emphasize standardization, miniaturization, and automation to enable scalable clinical translation of high-throughput exosomal screening platforms.

4.3 Integration with Omics Technologies

In the case of TB, blood and sputum are the main specimens, but urine and CSF are being investigated for extrapulmonary cases. Other difficulties include exosome heterogeneity, low concentrations of exosomes in dilute fluids, and the need for portable, inexpensive technologies in resource-poor environments such as low and middle-income countries (LMICs). A detailed summary of the origin of exosomes, isolation techniques, and identified cargo in studies on *M. tuberculosis* Infection is presented in Table 3.

Table 3: Summary of Exosomes' origin, isolation technique, and identified cargo in studies on *M. tuberculosis* Infection.

No.	Exosome Source	Isolation Method	Identified Cargo	Reference	Notes/Limitations
1	*M. tuberculosis*-infected macrophages	Sucrose-gradient ultracentrifugation, Ultracentrifugation	Lipoarabinomannan, 19-kDa lipoprotein	[69]	Possible co-isolation of heterogeneous EVs; traceability limited; negative controls not fully discussed
2	*M. tuberculosis*-infected macrophages	Ultracentrifugation	miRNA, mRNA	[70]	Purity concerns; potential loss of vesicles; negative controls not mentioned
3	Serum (LTBI individuals)	ExoQuick	*M. tuberculosis* peptides (Ag85C, DnaK, HspX, Ag85A, etc.)	[70]	Co-isolation of serum proteins is possible; limited discussion on contaminants; negative controls are limited
4	*M. tuberculosis*-infected and CFP-treated macrophages	Sucrose-gradient ultracentrifugation	Mycobacterial proteins (Antigen 85C, GlnA, 19-kDa LpqH, etc.)	[69]	Traceability limited; loss during gradient fractionation; negative controls not fully described
5	*M. tuberculosis*-infected macrophages	ExoQuick, Ultracentrifugation	19-kDa lipoprotein	[71]	Mixing ExoQuick with ultracentrifugation without discussion of contaminants; co-isolation likely; purity concerns
6	*M. tuberculosis*-infected macrophages	ExoQuick, Ultracentrifugation	*M. tuberculosis* RNA	[72]	Same as above: potential contaminants not addressed; heterogeneous EV populations; negative controls limited
7	Serum (TB patients)	ExoQuick, Sucrose-gradient ultracentrifugation	Host proteins (KYAT3, SERPINA1, HP, APOC3)	[73]	Purity issues; co-isolation of non-EV proteins; negative controls limited; traceability not detailed
8	Serum (TB patients)	ExoQuick	*M. tuberculosis* peptides (Ag85B, Ag85C, Apa, BfrB, GlcB, HspX, KatG, etc.)	[69]	Same as above: co-isolation likely; contaminants not discussed; negative controls limited
9	Urine (TB patients)	Centrifugation	Lipoarabinomannan, CFP-10	[74]	Potential loss of EVs; co-isolation with urine proteins; traceability and controls are limited
10	*M. tuberculosis*-infected macrophages	Sucrose-gradient ultracentrifugation	Lipoproteins	[75]	Loss during fractionation; limited negative controls; heterogeneous EVs possible
11	Serum (TB patients)	Ultracentrifugation	miRNAs (hsa-miR-1246, hsa-miR-2110, hsa-miR-370-3p, hsa-miR-28-3p, hsa-miR-193b-5p, etc.)	[74]	Purity and co-isolation concerns; negative controls not fully described
12	Serum (TB patients)	ExoQuick	miRNAs	[76]	Contaminant co-isolation likely; limited discussion on purity and traceability; negative controls limited

Elevating exosome analyses, Omics technologies such as proteomics, transcriptomics, and others examine their molecular intricacies, providing crucial information on TB pathogenesis and potential biomarkers. These high-dimensional approaches enable a systems-level understanding of host–pathogen

interactions that cannot be captured by single-analyte techniques. Using LC-MS/MS and shotgun proteomics, we not only map exosomal proteins but also track risk markers like HspX in LTBI and host markers such as IL-1β in active TB [77]. The integration of proteomics data into curated repositories such as UniProt enhances the refinement of TB-specific biomarker panels; however, their stringent data processing requirements necessitate advanced bioinformatics expertise, which can limit accessibility.

Transcriptomic analyses further expand the diagnostic and mechanistic potential of exosome research. RNA sequencing of exosomal nucleic acids (RNA-seq) uncovers regulatory microRNAs such as miR-155, *M. tuberculosis*–encoded RNAs, and host gene targets involved in immune modulation [78]. These RNA-based signatures offer valuable insights into disease activity and immune status. Although single-cell RNA sequencing of exosome-producing cells could theoretically enable precise discrimination of infection stages, the inherently low RNA content of exosomes poses technical challenges and increases susceptibility to analytical variability.

Greater insight can be achieved through multi-omics integration, where proteomic and transcriptomic datasets are analyzed in parallel to identify correlated molecular signatures. For instance, a TB exosome study demonstrated an association between miR-146a expression and IL-10 levels, highlighting coordinated regulation at the RNA and protein levels [79]. The application of machine learning algorithms to such integrated datasets has shown promise in developing predictive models of disease state or treatment response, with reported accuracies of 80–90% in preliminary studies. Although comparatively less explored, lipidomics may further enhance TB diagnostics by incorporating signatures of *M. tuberculosis*–derived lipids, including lipoarabinomannan (LAM).

Omics-based analyses provide compelling evidence of disease progression and severity, exemplified by the upregulation of exosomal miR-21 in multidrug-resistant TB [39]. Recent advances in cloud-based computing, user-friendly analytical pipelines, and simplified assay kits represent important steps toward broader implementation of omics approaches. These developments are particularly relevant for low- and middle-income countries, where resource limitations and restricted access to high-performance computing have historically constrained the adoption of omics-driven diagnostics.

5 Clinical Application in Tuberculosis Diagnosis

5.1 Diagnostic and Prognostic Application

Detecting and treating tuberculosis in patients as early as possible is crucial for reducing its transmission and improving health outcomes [80]. However, traditional approaches tend to overlook cases right before symptoms worsen. Exosomes, which are present in blood, sputum, urine, and saliva, act as markers of infection in its early stages [81]. These exosomes, which are shed by macrophages and dendritic cells during *M. tuberculosis* infection, contain bacterial antigens such as ESAT-6 and LAM, as well as inflammatory molecules like TNF-α and miR-155, signaling infection before bacterial loads become detectable in sputum [30].

Exostosomic biomarkers could help during the preclinical phase when sputum microscopy has low sensitivity (20–60%) [82]. For example, some researchers have identified *M. tuberculosis*-specific proteins in blood-derived exosomes from asymptomatic individuals who later progressed to active TB, indicating a predictive capability [83]. Exosomes found in urine are ideal for HIV-TB co-infection, where sputum is scarce. Their lipid bilayer encapsulation enhances stability and ensures that these markers are preserved, unlike free proteins that get degraded in circulation.

Exosomes have potential uses in extrapulmonary TB (~15–20% of cases) where sputum tests are irrelevant [84]. Exosomes in cerebrospinal fluid or blood containing Mtb antigens could accelerate the

diagnosis of TB meningitis and decrease the wait time associated with invasive biopsies [85]. However, while timely detection with exosomes is beneficial, population variability requires extensive validation and standardization in testing to establish consistency and reliability.

The sensitivity and specificity of exosomal biomarkers provide them with diagnostic credibility. In some contexts, initial research indicates that exosomal antigen biomarkers may outperform other approaches. For instance, detecting ESAT-6 and CFP-10 in the exosomes of active TB patients by ELISA has shown approximately 80–90% sensitivity in preliminary studies, which exceeds that of sputum smear microscopy (20–60%) and is comparable to GeneXpert (85–90% in smear-positive cases) [84,86]. With smear-negative TB more frequently seen in HIV patients, exosomal markers could originate from fluids other than sputum, providing potentially greater sensitivity (70–80%) than GeneXpert (50–60%) [87].

The specificity of *M. tuberculosis* is greatly enhanced by its unique antigens, such as ESAT-6, which is absent in BCG and non-tuberculous mycobacteria (NTM), although LAM shows cross-reactivity with some NTM species [88,89]. LAM demonstrates partial overlap with NTM mycobacterial species, which diminishes its specificity further (85–90% specificity) [90]. However, confirming that these antigens are indeed contained within exosomes, rather than free or transported by other particles, is essential. Proteomic analyses have shown that more exosomal markers, such as ESAT-6, Ag85B, and specific host miRNAs, can enhance specificity beyond 95%.

Exosomes in blood and sputum also show variation in both sensitivity and specificity depending on the stage of the disease. While blood exosomes might dilute weakly abundant antigens, those obtained from sputum carry higher concentrations but are more difficult to obtain from younger children. The paucibacillary nature of extrapulmonary TB also makes it potentially less sensitive [7]. While exosomes are less conclusive than culture tests (which demonstrate an 80–90% sensitivity and near 100% specificity), exosomes do offer a quicker alternative and could be useful for screening or early detection rather than definitive diagnosis.

Mycobacterial proteins culture filtrate protein 10 (CFP-10) and other antigenic determinants have been found in exosomes isolated in the plasma of TB patients, which are highly expressed in active and latent TB as compared to healthy controls and thus could serve as diagnostic biomarkers with high discriminative ability (e.g., area under the curve (AUC) values > 0.9) in preliminary studies.

Besides, aptamers and electrochemical biosensors to CFP-10 and early secreted antigenic target 6 (ESAT-6) (and their complexes) are highly sensitive and specific in clinical sputum samples, providing a pathway to portable, rapid detection that could overcome limitations of traditional smear microscopy, especially in smear-negative, extrapulmonary, and HIV-related TB.

New technologies like microfluidic exosome isolation, surface-enhanced Raman spectroscopy (SERS) also hold promise of providing sensitive, label-free detection of vesicle cargo. Even though standardized protocols of isolation and quantification are still a challenge, and ethical issues regarding patient consent and fair access to emerging diagnostics are paramount, the incorporation of the advances into point-of-care systems could significantly benefit TB detection in resource-constrained environments.

5.2 Applications in Latent vs. Active TB Diagnosis

The ability to differentiate latent TB infection from active TB is one of the core diagnostic capabilities of the test, as only 5–10% of the 2 billion people known to be latently infected actually progress to an active disease [91]. Current methods, including the TST and interferon-gamma release assay (IGRAs)—both of which only identify LTBI—fail to differentiate it from active TB, as well as make any predictive assessments

regarding progression [92]. They are also exosome-reliant, which offers the analysis of disease state through their cargo profiles.

In active tuberculosis (TB), exosomes from infected cells are associated with the bacterial virulence factors (e.g., ESAT-6, LAM) and pro-inflammatory miRNAs (like miR-155) proportional to immune response and bacterial replication [93,94]. There are reports of higher antigen loads in blood and sputum exosomes of actively infected cases compared to those with latent TB infection (LTBI), and these can be detected using ELISA or PCR. In LTBI, exosomes store dormant HspX latency-associated exosomes and anti-inflammatory mediators such as miR-146a, indicating dormancy [95]. Differential miRNA signatures—increased miR-155 for active TB versus elevated miR-223 for LTBI—serve as additional distinguishers [96].

5.3 Point-of-Care (POC) Applications and Future Directions

Translating exosome technologies into POC diagnostics is crucial for TB control in high-burden, low-resource settings. Current laboratory-based techniques (e.g., MS, ultracentrifugation) are not feasible for rural LMICs due to their cost, infrastructure requirements, and technical complexity. Consequently, significant efforts are being directed toward developing portable, low-cost, and user-friendly platforms that can maintain acceptable diagnostic performance outside of centralized laboratories.

Lateral flow assays (LFAs) are among the most advanced POC approaches for exosome-based TB detection. Prototypes have demonstrated sensitivities of 70–80%, comparable to smear microscopy, with costs under $5 per test [97]. Like rapid COVID-19 diagnostic tests, LFAs employ antibodies to identify exosomal TB antigens such as ESAT-6 in blood or urine samples, delivering results within 15–30 min. Their simplicity, affordability, and rapid turnaround time make LFAs particularly attractive for decentralized screening and early case detection.

In parallel, handheld biosensor-based devices are under active development and early clinical evaluation. These platforms integrate microfluidics with electrochemical or optical biosensors incorporating nanomaterials such as gold nanoparticles, enabling the detection of exosomal markers at picomolar concentrations [98]. Such devices are designed to achieve GeneXpert-like diagnostic speeds of approximately two hours without the need for sophisticated laboratory infrastructure. If successfully validated, biosensor technologies could bridge the gap between laboratory-grade sensitivity and true field deployability.

Paper-based microfluidic systems offer an additional low-cost alternative, using capillary action to isolate and detect exosomes on disposable, single-use chips suitable for sputum or saliva samples [99]. Early TB assays based on these platforms have reported a sensitivity of approximately 75%, and their simple design supports large-scale manufacturing. The scalability and minimal resource requirements of paper-based devices position them as promising candidates for mass screening programs in resource-limited settings.

Collectively, these emerging POC technologies underscore a shift toward decentralized TB diagnostics that prioritize accessibility, affordability, and speed. Continued optimization, large-scale clinical validation, and integration with existing TB control programs will be essential to realize their full potential in real-world settings.

Screening field tests could benefit from the addition of miniaturized MS and RNA-seq tools, but they must be lower in cost [100]. There is improved accuracy for predicting TB outcomes from multi-marker profiles as artificial intelligence enhances exosomal data interpretation [101]. These protocols are essential for gaining regulatory approval, as they ensure the consistency of isolation and detection measures across borders. Collaborative feasibility of POC trials in India, South Africa, and other regions considers issues of power, training, and supply chain [102].

6 Challenges, Ethics, Future Directions and Conclusion

6.1 Technical, Biological, and Standardization Challenges

There are several challenges to incorporating exosome technologies into TB care. Isolation techniques, such as ultracentrifugation, and detection Methods like mass spectrometry are laborious and expensive (costing $100–500 per sample), slow, and unsuited for LMIC clinics [103]. Furthermore, exosome heterogeneity as well as low yields of urine pose reliability issues. Most evidence stems from small, controlled studies, and diverse large-scale trials are lacking. Moreover, the sensitivity/specificity of 70–90% is significantly lower than the close to 100% feasibility of culture, and prognostic accuracy over time remains unvalidated [104].

Infrastructure: Regions with the highest burden lack the required power, labs, and trained personnel to perform exosome analysis. Controlled environments for simpler techniques, such as precipitation kits, are scarce in rural areas. Funds from LMICs do not meet the demand for advanced tools, such as GeneXpert cartridges costing $10–20. Without optimization, exosome assays could double that cost. There is an unmet demand for scaling production and reducing reagent costs. No protocols exist for the isolation of exosomes, antigen retrieval, or biomarker cutoff values. This creates potential for inconsistent outcomes in different settings. Social stigma affects testing and requires follow-up monitoring, while access refers to travel to the facilities. These issues are similar to those with the initial rollout of GeneXpert, indicating low-slope adoption [105].

There are regulatory and ethical issues to consider with the clinical application of exosome-based TB diagnostic tools:

6.2 Regulatory Barriers

There is a regulatory barrier to obtaining approval, as there is an expectation for extensive proof of safety, efficacy, and reproducibility. Large multi-center studies need to be conducted to demonstrate that the use of exosome-based diagnostics will meet or exceed the benchmarks set by GeneXpert [106]. Exosome therapeutics, which are considered biologics, face even more stringent requirements [107]. Before clinical testing, there needs to be preclinical safety data, such as immunogenicity, as well as compliance with Good Manufacturing Practices (GMP). In low and middle-income countries, these bodies do not have the resources and capacity to assess new technologies, which slows down the approval process.

6.3 Standardization Gaps

There is a need for compliance from these governing bodies through widely accepted documented processes, and protocols have to be validated, such as International Organization for Standardization (ISO) guidelines. However, there is a lack of consensus regarding exosomal processes.

6.4 Ethical Issues

Informed consent is particularly difficult to obtain in sensitive populations like the rural poor living with HIV, as they are not equipped to navigate intricate diagnostic processes. There are also notable privacy concerns that come with exosomal profiling due to the possibility of nucleic acids disclosing non-TB-related health information, such as cancer risk. Equitable access to diagnostics is one of the primary ethical concerns; expensive diagnostics may deepen inequity due to an increasing focus on rich regions and neglect of lower-middle-income countries.

6.5 Therapeutic Risks

Tailored exosomes are associated with effects on non-target sites of action or immune response complications, which need extensive monitoring for safety over time. In areas with a high incidence of TB, there is a great need for ethical community involvement to prevent their use for exploitation within trial settings. There is a need to create a balance between the regulation of exosome technologies and the ethical considerations concerning patients and innovation to ensure that frameworks for patents are in place.

6.6 Emerging Research Directions

The development of TB solutions through exosomes has been made possible by novel research breakthroughs. Focusing on the small RNAs Mtb is known to excrete, as well as some of the proteins produced by the host, like cathelicidin, researchers are attempting to add more specificity to other existing biomarkers, such as ESAT-6 and LAM, particularly for MDR-TB or LTBI progression. Moreover, lipidomics aims to discover distinct signatures where Mtb cell wall lipids are targeted. There is ongoing pre-clinical research on genetically modifying exosomes so that they can carry some anti-TB drugs, like rifampicin, and immune enhancers like IFN-γ to reduce the toxic effects while sharpening the effectiveness of the drugs. Currently, mouse models show a 30% increase in bacterial clearance, with human trials anticipated in the near future. Some pilot studies have demonstrated that with the aid of AI, integrating data from exosomal proteomics and miRNA offers the ability to predict the outcome of TB with 80–90% accuracy. Rapid point-of-care testing could become a reality due to AI-powered biosensors. The focus of research now moves to urine and salivary exosomes, bypassing the issues posed by sputum collection in children or extrapulmonary TB. Assays based on urine from HIV-TB cohorts demonstrate 70% sensitivity, thus strengthening the potential for point-of-care use.

Studies investigate how Mtb exploits exosome release, for instance, through the ESX-1 secretion system, shedding light on intervention targets. The involvement of exosomes in granuloma upkeep and reactivation has become an area of increasing interest. Exosome research continues to advance, aided by support from the NIH and the Gates Foundation. However, the development of clinical applications remains a primary challenge.

7 Conclusions

In order to reach the desired outcomes, purposeful research on exosomes is critical. A central requirement for progress is rigorous validation supported by standardization. The existing proof for smaller cohorts of 20–100 participants is insufficient. It is imperative to conduct multi-center trials in TB high-prevalence (LMICs) such as India and South Africa, where exosomal diagnostics and prognostics are tested against gold standards (culture, GeneXpert). These milestones need to be reached with age and ethnic diversity: HIV and diabetes as co-morbidities alongside TB, with an expectation of above 90% sensitivity/specificity. It is elementary and pressing to establish universal isolation protocols like microfluidics, biosensor detection, and define a set of candidate biomarkers, ESAT-6 level thresholds. Cross-border collaboration is therefore essential, building on MISEV guidelines, TB standard benchmarks for regulatory endorsement, and consistency in clinical endorsement.

Beyond validation, translation into real-world settings depends on simplifying and integrating technologies. Research must focus on portable and low-cost diagnostic tools like lateral flow assays (which cost $5 per test) or handheld biosensors, suited for clinics in low and middle-income countries (LMICs). Streamlining omics workflows, such as a miniaturized form of mass spectrometry, could improve access to multi-marker analysis.

Therapeutic development represents an additional and promising frontier. Preclinical results from engineered exosomes, such as 30% enhanced bacterial clearance in mice, justify human trials to evaluate the safety, efficacy, and scalability of exosome-based drug delivery and immunotherapy approaches.

Sustained funding and coordinated collaboration act as the enabling foundation for all these advances. International health organizations like WHO and NIH, as well as the private sector, need to support this work and facilitate collaboration among researchers, clinicians, and policymakers to develop solutions oriented towards LMICs. By aligning validation, technological simplification, combination strategies, and therapeutic exploration, exosome research can move from a concept of great promise into practical application, helping to narrow the gap between the global burden of tuberculosis and its eventual eradication.

Acknowledgement: The authors are thankful to the Deanship of Graduate Studies and Scientific Research at the University of Bisha for supporting this work through the Fast-Track Research Support Program.

Funding Statement: The authors received no specific funding for this study.

Author Contributions: The authors confirm their contributions to the paper as follows: Study conception and design: Rashid Mir; manuscript preparation: Mohammad Muzaffar Mir, Rashid Mir, Ulfat Jan, Badr A. Alsayed, Mohammed M. Jalal, Malik A. Altayar, Hanan M. Aljammaz, Zinab Alatawi; review and editing: Mohammad Muzaffar Mir, Rashid Mir, Ulfat Jan Badr A. Alsayed, Mohammed M. Jalal, Malik A. Altayar, Hanan M. Aljammaz, Zinab Alatawi; visualization: Khalid A. Alfifi, Basmah M. Alenzi, Mohammad Tanveer Khaji, Umair Ismail, Ghada Mohamed, Nada Zai Sageer, Abdullatif Taha Babakr, Saba M. Mir, Ulfat Jan; supervision: Mohammad Muzaffar Mir, Rashid Mir, Badr A. Alsayed, Mohammed M. Jalal, Malik A. Altayar, Hanan M. Aljammaz, Zinab Alatawi. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: Not applicable.

Ethics Approval: Not Applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Falzon D, Zignol M, Bastard M, Floyd K, Kasaeva T. The impact of the COVID-19 pandemic on the global tuberculosis epidemic. Front Immunol. 2023;14:1234785. [CrossRef].
2. Sossen B, Kubjane M, Meintjes G. Tuberculosis and HIV coinfection: Progress and challenges towards reducing incidence and mortality. Int J Infect Dis. 2025;155:107876. [CrossRef].
3. Villar-Hernandez R, Ghodousi A, Konstantynovska O, Duarte R, Lange C, Raviglione M. Tuberculosis: Current challenges and beyond. Breathe. 2023;19(1):220166. [CrossRef].
4. Dheda K, Schwander SK, Zhu B, van Zyl-Smit RN, Zhang Y. The immunology of tuberculosis: From bench to bedside. Respirology. 2010;15(3):433–50. [CrossRef].
5. Kiazyk S, Ball TB. Latent tuberculosis infection: An overview. Can Commun Dis Rep. 2017;43(3–4):62–6. [CrossRef].
6. Chandra P, Grigsby SJ, Philips JA. Immune evasion and provocation by *Mycobacterium tuberculosis*. Nat Rev Microbiol. 2022;20(12):750–66. [CrossRef].
7. Chen Y, Fan L, Ren Z, Yu Y, Sun J, Wang M, et al. Sensitive diagnosis of paucibacillary tuberculosis with targeted next-generation sequencing: A molecular diagnostic study. BMC Med. 2025;23(1):178. [CrossRef].
8. Zhuang HH, Qu Q, Long WM, Hu Q, Wu XL, Chen Y, et al. Ceftazidime/avibactam versus polymyxin B in carbapenem-resistant *Klebsiella pneumoniae* infections: A propensity score-matched multicenter real-world study. Infection. 2025;53(1):95–106. [CrossRef].
9. Lv K, Zhang Y, Yin G, Li X, Zhong M, Zhu X, et al. Extracellular vesicles derived from lung M2 macrophages enhance group 2 innate lymphoid cells function in allergic airway inflammation. Exp Mol Med. 2025;57(6):1202–15. [CrossRef].

10. Di Bella MA. Overview and update on extracellular vesicles: Considerations on exosomes and their application in modern medicine. Biology. 2022;11(6):804. [CrossRef].
11. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;367(6478):eaau6977. [CrossRef].
12. Corrado C, Raimondo S, Chiesi A, Ciccia F, De Leo G, Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: Basic science and clinical applications. Int J Mol Sci. 2013;14(3):5338–66. [CrossRef].
13. Osaid Z, Haider M, Hamoudi R, Harati R. Exosomes interactions with the blood-brain barrier: Implications for cerebral disorders and therapeutics. Int J Mol Sci. 2023;24(21):15635. [CrossRef].
14. Tai YL, Chen KC, Hsieh JT, Shen TL. Exosomes in cancer development and clinical applications. Cancer Sci. 2018;109(8):2364–74. [CrossRef].
15. Li T, Li X, Han G, Liang M, Yang Z, Zhang C, et al. The therapeutic potential and clinical significance of exosomes as carriers of drug delivery system. Pharmaceutics. 2022;15(1):21. [CrossRef].
16. Jankovičová J, Sečová P, Michalková K, Antalíková J. Tetraspanins, more than markers of extracellular vesicles in reproduction. Int J Mol Sci. 2020;21(20):7568. [CrossRef].
17. Shen YQ, Sun L, Wang SM, Zheng XY, Xu R. Exosomal integrins in tumor progression, treatment and clinical prediction (review). Int J Oncol. 2024;65(6):1–5. [CrossRef].
18. Yang J, Zhu J, Lu S, Qin H, Zhou W. Transdermal psoriasis treatment inspired by tumor microenvironment-mediated immunomodulation and advanced by exosomal engineering. J Control Release. 2025;382:113664. [CrossRef].
19. Mosquera-Heredia MI, Morales LC, Vidal OM, Barceló E, Silvera-Redondo C, Vélez JI, et al. Exosomes: Potential disease biomarkers and new therapeutic targets. Biomedicines. 2021;9(8):1061. [CrossRef].
20. Li W, Wang S, Xu J, Xiang J. Inferring latent microRNA-disease associations on a gene-mediated tripartite heterogeneous multiplexing network. IEEE/ACM Trans Comput Biol Bioinform. 2022;19(6):3190–201. [CrossRef].
21. Ghoshal A, Verma A, Bhaskar A, Dwivedi VP. The uncharted territory of host-pathogen interaction in tuberculosis. Front Immunol. 2024;15:1339467. [CrossRef].
22. Lau NCH, Yam JWP. From exosome biogenesis to absorption: Key takeaways for cancer research. Cancers. 2023;15(7):1992. [CrossRef].
23. Xu M, Ji J, Jin D, Wu Y, Wu T, Lin R, et al. The biogenesis and secretion of exosomes and multivesicular bodies (MVBs): Intercellular shuttles and implications in human diseases. Genes Dis. 2022;10(5):1894–907. [CrossRef].
24. Sun YF, Pi J, Xu JF. Emerging role of exosomes in tuberculosis: From immunity regulations to vaccine and immunotherapy. Front Immunol. 2021;12:628973. [CrossRef].
25. Jiang H, Zhao H, Zhang M, He Y, Li X, Xu Y, et al. Hypoxia induced changes of exosome cargo and subsequent biological effects. Front Immunol. 2022;13:824188. [CrossRef].
26. Sun X, Li W, Zhao L, Fan K, Qin F, Shi L, et al. Current landscape of exosomes in tuberculosis development, diagnosis, and treatment applications. Front Immunol. 2024;15:1401867. [CrossRef].
27. Cohen SB, Gern BH, Delahaye JL, Adams KN, Plumlee CR, Winkler JK, et al. Alveolar macrophages provide an early *Mycobacterium tuberculosis* niche and initiate dissemination. Cell Host Microbe. 2018;24(3):439–46. [CrossRef].
28. Huang Y, Mei M, Fan T, Liu J, Li X, Chen X, et al. Exosomes in cerebrospinal fluid as biomarkers of bacterial infections of the central nervous system: A pilot study. Front Cell Infect Microbiol. 2026;16:1760073. [CrossRef].
29. Palacios A, Gupta S, Rodriguez GM, Prados-Rosales R. Extracellular vesicles in the context of *Mycobacterium tuberculosis* infection. Mol Immunol. 2021;133:175–81. [CrossRef].
30. Athman JJ, Wang Y, McDonald DJ, Boom WH, Harding CV, Wearsch PA. Bacterial membrane vesicles mediate the release of *Mycobacterium tuberculosis* lipoglycans and lipoproteins from infected macrophages. J Immunol. 2015;195(3):1044–53. [CrossRef].
31. Arya R, Jit BP, Kumar V, Kim JJ. Exploring the potential of exosomes as biomarkers in tuberculosis and other diseases. Int J Mol Sci. 2024;25(5):2885. [CrossRef].
32. Bhatnagar S, Shinagawa K, Castellino FJ, Schorey JS. Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response *in vitro* and *in vivo*. Blood. 2007;110(9):3234–44. [CrossRef].

33. Luo G, Zhou Z, Cao Z, Huang C, Li C, Li X, et al. M2 macrophage-derived exosomes induce angiogenesis and increase skin flap survival through HIF1AN/HIF-1α/VEGFA control. Arch Biochem Biophys. 2024;751:109822. [CrossRef].
34. Cronan MR. In the thick of it: Formation of the tuberculous granuloma and its effects on host and therapeutic responses. Front Immunol. 2022;13:820134. [CrossRef].
35. Masood KI, Irfan M, Masood Q, Yameen M, Jamil B, Ram N, et al. Latent *M. tuberculosis* infection is associated with increased inflammatory cytokine and decreased suppressor of cytokine signalling (SOCS)-3 in the diabetic host. Scand J Immunol. 2022;95(4):e13134. [CrossRef].
36. Kaushal D, Singh DK, Mehra S. Immune responses in lung granulomas during mtb/HIV co-infection: Implications for pathogenesis and therapy. Pathogens. 2023;12(9):1120. [CrossRef].
37. Kim JK, Silwal P, Jo EK. Host-pathogen dialogues in autophagy, apoptosis, and necrosis during mycobacterial infection. Immune Netw. 2020;20(5):e37. [CrossRef].
38. Testa U, Pelosi E, Castelli G, Labbaye C. miR-146 and miR-155: Two key modulators of immune response and tumor development. Noncoding RNA. 2017;3(3):22. [CrossRef].
39. Wang J, Li Y, Wang N, Wu J, Ye X, Jiang Y, et al. Functions of exosomal non-coding RNAs to the infection with *Mycobacterium tuberculosis*. Front Immunol. 2023;14:1127214. [CrossRef].
40. Mukhtar F, Guarnieri A, Brancazio N, Falcone M, Di Naro M, Azeem M, et al. The role of *Mycobacterium tuberculosis* exosomal miRNAs in host pathogen cross-talk as diagnostic and therapeutic biomarkers. Front Microbiol. 2024;15:1441781. [CrossRef].
41. Zhang D, Yi Z, Fu Y. Downregulation of miR-20b-5p facilitates *Mycobacterium tuberculosis* survival in RAW 264.7 macrophages via attenuating the cell apoptosis by Mcl-1 upregulation. J Cell Biochem. 2019;120(4):5889–96. [CrossRef].
42. Zhan X, Yuan W, Zhou Y, Ma R, Ge Z. Small RNA sequencing and bioinformatics analysis of RAW264.7-derived exosomes after Mycobacterium Bovis Bacillus Calmette-Guérin infection. BMC Genom. 2022;23(1):355. [CrossRef].
43. Kaushik AC, Wu Q, Lin L, Li H, Zhao L, Wen Z, et al. Exosomal ncRNAs profiling of mycobacterial infection identified miRNA-185-5p as a novel biomarker for tuberculosis. Brief Bioinform. 2021;22(6):bbab210. [CrossRef].
44. Tu H, Yang S, Jiang T, Wei L, Shi L, Liu C, et al. Elevated pulmonary tuberculosis biomarker miR-423-5p plays critical role in the occurrence of active TB by inhibiting autophagosome-lysosome fusion. Emerg Microbes Infect. 2019;8(1):448–60. [CrossRef].
45. Lyu L, Zhang X, Li C, Yang T, Wang J, Pan L, et al. Small RNA profiles of serum exosomes derived from individuals with latent and active tuberculosis. Front Microbiol. 2019;10:1174. [CrossRef].
46. Alipoor SD, Tabarsi P, Varahram M, Movassaghi M, Dizaji MK, Folkerts G, et al. Serum exosomal miRNAs are associated with active pulmonary tuberculosis. Dis Markers. 2019;2019:1907426. [CrossRef].
47. Wang Y, Xu YM, Zou YQ, Lin J, Huang B, Liu J, et al. Identification of differential expressed PE exosomal miRNA in lung adenocarcinoma, tuberculosis, and other benign lesions. Medicine. 2017;96(44):e8361. [CrossRef].
48. Zhang X, Bao L, Yu G, Wang H. Exosomal miRNA-profiling of pleural effusion in lung adenocarcinoma and tuberculosis. Front Surg. 2023;9:1050242. [CrossRef].
49. Guio H, Aliaga-Tobar V, Galarza M, Pellon-Cardenas O, Capristano S, Gomez HL, et al. Comparative profiling of circulating exosomal small RNAs derived from Peruvian patients with tuberculosis and pulmonary adenocarcinoma. Front Cell Infect Microbiol. 2022;12:909837. [CrossRef].
50. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: Is size exclusion chromatography the best option? Int J Mol Sci. 2020;21(18):6466. [CrossRef].
51. Panwar D, Shrivastava D, Kumar A, Gupta LK, Sampath Kumar NS, Chintagunta AD. Efficient strategy to isolate exosomes using anti-CD63 antibodies conjugated to gold nanoparticles. AMB Express. 2023;13(1):90. [CrossRef].
52. Yakubovich EI, Polischouk AG, Evtushenko VI. Principles and problems of exosome isolation from biological fluids. Biochem. 2022;16(2):115–26. [CrossRef].
53. Dilsiz N. A comprehensive review on recent advances in exosome isolation and characterization: Toward clinical applications. Transl Oncol. 2024;50:102121. [CrossRef].
54. Wu Y, Wang Y, Lu Y, Luo X, Huang Y, Xie T, et al. Microfluidic technology for the isolation and analysis of exosomes. Micromachines. 2022;13(10):1571. [CrossRef].

55. Kozhevnikova D, Chernyshev V, Yashchenok A. Progress in isolation and molecular profiling of small extracellular vesicles via bead-assisted platforms. Biosensors. 2023;13(7):688. [CrossRef].
56. Ku A, Lim HC, Evander M, Lilja H, Laurell T, Scheding S, et al. Acoustic enrichment of extracellular vesicles from biological fluids. Anal Chem. 2018;90(13):8011–9. [CrossRef].
57. Coughlan C, Bruce KD, Burgy O, Boyd TD, Michel CR, Garcia-Perez JE, et al. Exosome isolation by ultracentrifugation and precipitation and techniques for downstream analyses. Curr Protoc Cell Biol. 2020;88(1):e110. [CrossRef].
58. Comfort N, Cai K, Bloomquist TR, Strait MD, Ferrante AW Jr, Baccarelli AA. Nanoparticle tracking analysis for the quantification and size determination of extracellular vesicles. J Vis Exp. 2021;(169):e62447. [CrossRef].
59. Lai JJ, Chau ZL, Chen SY, Hill JJ, Korpany KV, Liang NW, et al. Exosome processing and characterization approaches for research and technology development. Adv Sci. 2022;9(15):2103222. [CrossRef].
60. Omar RA, Verma N, Arora PK. Development of ESAT-6 based immunosensor for the detection of *Mycobacterium tuberculosis*. Front Immunol. 2021;12:653853. [CrossRef].
61. Giri PK, Kruh NA, Dobos KM, Schorey JS. Proteomic analysis identifies highly antigenic proteins in exosomes from *M. tuberculosis*-infected and culture filtrate protein-treated macrophages. Proteomics. 2010;10(17):3190–202. [CrossRef].
62. Kruh-Garcia NA, Murray M, Prucha JG, Dobos KM. Antigen 85 variation across lineages of *Mycobacterium tuberculosis*-implications for vaccine and biomarker success. J Proteom. 2014;97:141–50. [CrossRef].
63. Arya R, Dabral D, Faruquee HM, Mazumdar H, Patgiri SJ, Deka T, et al. Serum small extracellular vesicles proteome of tuberculosis patients demonstrated deregulated immune response. Proteom Clin Appl. 2020;14(1):e1900062. [CrossRef].
64. Sun J, Zhou X, Yu J, Fang S, Duan S, Liu F. Diagnostic value of tuberculosis-specific antigens Ag85B, ESAT-6 and CFP10 in pulmonary tuberculosis. J Clin Tuberc Other Mycobact Dis. 2024;37:100486. [CrossRef].
65. Liu B, Du H, Zhang J, Jiang J, Zhang X, He F, et al. Developing a new sepsis screening tool based on lymphocyte count, international normalized ratio and procalcitonin (LIP score). Sci Rep. 2022;12(1):20002. [CrossRef].
66. Karpievitch YV, Polpitiya AD, Anderson GA, Smith RD, Dabney AR. Liquid chromatography mass spectrometry-based proteomics: Biological and technological aspects. Ann Appl Stat. 2010;4(4):1797–823. [CrossRef].
67. Zhao Z, Yang Y, Zeng Y, He M. A microfluidic ExoSearch chip for multiplexed exosome detection towards blood-based ovarian cancer diagnosis. Lab Chip. 2016;16(3):489–96. [CrossRef].
68. Fang Z, Feng X, Tang F, Jiang H, Han S, Tao R, et al. Aptamer screening: Current methods and future trend towards non-SELEX approach. Biosensors. 2024;14(7):350. [CrossRef].
69. Guerrini L, Garcia-Rico E, O'Loghlen A, Giannini V, Alvarez-Puebla RA. Surface-enhanced Raman scattering (SERS) spectroscopy for sensing and characterization of exosomes in cancer diagnosis. Cancers. 2021;13(9):2179. [CrossRef].
70. Lee JY. Diagnosis and treatment of extrapulmonary tuberculosis. Tuberc Respir Dis. 2015;78(2):47–55. [CrossRef].
71. Singh PP, Li L, Schorey JS. Exosomal RNA from *Mycobacterium tuberculosis*-infected cells is functional in recipient macrophages. Traffic. 2015;16(6):555–71. [CrossRef].
72. Cheng Y, Schorey JS. Exosomes carrying mycobacterial antigens can protect mice against *Mycobacterium tuberculosis* infection. Eur J Immunol. 2013;43(12):3279–90. [CrossRef].
73. Cheng Y, Schorey JS. Extracellular vesicles deliver *Mycobacterium* RNA to promote host immunity and bacterial killing. EMBO Rep. 2019;20(3):e46613. [CrossRef].
74. Kruh-Garcia NA, Wolfe LM, Chaisson LH, Worodria WO, Nahid P, Schorey JS, et al. Detection of *Mycobacterium tuberculosis* peptides in the exosomes of patients with active and latent *M. tuberculosis* infection using MRM-MS. PLoS One. 2014;9(7):e103811. [CrossRef].
75. Dahiya B, Khan A, Mor P, Kamra E, Singh N, Gupta KB, et al. Detection of *Mycobacterium tuberculosis* lipoarabinomannan and CFP-10 (Rv3874) from urinary extracellular vesicles of tuberculosis patients by immuno-PCR. Pathog Dis. 2019;77(5):ftz049. [CrossRef].
76. Singh PP, LeMaire C, Tan JC, Zeng E, Schorey JS. Exosomes released from *M. tuberculosis* infected cells can suppress IFN-γ mediated activation of naïve macrophages. PLoS One. 2011;6(4):e18564. [CrossRef].

77. Mehaffy C, Kruh-Garcia NA, Graham B, Jarlsberg LG, Willyerd CE, Borisov A, et al. Identification of *Mycobacterium tuberculosis* peptides in serum extracellular vesicles from persons with latent tuberculosis infection. J Clin Microbiol. 2020;58(6):e00393–20. [CrossRef].
78. Zhang M, Xie Y, Li S, Ye X, Jiang Y, Tang L, et al. Proteomics analysis of exosomes from patients with active tuberculosis reveals infection profiles and potential biomarkers. Front Microbiol. 2022;12:800807. [CrossRef].
79. Mirzaei R, Babakhani S, Ajorloo P, Ahmadi RH, Hosseini-Fard SR, Keyvani H, et al. The emerging role of exosomal miRNAs as a diagnostic and therapeutic biomarker in *Mycobacterium tuberculosis* infection. Mol Med. 2021;27(1):34. [CrossRef].
80. Musisi E, Sessolo A, Kaswabuli S, Zawedde J, Byanyima P, Kasinga S, et al. High *Mycobacterium tuberculosis* bacillary loads detected by tuberculosis molecular bacterial load assay in patient stool: A potential alternative for nonsputum diagnosis and treatment response monitoring of tuberculosis. Microbiol Spectr. 2022;10(1):e0210021. [CrossRef].
81. Yayan J, Franke KJ, Berger M, Windisch W, Rasche K. Early detection of tuberculosis: A systematic review. Pneumonia. 2024;16(1):11. [CrossRef].
82. Gouhar SA, Abd Elmageed ZY, El-Daly SM. Extracellular vesicles as dynamic biomarkers: Monitoring therapeutic responses and drug resistance in cancer. Int Rev Cell Mol Biol. 2026;399:43–112. [CrossRef].
83. Rahmati S, Bahrampour A, Nasehi M, Mirzazadeh A, Ghaderi H, Shahesmaeili A. An evaluation of the diagnostic value of sputum smears microscopy and pcr relative to sputum culture in the diagnosis of pulmonary tuberculosis: A systematic review and meta-analysis in Iran. Med J Islam Repub Iran. 2022;36:112. [CrossRef].
84. Wang N, Yao Y, Qian Y, Qiu D, Cao H, Xiang H, et al. Cargoes of exosomes function as potential biomarkers for *Mycobacterium tuberculosis* infection. Front Immunol. 2023;14:1254347. [CrossRef].
85. Biadglegne F, König B, Rodloff AC, Dorhoi A, Sack U. Composition and clinical significance of exosomes in tuberculosis: A systematic literature review. J Clin Med. 2021;10(1):145. [CrossRef].
86. Biadglegne F, Schmidt JR, Engel KM, Lehmann J, Lehmann RT, Reinert A, et al. *Mycobacterium tuberculosis* affects protein and lipid content of circulating exosomes in infected patients depending on tuberculosis disease state. Biomedicines. 2022;10(4):783. [CrossRef].
87. Gey van Pittius NC, Warren RM, van Helden PD. ESAT-6 and CFP-10: What is the diagnosis? Infect Immun. 2002;70(11):6509–11. [CrossRef].
88. Peter JG, Theron G, Muchinga TE, Govender U, Dheda K. The diagnostic accuracy of urine-based Xpert MTB/RIF in HIV-infected hospitalized patients who are smear-negative or sputum scarce. PLoS One. 2012;7(7):e39966. [CrossRef].
89. To KW, Zhang R, Lee SS. Is the new tuberculous antigen-based skin test ready for use as an alternative to tuberculin skin test/interferon-gamma release assay for tuberculous diagnosis? A narrative review. Int J Infect Dis. 2024;141:106992. [CrossRef].
90. Chen C, Huang H. Methyltransferase 3-mediated m6A modification of Switch/sucrose non-fermenting-related matrix-associated actin-dependent regulator of chromatin subfamily a member 5 promotes *Mycobacterium tuberculosis*-infected macrophage M1 polarization and inflammation. Cytojournal. 2025;22:38. [CrossRef].
91. Tran T, Bonham AJ, Chan ED, Honda JR. A paucity of knowledge regarding nontuberculous mycobacterial lipids compared to the tubercle *Bacillus*. Tuberculosis. 2019;115:96–107. [CrossRef].
92. Frahm M, Goswami ND, Owzar K, Hecker E, Mosher A, Cadogan E, et al. Discriminating between latent and active tuberculosis with multiple biomarker responses. Tuberculosis. 2011;91(3):250–6. [CrossRef].
93. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, et al. Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. Clin Microbiol Rev. 2014;27(1):3–20. [CrossRef].
94. Qu M, Zhu H, Zhang X. Extracellular vesicle-mediated regulation of macrophage polarization in bacterial infections. Front Microbiol. 2022;13:1039040. [CrossRef].
95. Sinigaglia A, Peta E, Riccetti S, Venkateswaran S, Manganelli R, Barzon L. Tuberculosis-associated microRNAs: From pathogenesis to disease biomarkers. Cells. 2020;9(10):2160. [CrossRef].
96. Wang H, Zhang Y, Zhang C, Zhao Y, Shu J, Tang X. Exosomes derived from miR-146a-overexpressing fibroblast-like synoviocytes in cartilage degradation and macrophage M1 polarization: A novel protective agent for osteoarthritis? Front Immunol. 2024;15:1361606. [CrossRef].

97. Omidfar K, Riahi F, Kashanian S. Lateral flow assay: A summary of recent progress for improving assay performance. Biosensors. 2023;13(9):837. [CrossRef].
98. Koczula KM, Gallotta A. Lateral flow assays. Essays Biochem. 2016;60(1):111–20. [CrossRef].
99. Ma X, Hao Y, Liu L. Progress in nanomaterials-based optical and electrochemical methods for the assays of exosomes. Int J Nanomed. 2021;16:7575–608. [CrossRef].
100. Jin Y, Aziz AUR, Wu B, Lv Y, Zhang H, Li N, et al. The road to unconventional detections: Paper-based microfluidic chips. Micromachines. 2022;13(11):1835. [CrossRef].
101. Dai X, Shen L. Advances and trends in omics technology development. Front Med. 2022;9:911861. [CrossRef].
102. Shanmuga Priya K, Parivakkam Mani A, Geethalakshmi S, Yadav S. Advancements in artificial intelligence for the diagnosis of multidrug resistance and extensively drug-resistant tuberculosis: A comprehensive review. Cureus. 2024;16(5):e60280. [CrossRef].
103. Diaz G, Wolfe LM, Kruh-Garcia NA, Dobos KM. Changes in the membrane-associated proteins of exosomes released from human macrophages after *Mycobacterium tuberculosis* infection. Sci Rep. 2016;6:37975. [CrossRef].
104. Huang C, Pan L, Shen X, Tian H, Guo L, Zhang Z, et al. Hsp16.3 of *Mycobacterium tuberculosis* in exosomes as a biomarker of tuberculosis. Eur J Clin Microbiol Infect Dis. 2021;40(11):2427–30. [CrossRef].
105. Williams V, Calnan M, Edem B, Onwuchekwa C, Okoro C, Candari C, et al. GeneXpert rollout in three high-burden tuberculosis countries in Africa: A review of pulmonary tuberculosis diagnosis and outcomes from 2001 to 2019. Afr J Lab Med. 2022;11(1):1811. [CrossRef].
106. Chen H, Liang S, Li W, Cao T, Geng S, Liu J. Exosomal cargoes as potential biomarkers for latent tuberculosis infection: A promising frontier in diagnosis? Clin Chim Acta. 2026;578:120523. [CrossRef].
107. Deng T, Zhang Y, Yao Y, Ye P, Zhang D, Cheng F, et al. Exosome therapeutics: A paradigm shift in skin repair through multidimensional immunomodulation and biomaterial-driven delivery. Biomed Pharmacother. 2025;193:118830. [CrossRef].