Field-deployable immunodiagnostics for poverty-associated infectious diseases: development and use cases in public health

Pierneef, L.

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General introduction

Louise Pierneef[1]

[1]Leiden University Center for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

Leprosy: a neglected tropical disease of ongoing global concern

Leprosy, also known as Hansen disease, is a chronic, poverty-associated infectious disease caused primarily by *Mycobacterium leprae (M. leprae)* [1], and in some cases by *Mycobacterium lepromatosis (M. lepromatosis)* [2, 3]. The disease predominantly affects the skin, peripheral nerves, mucosa of the upper respiratory tract, and eyes, leading to a wide spectrum of clinical manifestations ranging from localized skin lesions to systemic deformities and permanent disabilities [1, 4]. In addition to its physical manifestations, leprosy imposes a considerable psychosocial burden [5, 6]. Affected individuals frequently face stigma, discrimination, and social exclusion, which can delay diagnosis and treatment, exacerbate disability, and reduce quality of life [7-9].

Despite being one of the oldest known human diseases [10, 11], leprosy continues to pose a substantial public health challenge, particularly in low- and middle-income countries (LMICs) where healthcare infrastructure is often limited [12]. It is the second most prevalent mycobacterial disease after tuberculosis (TB) [13, 14]. According to the World Health Organization (WHO), 172,717 new leprosy cases were reported globally in 2024 [15, 16]. A large proportion of these cases are concentrated in a few endemic countries, with India, Brazil, and Indonesia consistently bearing the highest burden (Figure 1).

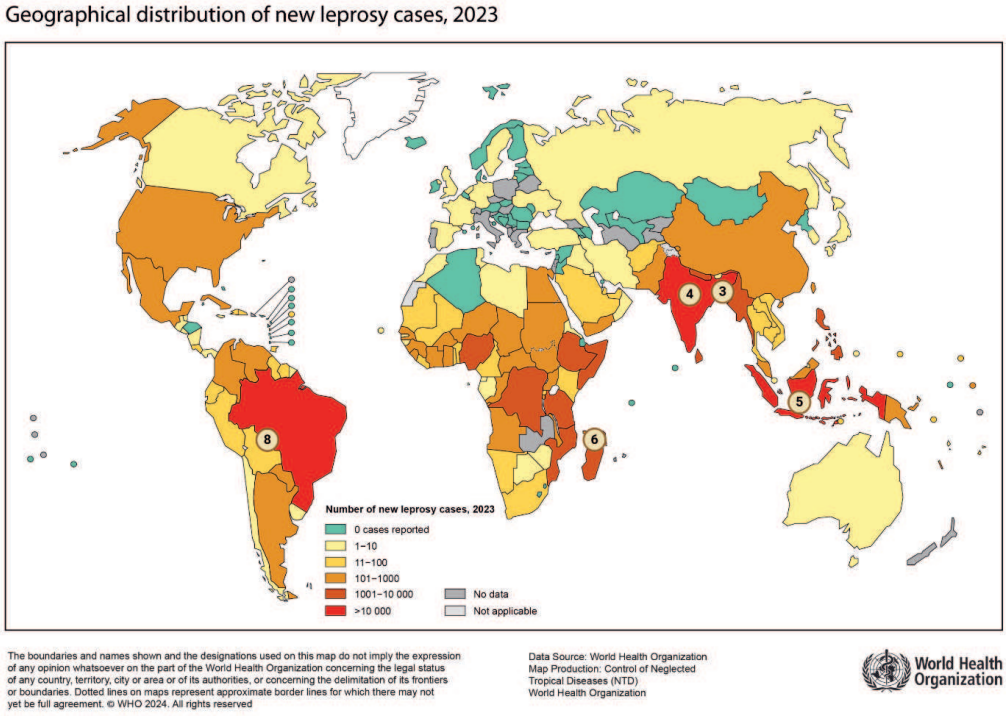

Figure 1. Geographical distribution of new leprosy cases in 2023, with study locations highlighted by chapter.

The map displays the number of newly reported leprosy cases by country in 2023. Countries are color-coded by case count, ranging from 1-10 (light yellow) to over 10,000 (dark red). Locations associated with fieldwork in this thesis are marked with chapter numbers: Bangladesh (Chapter 3), India (Chapter 4), Indonesia (Chapter

5), Comoros and Madagascar (Chapter 6). Brazil and Bolivia are additionally discussed in Chapter 8 in the context of broader implementation experiences and public health implications. Data source: WHO (2024). Figure created using BioRender.

Leprosy is curable with multidrug therapy (MDT) which includes a combination of dapsone, rifampicin and clofazimine [17]. The introduction of MDT in the mid-1980s led to a marked reduction in global prevalence, prompting the WHO to declare leprosy "eliminated as a public health problem" in 2000, defined as a prevalence of fewer than 1 case per 10,000 population [18]. However, mathematical modelling suggests that a significant number of cases - potentially thousands - continue to go undetected each year. The declaration was followed by a decline in funding, political commitment, and programmatic focus, which may have contributed to the observed stagnation in progress [19]. Consequently, diminished clinical expertise of leprosy in both endemic and non-endemic regions often leads to delayed or misdiagnosis [20-22].

Early detection and timely initiation of MDT remain fundamental to effective disease control. However, the continued emergence of new cases among children - who represent approximately 10% of new diagnoses - prove that transmission is ongoing and underscore the need for improved surveillance and case-finding strategies [15].

Transmission and risk factors for leprosy

M. leprae cannot be cultured *in vitro* [23], and its transmission remains incompletely understood [24]. The bacterium is believed to be transmitted primarily via aerosolized droplets through prolonged close contact with untreated patients [25] or alternatively, skin-to-skin contact [26]. The long incubation period of *M. leprae* - typically 2 to 10 years [1], and occasionally exceeding several decades [27, 28] - further complicates efforts to identify sources of infection and interrupt transmission early.

M. leprae DNA has been detected in nasal swabs (NS) of healthy individuals residing in endemic areas, suggesting that they may serve as unrecognized reservoirs and facilitate continued transmission [29]. Household contacts of leprosy patients (with high bacterial loads in particular) are at the highest risk of infection [30-33]. Nonetheless, the majority of exposed individuals are believed to clear the bacterium naturally without developing clinical disease [34]. Susceptibility to leprosy is influenced by a range of factors, including genetic predisposition [35, 36], nutritional status [37, 38], hygiene [39], helminth co-infections [40], and socioeconomic conditions [41].

The leprosy disease spectrum

Two primary classification systems are used to support diagnosis and guide treatment: the Ridley-Jopling (RJ) classification and the WHO classification.

The RJ classification defines five forms: tuberculoid (TT), borderline tuberculoid (BT), mid borderline (BB), borderline lepromatous (BL), and lepromatous (LL) [42]. At one end, TT leprosy is associated with a strong Th1/Th17-mediated immune response, low bacterial

load, and localized lesions with granuloma formation [43, 44]. At the other extreme, LL leprosy features a Th2-skewed immune response, high bacterial burden, disseminated lesions, and bacilli-laden foamy macrophages [45]. The borderline forms are immunologically unstable and account for the majority of cases.

Due to the complexity of the RJ classification and the need for histopathological facilities, the WHO classification is more commonly used in field settings [46]. It divides leprosy into two categories based on the number of skin lesions and slit skin smear (SSS) results: paucibacillary (PB) with up to five skin lesions and negative SSS and multibacillary (MB) with more than five lesions and/or positive SSS [47]. This simplified classification also guides treatment. PB leprosy is treated with MDT for 6 months, while MB leprosy requires 12 months of treatment.

Challenges and advances in leprosy diagnosis

Leprosy diagnosis remains predominantly clinical, relying on the identification of one or more of the three cardinal signs defined by the WHO [47]:

1. Definite loss of sensation in a pale (hypopigmented) or reddish skin patch
2. Thickened or enlarged peripheral nerve, with sensory loss and/or muscle weakness
3. Microscopic detection of acid-fast bacilli (AFB) in a slit-skin smear (SSS)

Traditionally, microscopic detection of AFB via Ziehl-Neelsen (ZN) staining has been utilized, with the bacteriological index (BI) quantifying bacillary load on a logarithmic scale [48]. However, microscopy lacks the sensitivity required for early detection and PB cases where bacterial loads are low [49] and cannot differentiate *M. leprae* from other mycobacteria. Furthermore, clinical signs can overlap with other dermatological or neurological conditions such as psoriasis [50] or dermal leishmaniasis [51], increasing the risk of misdiagnosis [52].

Molecular diagnostic tools have improved the detection of *M. leprae* [53-57]. They enable detection of *M. leprae* DNA in a variety of clinical specimens such as SSS [29], biopsies [58], NS [29], blood [59], and even environmental [23, 60] and archaeological samples [11, 61]. Despite these advances, these methods require specialized equipment and trained personnel; resources that are often unavailable in regions where the majority of leprosy cases occur [62].

Given the critical role of the immune response in determining the clinical outcome of *M. leprae* infection, immunodiagnostic tests present a promising avenue for improving or aiding leprosy diagnostics [63]. Detection of antibodies against *M. leprae*-specific antigens, particularly phenolic glycolipid-I (PGL-I), has been extensively investigated. PGL-I, a unique *M. leprae* cell wall component, induces strong IgM responses, especially in MB patients. Moreover, it has been amply shown that anti-PGL-I antibodies strongly correlate with the BI of patients and with *M. leprae* DNA present in SSS of patients and contacts [29, 64, 65], therefore representing a useful marker for past or present *M. leprae* infection. Enzyme-linked immunosorbent assays (ELISAs) targeting anti-PGL-I IgM are available and highly specific [64, 66], but require well-equipped laboratories, stable electricity, and trained personnel. To improve accessibility, rapid lateral flow tests have been developed to detect antibodies

against PGL-I [67, 68]. These point-of-care (POC) assays provide qualitative, visual results; however, their reliance on operator interpretation introduces variability and potential inconsistency in results.

Leprosy control efforts and the need for novel tools

Passive case detection and treatment with MDT alone have proven insufficient to interrupt transmission of *M. leprae*. To enhance control efforts, the WHO recommends contact tracing - including household, neighborhood, and social contacts - combined with the administration of a single dose of rifampicin as post-exposure prophylaxis (SDR-PEP) [69]. This strategy aims to prevent progression from subclinical infection to active disease. Other chemoprophylactic strategies - such as multi-dose regimens and combination therapies - are under investigation [70-72].

Alongside chemoprophylaxis, immunoprophylaxis has also played a role in leprosy control. The Bacillus Calmette–Guérin (BCG) vaccine, originally developed for TB [73], is widely administered as part of neonatal immunization programs in many leprosy- and TB-endemic countries [74]. While BCG remains a valuable component of leprosy control, it does not offer complete or durable protection [75, 76]. In response to these limitations, a promising new vaccine candidate is currently under investigation [77].

The WHO has set the ambitious goal of moving Towards Zero Leprosy in The Global Leprosy Strategy 2021–2030 [78, 79]. Recently, the Maldives has achieved elimination of leprosy as a public health problem through sustained efforts in early case detection, prompt treatment, and contact tracing [80]. This success underscores the potential of existing strategies when effectively implemented.

However, to reach complete global elimination, transmission needs to be halted. The current primary indicator for assessing the success of interventions - the annual number of newly diagnosed cases – is delayed and does not provide accurate insight into transmission. It is influenced by leprosy's long incubation period and fails to account for asymptomatic infections. Consequently, there is a pressing need for novel tools capable of detecting *M. leprae* infection in asymptomatic individuals. Such monitoring tools would provide more insights into ongoing transmission and enable more targeted deployment of prophylaxis.

In this respect, the introduction of rapid tests that enable large-scale field testing could provide a practical and effective means. Serosurveys, which have demonstrated significant value in mapping other infectious disease epidemiology [81-85], similarly hold promise for leprosy control by identifying infection prevalence and revealing potential transmission hotspots within populations. Although antibodies in leprosy do not appear to confer protective immunity [12], their presence can serve as an important marker of current or past infection, offering valuable insights into infection rates within communities.

Shared challenges in TB and leprosy diagnosis

Leprosy and TB share several diagnostic hurdles. Both diseases are caused by mycobacteria [14, 86], affect vulnerable populations in low-resource settings [87, 88], and involve complex host-pathogen interactions [89]. Globally, TB remains the top infectious disease killer [90], with an estimated 10.8 million new cases and 1.25 million deaths reported in 2023 [91].

TB diagnosis relies primarily on detecting *Mycobacterium tuberculosis* (*M. tuberculosis*) – the causative agent of TB - in sputum through microscopy, culture, or nucleic acid amplification tests such as GeneXpert [92, 93]. These methods face important limitations: they require good quality sputum samples, which are difficult to obtain from children and immunocompromised individuals and depend on laboratory infrastructure and trained personnel [94, 95]. The emergence of COVID-19, caused by the respiratory virus SARS-CoV-2, further disrupted TB control programs globally, leading to reductions in TB diagnosis and treatment [96-98]. Additionally, COVID-19 partly shares respiratory symptoms with TB, creating potential for misdiagnosis.

Host immune response-based tests, such as the tuberculin skin test (TST) and the interferon-gamma release assay (IGRA; or QuantiFERON [99]), offer indirect means to detect infection [100]. However, these immunodiagnostics cannot reliably differentiate active from latent infection and cross-reactivity with environmental mycobacteria or prior BCG vaccination further limits their specificity [101]. Exploratory proteomics previously identified a promising host protein signature that distinguished active TB patients from other respiratory diseases with signs and symptoms suggestive of TB in an African setting [102], but such approaches still require further research and innovation before they can be widely applied.

The limitations of current diagnostic tools highlight critical gaps in the timely and accurate diagnosis of TB (and leprosy) and underscore the urgent need for novel diagnostic tests that are suitable for use in low-resource, high-burden settings.

Guiding diagnostic development: WHO target product profiles for TB and leprosy

Not only technological innovation but also alignment with clear, standardized criteria that guide the development of new tools are required. The WHO has established target product profiles (TPPs) that outline the desired characteristics for next-generation diagnostics, emphasizing accessibility, accuracy, and applicability in resource-limited settings [103]. These TPPs serve as crucial benchmarks to drive the creation of tests for detection assays for *M. leprae* infection and triage tests for TB.

In the context of leprosy, the WHO has outlined a TPP for a diagnostic test capable of detecting *M. leprae* infection in asymptomatic contacts [104]. The ideal test should be field-friendly, rapid, affordable, usable by minimally trained personnel and applicable in combination with a low-invasive sample such as fingerstick blood (FSB). It should detect biomarkers indicative of infection or immune response with sufficient sensitivity (minimum: ≥81%; ideal: ≥94%) and specificity (minimum: ≥99.5%; ideal: ≥99.9) to differentiate infected from uninfected individuals.

For TB, the WHO emphasizes the need for a triage test that can rapidly and accurately identify individuals who require further confirmatory testing with a minimum sensitivity of 90% and specificity of 70% [105]. Such a test should be affordable, non-sputum based, simple to use at the POC, and capable of delivering results quickly.

Upconverting reporter particle lateral flow assays (UCP-LFAs)

To address the diagnostic shortcomings of conventional tools, upconverting reporter particle lateral flow assays (UCP-LFAs) offer a powerful and versatile diagnostic platform tailored to meet the needs of POC and community-based screening. UCP-LFAs combine the simplicity and accessibility of traditional low-cost lateral flow tests with the use of UCP particles: luminescent particles that emit visible green light (~550 nm) when excited by near-infrared light [106]. Since this upconversion process does not occur naturally in biological specimens, it avoids issues with autofluorescence and provides high signal-to-noise ratios. The technology allows for stable, quantitative fluorescence-based readout, which is captured by a portable, battery-operated reader, eliminating the need for advanced laboratory equipment or highly trained personnel.

Using this platform, quantitative UCP-LFAs for a wide range of analytes, including cytokines, acute phase proteins, and antibodies have been developed and field-evaluated [64, 107-111]. This user-friendly setup is highly suitable for use in remote or underserved communities, with advantages including ambient storage, rapid turnaround time, and compatibility with minimally invasive samples such as FSB and urine.

Thesis outline

This thesis explores the development, validation, and implementation of field-deployable immunodiagnostic tools based on the UCP-LFA format, for the detection and monitoring of poverty-associated infectious diseases, with a primary focus on leprosy, and additional applications for TB and COVID-19 (Figure 2). By combining test innovation with large-scale field evaluations, this work aims to advance public health strategies for disease control and surveillance in resource-limited settings.

Chapter 2 presents a systematic review of the available literature on serological testing for anti-*M. leprae* antibodies in children without clinical leprosy living in endemic areas. Given that antibody responses in young children per definition reflect recent infection, this chapter examines the potential of using anti-PGL-I seroprevalence as a proxy to monitor ongoing *M. leprae* transmission.

Chapter 3 describes the evaluation of the final version of a quantitative rapid test for detection of *M. leprae* infection, the PGL-I QURapid. The test - based on UCP-LFA technology - measures anti-PGL-I IgM antibodies in FSB and serum samples. The assay's diagnostic performance, reproducibility, and field usability are evaluated.

Chapters 4-5 cover the application of the PGL-I QURapid, presented in Chapter 3, for the use case of assessing *M. leprae* transmission through two serosurveys performed among school-aged children in different leprosy endemic areas. In **Chapter 4**, a field-based serosurvey was conducted in Bihar, India. Using the UCP-LFA, anti-PGL-I IgM seroprevalence was measured in over 1,800 children. **Chapter 5** presents a second serosurvey, conducted in rural and urban regions of Indonesia. Anti-PGL-I IgM seroprevalence was assessed among 637 school-aged children, with additional investigation of socioeconomic factors and helminth coinfection.

Chapter 6 describes the implementation of large-scale serological monitoring within the *Post-ExpOsure Prophylaxis for LEprosy (PEOPLE)* trial in the Comoros and Madagascar (2019-2023). Anti-PGL-I IgM levels were measured in newly diagnosed leprosy patients and over 17,000 screened contacts over three consecutive years. FSB samples were tested on-site by local staff using the field-friendly, quantitative UCP-LFA. This chapter explores the use of the rapid test as a tool to monitor the population-level impact of public health interventions aimed at reducing *M. leprae* transmission.

Chapter 7 expands the scope of the thesis beyond leprosy by examining the application of host biomarker-based UCP-LFAs for another mycobacterial disease: TB. The chapter presents data on the diagnostic performance of these assays in distinguishing between active and latent TB, as well as COVID-19 patients and healthy controls and discusses its usefulness for triage purposes.

Chapter 8 provides a general discussion and synthesis of the findings. The chapter reflects on the scientific, technical, and public health implications of deploying quantitative immunodiagnostics in endemic (and non-endemic) settings. Strengths and limitations are discussed in relation to test design, field implementation, and health system integration. Finally, the chapter outlines future directions for research and development, including assay optimization, expansion to other diseases, and integration within disease control activities.

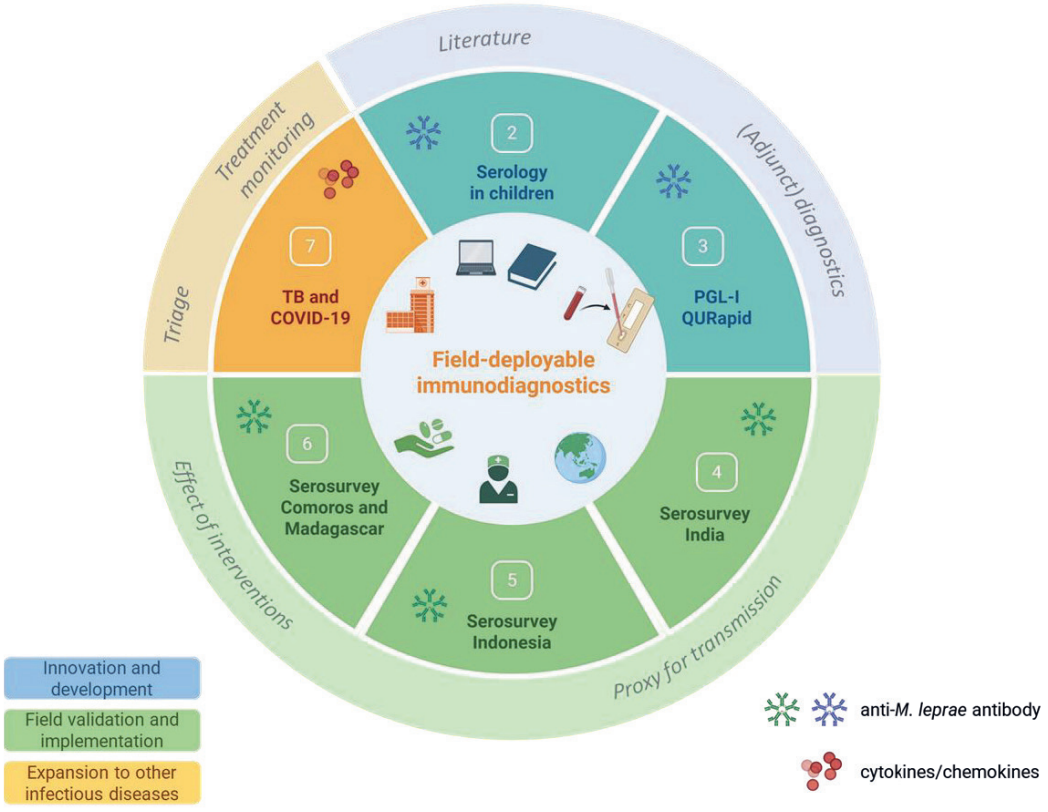

Figure 2. Chapter alignment through field-deployable immunodiagnostics for leprosy, TB, and COVID-19.

The central UCP-LFA platform connects studies across three domains: (1) Innovation and development (blue) – assay development and conceptual groundwork; (2) Field validation (green) – serosurveys in endemic regions; (3) Expansion (orange) – application to TB and COVID-19. Icons indicate biomarker types: green for anti-*M. leprae* antibodies, red for cytokines/chemokines. Use cases are shown in grey italics.

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