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

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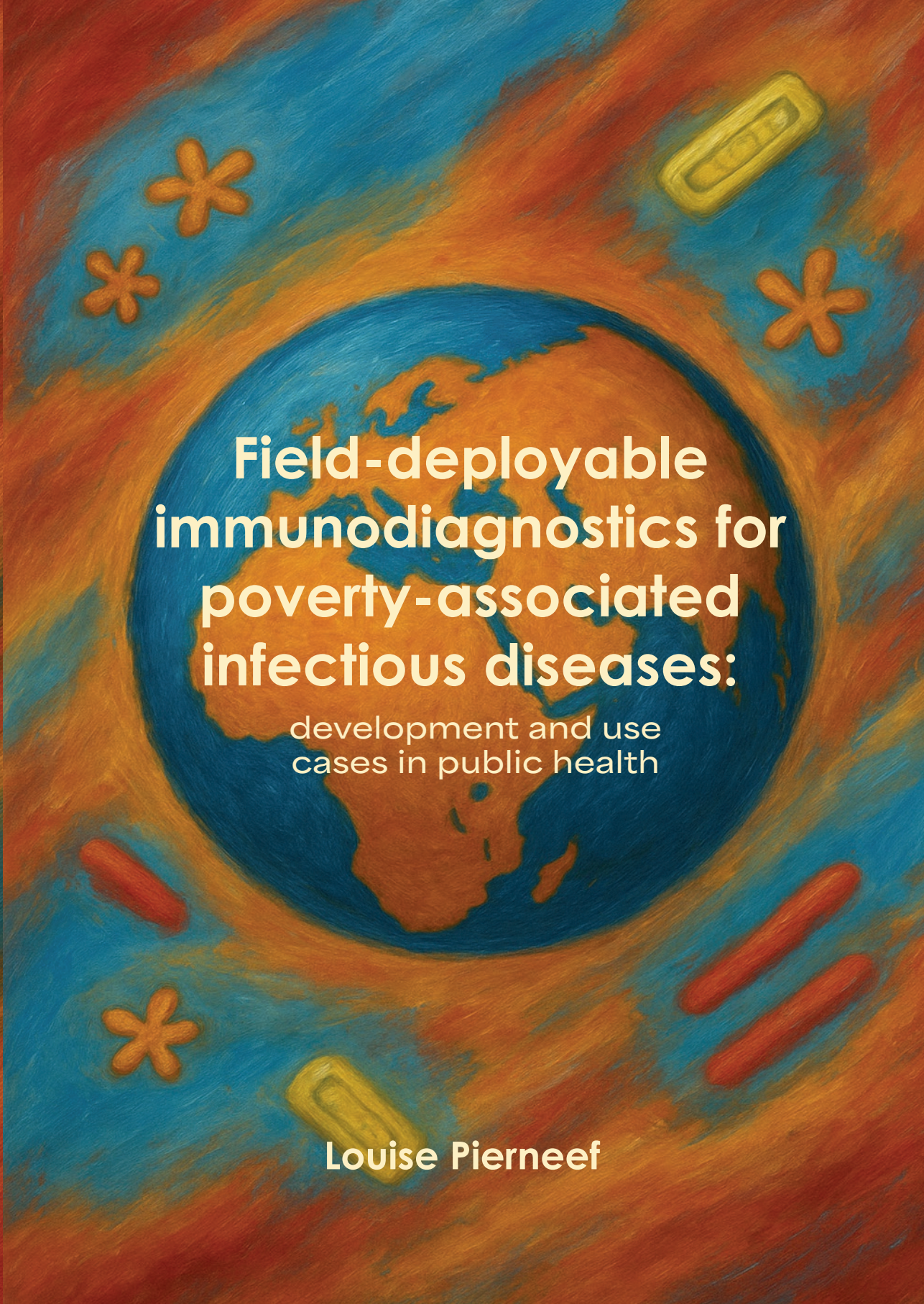
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# Field-deployable immunodiagnostics for poverty-associated infectious diseases:

development and use  
cases in public health

Louise Pierneef

## **Stellingen behorende bij het proefschrift getiteld Field-deployable immunodiagnostics for poverty-associated infectious diseases: development and use cases in public health**

1. Disease elimination is defined not only by reduced incidence but also by sustained interruption of transmission (*modified from WHO leprosy elimination framework*).
2. Quantitative anti-PGL-I serology holds promise as a screening test to assess *M. leprae* infection and may be applied as a proxy for transmission when measured in young children (*this thesis*).
3. The PGL-I QURapid meets the WHO target product profile criteria and is a field-ready tool applicable for multiple public health use cases, including diagnosis, treatment monitoring, transmission surveillance, evaluation of interventions, and One Health applications (*this thesis*).
4. Serosurveillance in children can reveal hidden *M. leprae* transmission hotspots that remain undetected by routine case detection and offers a promising tool for targeted prophylaxis guidance (*this thesis*).
5. The impact of leprosy control interventions may become apparent earlier through serological trends than through clinical incidence data (*this thesis*).
6. Host biomarker-based diagnostics provide a promising adjunct diagnostic tool for tuberculosis triage (*this thesis*).
7. Many infectious diseases, including neglected tropical diseases such as leprosy, are deeply rooted in structural determinants such as poverty, inadequate sanitation and overcrowded living conditions.
8. The UCP-LFA platform provides a robust foundation for integrated disease diagnostics across multiple infectious diseases in resource-limited settings.
9. The pursuit of perfect diagnostics should not delay the implementation of effective field-ready tools in public health practice.
10. Effective leprosy control requires not only technological innovation and treatment access, but also attention to stigma, mental wellbeing, implementation, and sustained commitment from local healthcare workers and governments.
11. The most valuable lessons in global health are often learned outside the laboratory and the office.
12. In global health, alone we can do so little, together we can do so much (*modified from Helen Keller*).

**Field-deployable immunodiagnostics for  
poverty-associated infectious diseases:  
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Louise Pierneef



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

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# 1

## General introduction

Louise Pierneef<sup>1</sup>

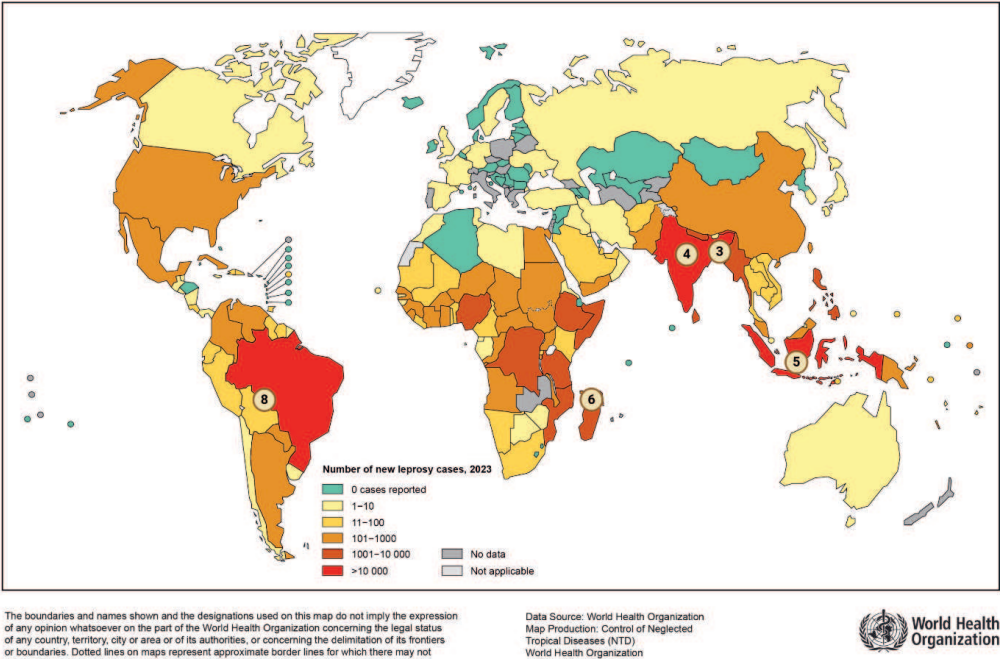
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# Leprosy: a neglected tropical disease of ongoing global concern

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).

Geographical distribution of new leprosy cases, 2023



**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 immunodiagnosics 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.

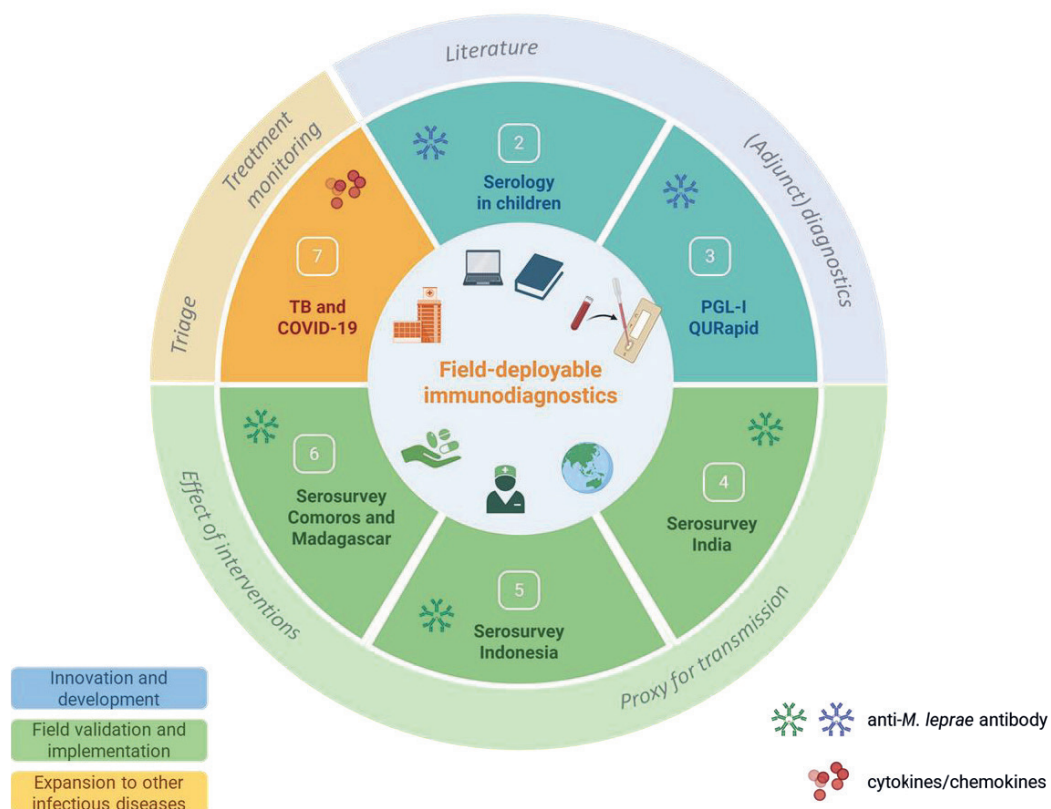
**Chapter 3** describes the evaluation of the final version of a quantitative rapid test for detection of *M. leprae* infection, the PGL-I QURapid. The test - based on UCP-LFA technology - measures anti-PGL-I IgM antibodies in FSB and serum samples. The assay's diagnostic performance, reproducibility,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

## References

1. Scollard, D.M., et al., *The continuing challenges of leprosy*. Clin Microbiol Rev, 2006. 19(2): p. 338–81.
2. Han, X.Y., et al., *A new Mycobacterium species causing diffuse lepromatous leprosy*. Am J Clin Pathol, 2008. 130(6): p. 856–64.
3. Han, X.Y., et al., *Analysis of the leprosy agents Mycobacterium leprae and Mycobacterium lepromatosis in four countries*. Am J Clin Pathol, 2014. 142(4): p. 524–32.
4. Geluk, A., *Correlates of immune exacerbations in leprosy*. Semin Immunol, 2018. 39: p. 111–118.
5. Somar, P., M.M. Waltz, and W.H. van Brakel, *The impact of leprosy on the mental wellbeing of leprosy-affected persons and their family members - a systematic review*. Glob Ment Health (Camb), 2020. 7: p. e15.
6. Govindasamy, K., et al., *Burden of depression and anxiety among leprosy affected and associated factors-A cross sectional study from India*. PLoS Negl Trop Dis, 2021. 15(1): p. e0009030.
7. van Brakel, W.H., et al., *Disability in people affected by leprosy: the role of impairment, activity, social participation, stigma and discrimination*. Glob Health Action, 2012. 5.
8. Pierneef, L., et al., *Factors influencing the mental wellbeing of persons affected by leprosy in Far-Western Nepal*. Leprosy Review, 2022. 93(1): p. 12–25.

9. Barcelos, R., et al., *Leprosy patients quality of life: a scoping review*. Rev Esc Enferm USP, 2021. 55: p. e20200357.
10. Monot, M., et al., *On the origin of leprosy*. Science, 2005. 308(5724): p. 1040–2.
11. Robbins, G., et al., *Ancient skeletal evidence for leprosy in India (2000 B.C.)*. PLoS One, 2009. 4(5): p. e5669.
12. Grijnsen, M.L., et al., *Leprosy*. Nat Rev Dis Primers, 2024. 10(1): p. 90.
13. Dallmann-Sauer, M., W. Correa-Macedo, and E. Schurr, *Human genetics of mycobacterial disease*. Mamm Genome, 2018. 29(7-8): p. 523–538.
14. Geluk, A., *All mycobacteria are inventive, but some are more Daedalean than others*. Immunol Rev, 2021. 301(1): p. 5–9.
15. World Health Organization, *Global leprosy (Hansen disease) update, 2022: new paradigm - control to elimination*, in *Wkly Epidemiol. Rec*. 2023.
16. World Health Organization, *Global leprosy (Hansen disease) update, 2023: elimination of leprosy disease is possible - time to act!*, in *Wkly Epidemiol. Rec*. 2024.
17. World Health Organization. *Control of Neglected Tropical Diseases*. Leprosy treatment 2025 [cited 2025 11th of September]; Available from: <https://www.who.int/teams/control-of-neglected-tropical-diseases/leprosy/treatment>.
18. Smith, W.C., et al., *The missing millions: a threat to the elimination of leprosy*. PLoS Negl Trop Dis, 2015. 9(4): p. e0003658.
19. Smith, C.S., et al., *Time to define leprosy elimination as zero leprosy transmission?* Lancet Infect Dis, 2016. 16(4): p. 398–9.
20. Dharmawan, Y., et al., *Delayed detection of leprosy cases: A systematic review of healthcare-related factors*. PLoS Negl Trop Dis, 2022. 16(9): p. e0010756.
21. Siddiqui, M.R., et al., *Integration of leprosy elimination into primary health care in orissa, India*. PLoS One, 2009. 4(12): p. e8351.
22. Lockwood, D.N., et al., *Diagnosing and treating leprosy in a non-endemic setting in a national centre, London, United Kingdom 1995-2018*. PLoS Negl Trop Dis, 2022. 16(10): p. e0010799.
23. Turankar, R.P., et al., *Survival of Mycobacterium leprae and association with Acanthamoeba from environmental samples in the inhabitant areas of active leprosy cases: A cross sectional study from endemic pockets of Purulia, West Bengal*. Infect Genet Evol, 2019. 72: p. 199–204. Formatted: Dutch (Netherlands)9
24. Bratschi, M.W., et al., *Current knowledge on Mycobacterium leprae transmission: a systematic literature review*. Lepr Rev, 2015. 86(2): p. 142–55.
25. Araujo, S., et al., *Molecular Evidence for the Aerial Route of Infection of Mycobacterium leprae and the Role of Asymptomatic Carriers in the Persistence of Leprosy*. Clin Infect Dis, 2016. 63(11): p. 1412–1420.
26. Girdhar, B.K., *Skin to skin transmission of leprosy*. Indian J Dermatol Venereol Leprol, 2005. 71(4): p. 223–5.
27. Taggart, M., et al., *Multibacillary leprosy with an incubation period exceeding 50 years*. BMJ Case Rep, 2022. 15(7).
28. Pelser, S., et al., *A case of leprosy in an immunocompromised traveller*. J Travel Med, 2024. 31(4).
29. Tio-Coma, M., et al., *Genomic Characterization of Mycobacterium leprae to Explore Transmission Patterns Identifies New Subtype in Bangladesh*. Front Microbiol, 2020. 11: p. 1220.
30. Quilter, E.E.V., et al., *Patients with skin smear positive leprosy in Bangladesh are the main risk factor for leprosy development: 21-year follow-up in the household contact study (COCOA)*. PLoS Negl Trop Dis, 2020. 14(10): p. e0008687.
31. Barreto, J.G., et al., *Spatial epidemiology and serologic cohorts increase the early detection of leprosy*. BMC Infect Dis, 2015. 15: p. 527.
32. Goulart, I.M., et al., *Risk and protective factors for leprosy development determined by epidemiological surveillance of household contacts*. Clin Vaccine Immunol, 2008. 15(1): p. 101–5.
33. Fine, P.E., et al., *Household and dwelling contact as risk factors for leprosy in northern Malawi*. Am J Epidemiol, 1997. 146(1): p. 91–102.
34. Joyce, M.P., *Historic aspects of human susceptibility to leprosy and the risk of conjugal transmission*. Mem Inst Oswaldo Cruz, 2012. 107 Suppl 1: p. 17–21.
35. Zhang, F.R., et al., *Genomewide association study of leprosy*. N Engl J Med, 2009. 361(27): p. 2609–18.
36. Mira, M.T., et al., *Susceptibility to leprosy is associated with PARK2 and PACRG*. Nature, 2004. 427(6975): p. 636–40.
37. Dwivedi, V.P., et al., *Diet and nutrition: An important risk factor in leprosy*. Microb Pathog, 2019. 137: p. 103714.
38. Feenstra, S.G., et al., *Recent food shortage is associated with leprosy disease in Bangladesh: a case-control study*. PLoS Negl Trop Dis, 2011. 5(5): p. e1029.
39. Emerson, L.E., et al., *Poor WASH (Water, Sanitation, and Hygiene) Conditions Are Associated with Leprosy in North Gondar, Ethiopia*. Int J Environ Res Public Health, 2020. 17(17).

40. Hagge, D.A., et al., *Opening a Can of Worms: Leprosy Reactions and Complicit Soil-Transmitted Helminths*. EBioMedicine, 2017. 23: p. 119–124.
41. Nery, J.S., et al., *Socioeconomic determinants of leprosy new case detection in the 100 Million Brazilian Cohort: a population-based linkage study*. Lancet Glob Health, 2019. 7(9): p. e1226–e1236.
42. Ridley, D.S. and W.H. Jopling, *Classification of leprosy according to immunity. A five-group system*. Int J Lepr Other Mycobact Dis, 1966. 34(3): p. 255–73.
43. Kaplan, G. and Z.A. Cohn, *Leprosy and cell-mediated immunity*. Curr Opin Immunol, 1991. 3(1): p. 91–6.
44. Modlin, R.L., et al., *Learning from lesions: patterns of tissue inflammation in leprosy*. Proc Natl Acad Sci U S A, 1988. 85(4): p. 1213–7.
45. Rodrigues, L.C. and D. Lockwood, *Leprosy now: epidemiology, progress, challenges, and research gaps*. Lancet Infect Dis, 2011. 11(6): p. 464–70. Formatted: Portuguese (Brazil)10
46. Lockwood, D.N., et al., *Comparing the clinical and histological diagnosis of leprosy and leprosy reactions in the INFIR cohort of Indian patients with multibacillary leprosy*. PLoS Negl Trop Dis, 2012. 6(6): p. e1702.
47. World Health Organization. *Leprosy*. Fact sheets 2025; Available from: <https://www.who.int/news-room/fact-sheets/detail/leprosy>.
48. Jopling, W.H., McDougall A. C., *Handbook of leprosy*. 1996, UK: Heinemann Professional Publishing Ltd.
49. Gurung, P., et al., *Diagnostic accuracy of tests for leprosy: a systematic review and meta-analysis*. Clin Microbiol Infect, 2019. 25(11): p. 1315–1327.
50. Vora, R.V., et al., *Leprosy Mimicking Psoriasis*. J Clin Diagn Res, 2015. 9(9): p. WJ01–2.
51. Scollard, D.M., Gillis, T. P., *Differential diagnosis of leprosy*, in *International textbook of leprosy*. 2016, American Leprosy Missions: Greenville, SC.
52. Chatterjee, K., V.S. Yadav, and V. Ramesh, *Slit-skin smear in post kala-azar dermal leishmaniasis and leprosy: How a negative report for Leishman-Donovan bodies in Giemsa stain may indicate leprosy*. JAAD Int, 2023. 13: p. 15–16.
53. Cole, S.T., et al., *Massive gene decay in the leprosy bacillus*. Nature, 2001. 409(6823): p. 1007–11.
54. Donoghue, H.D., J. Holton, and M. Spigelman, *PCR primers that can detect low levels of Mycobacterium leprae DNA*. J Med Microbiol, 2001. 50(2): p. 177–182.
55. Truman, R.W., et al., *Enumeration of Mycobacterium leprae using real-time PCR*. PLoS Negl Trop Dis, 2008. 2(11): p. e328.
56. Martinez, A.N., et al., *Evaluation of qPCR-based assays for leprosy diagnosis directly in clinical specimens*. PLoS Negl Trop Dis, 2011. 5(10): p. e1354.
57. Braet, S., et al., *The Repetitive Element RLEP Is a Highly Specific Target for Detection of Mycobacterium leprae*. J Clin Microbiol, 2018. 56(3).
58. Goulart, I.M., et al., *Detection of Mycobacterium leprae DNA in skin lesions of leprosy patients by PCR may be affected by amplicon size*. Arch Dermatol Res, 2007. 299(5-6): p. 267–71.
59. Diana, D. and M.C. Harish, *qPCR detection of DNA in urine samples of leprosy patients using the gene target*. Frontiers in Molecular Biosciences, 2024. 11.
60. Tio-Coma, M., et al., *Detection of Mycobacterium leprae DNA in soil: multiple needles in the haystack*. Sci Rep, 2019. 9(1): p. 3165.
61. Suzuki, K., et al., *Detection of Mycobacterium leprae DNA from archaeological skeletal remains in Japan using whole genome amplification and polymerase chain reaction*. PLoS One, 2010. 5(8): p. e12422.
62. Silva, M.J.A., et al., *Comparative analysis of the leprosy detection rate regarding its clinical spectrum through PCR using the 16S rRNA gene: a scientometrics and meta-analysis*. Front Microbiol, 2024. 15: p. 1497319.
63. van Hooij, A., *Development of immunodiagnostic tests for leprosy: from biomarker discovery to application in endemic areas*. 2021, Leiden University: Leiden.
64. van Hooij, A., et al., *Field-friendly serological tests for determination of M. leprae-specific antibodies*. Sci Rep, 2017. 7(1): p. 8868.
65. Zenha, E.M., M.A. Ferreira, and N.T. Foss, *Use of anti-PGL-1 antibodies to monitor therapy regimes in leprosy patients*. Braz J Med Biol Res, 2009. 42(10): p. 968–72.
66. Lima, M.I.S., et al., *Biotechnological and Immunological Platforms Based on PGL-I Carbohydrate-Like Peptide of Mycobacterium leprae for Antibodies Detection Among Leprosy Clinical Forms*. Front Microbiol, 2020. 11: p. 429. 11
67. Duthie, M.S., et al., *Rapid quantitative serological test for detection of infection with Mycobacterium leprae, the causative agent of leprosy*. J Clin Microbiol, 2014. 52(2): p. 613–9.
68. Stefani, M.M., et al., *Comparison of two rapid tests for anti-phenolic glycolipid-I serology in Brazil and Nepal*. Mem Inst Oswaldo Cruz, 2012. 107 Suppl 1: p. 124–31.
69. World Health Organization, *Leprosy/Hansen disease: Contact tracing and post-exposure prophylaxis. Technical guidance*. 2017, World Health Organization, Regional Office for South-East Asia: New Delhi.

70. Mieras, L.F., et al., *An enhanced regimen as post-exposure chemoprophylaxis for leprosy: PEP+*. BMC Infect Dis, 2018. 18(1): p. 506.
71. Hasker, E., et al., *Post-exposure prophylaxis in leprosy (PEOPLE): a cluster randomised trial*. Lancet Glob Health, 2024. 12(6): p. e1017–e1026.
72. de Jong, B.C., et al., *Safety of single-dose bedaquiline combined with rifampicin for leprosy post-exposure prophylaxis: A Phase 2 randomized non-inferiority trial in the Comoros Islands*. PLoS Med, 2024. 21(10): p. e1004453.
73. Calmette, A., *Preventive Vaccination Against Tuberculosis with BCG*. Proc R Soc Med, 1931. 24(11): p. 1481–90.
74. Setia, M.S., et al., *The role of BCG in prevention of leprosy: a meta-analysis*. Lancet Infect Dis, 2006. 6(3): p. 162–70.
75. Mangtani, P., et al., *The duration of protection of school-aged BCG vaccination in England: a population-based case-control study*. Int J Epidemiol, 2018. 47(1): p. 193–201.
76. Rodrigues, L.C., et al., *Long lasting BCG protection against leprosy*. Vaccine, 2007. 25(39-40): p. 6842–4.
77. Duthie, M.S., et al., *A phase 1 antigen dose escalation trial to evaluate safety, tolerability and immunogenicity of the leprosy vaccine candidate LepVax (LEP-F1 + GLA-SE) in healthy adults*. Vaccine, 2020. 38(7): p. 1700–1707.
78. Richardus, J.H., *Towards zero leprosy: Dream or vision?* Indian J Med Res, 2021. 153(4): p. 401–403.
79. World Health Organization, *Towards zero leprosy. Global leprosy (Hansen's Disease) strategy 2021–2030*. 2021, World Health Organization, Regional Office for South-East Asia: New Delhi.
80. World Health Organization. *World Health Organization honors Maldives for achieving five consecutive years of zero child Leprosy case detection and Interruption of transmission*. 2023.
81. Ward, S., et al., *Integrated Serosurveillance of Infectious Diseases Using Multiplex Bead Assays: A Systematic Review*. Trop Med Infect Dis, 2025. 10(1).
82. Carreno, J.M., et al., *SARS-CoV-2 serosurvey across multiple waves of the COVID-19 pandemic in New York City between 2020-2023*. Nat Commun, 2024. 15(1): p. 5847.
83. Haselbeck, A.H., et al., *Serology as a Tool to Assess Infectious Disease Landscapes and Guide Public Health Policy*. Pathogens, 2022. 11(7).
84. Guiomar, R., et al., *Seroprevalence of Protective Antibodies Against Influenza and the Reduction of the Influenza Incidence Rate: An Annual Repeated Cross-Sectional Study From 2014 to 2019*. Influenza Other Respir Viruses, 2024. 18(5): p. e13307.
85. Tiamiyu, A.B., et al., *Seroprevalence and risk factors for Lassa virus infection in South-West and North-Central Nigeria: a community-based cross-sectional study*. BMC Infect Dis, 2024. 24(1): p. 1118.
86. Miggiano, R., M. Rizzi, and D.M. Ferraris, *Mycobacterium tuberculosis Pathogenesis, Infection Prevention and Treatment*. Pathogens, 2020. 9(5).
87. Fine, P., *Leprosy and tuberculosis—an epidemiological comparison*. Tubercle, 1984. 65(2): p. 137–53.
88. Barter, D.M., et al., *Tuberculosis and poverty: the contribution of patient costs in sub-Saharan Africa—a systematic review*. BMC Public Health, 2012. 12: p. 980.
89. Luo, Y., et al., *Paired analysis of host and pathogen genomes identifies determinants of human tuberculosis*. Nat Commun, 2024. 15(1): p. 10393.
90. World Health Organization, *Tuberculosis resurges as top infectious disease killer*. 2024: Washington D.C., USA.
91. World Health Organization. *Tuberculosis*. 2025; Available from: <https://www.who.int/news-room/fact-sheets/detail/tuberculosis>.
92. Cazabon, D., et al., *Market penetration of Xpert MTB/RIF in high tuberculosis burden countries: A trend analysis from 2014 - 2016*. Gates Open Res, 2018. 2: p. 35.
93. Bartolomeu-Goncalves, G., et al., *Tuberculosis Diagnosis: Current, Ongoing, and Future Approaches*. Diseases, 2024. 12(9).
94. Brent, A.J., et al., *Author Correction: Bacteriological diagnosis of childhood TB: a prospective observational study*. Sci Rep, 2018. 8(1): p. 7223.
95. Johnson, J.L., et al., *Impact of human immunodeficiency virus type-1 infection on the initial bacteriologic and radiographic manifestations of pulmonary tuberculosis in Uganda. Makerere University-Case Western Reserve University Research Collaboration*. Int J Tuberc Lung Dis, 1998. 2(5): p. 397–404.
96. Migliori, G.B., et al., *Gauging the impact of the COVID-19 pandemic on tuberculosis services: a global study*. Eur Respir J, 2021. 58(5).
97. Visca, D., et al., *Tuberculosis and COVID-19 interaction: A review of biological, clinical and public health effects*. Pulmonology, 2021. 27(2): p. 151–165.
98. Gao, Y., et al., *Association between tuberculosis and COVID-19 severity and mortality: A rapid systematic review and meta-analysis*. J Med Virol, 2021. 93(1): p. 194–196.

99. Yancey, J.R. and V.E. Melchert, *QuantiFERON-TB Gold+ for the Diagnosis of Mycobacterium tuberculosis Infection*. Am Fam Physician, 2021. 103(3): p. 177–178.
100. Starke, J.R., *Tuberculin Skin Test Versus the Interferon-gamma Release Assays: Out With the Old, In With the New*. Pediatrics, 2020. 145(1).
101. Pai, M., et al., *Gamma interferon release assays for detection of Mycobacterium tuberculosis infection*. Clin Microbiol Rev, 2014. 27(1): p. 3–20.
102. Chegou, N.N., et al., *Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB*. Thorax, 2016. 71(9): p. 785–94.
103. World Health Organization. *Target product profile directory*. 2023; Available from: <https://www.who.int/tools/target-product-profile-database>.
104. World Health Organization, *TPP: For a diagnostic test to detect Mycobacterium leprae infection among asymptomatic household and familial contacts of leprosy patients*. 2023, World Health Organization.
105. Denking, C.M., et al., *Guidance for the Evaluation of Tuberculosis Diagnostics That Meet the World Health Organization (WHO) Target Product Profiles: An Introduction to WHO Process and Study Design Principles*. J Infect Dis, 2019. 220 Suppl 3(Suppl 3): p. S91–98.
106. Corstjens, P.L., et al., *Infrared up-converting phosphors for bioassays*. IEE Proc Nanobiotechnol, 2005. 152(2): p. 64–72.
107. Corstjens, P., et al., *Fingerstick test quantifying humoral and cellular biomarkers indicative for M. leprae infection*. Clin Biochem, 2019. 66: p. 76–82.
108. van Hooij, A., et al., *Quantitative lateral flow strip assays as User-Friendly Tools To Detect Biomarker Profiles For Leprosy*. Sci Rep, 2016. 6: p. 34260.
109. van Hooij, A., et al., *Evaluation of Immunodiagnostic Tests for Leprosy in Brazil, China and Ethiopia*. Sci Rep, 2018. 8(1): p. 17920.
110. van Hooij, A., et al., *Application of new host biomarker profiles in quantitative point-of-care tests facilitates leprosy diagnosis in the field*. EBioMedicine, 2019. 47: p. 301–308. 13
111. van Hooij, A., et al., *Prototype multi-biomarker test for point-of-care leprosy diagnostics*. iScience, 2021. 24(1): p. 102006.



# 2

## **Detection of anti-*M. leprae* antibodies in children in leprosy-endemic areas: A systematic review**

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## Abstract

Leprosy elimination primarily targets transmission of *Mycobacterium leprae* which is not restricted to patients' households. As interruption of transmission is imminent in many countries, a test to detect infected asymptomatic individuals who can perpetuate transmission is required. Antibodies directed against *M. leprae* antigens are indicative of *M. leprae* infection but cannot discriminate between active and past infection. Seroprevalence in young children, however, reflects recent *M. leprae* infection and may thus be used to monitor transmission in an area. Therefore, this literature review aimed to evaluate what has been reported on serological tests measuring anti-*M. leprae* antibodies in children without leprosy below the age of 15 in leprosy-endemic areas. A literature search was performed in the databases Pubmed, Infolep, Web of Science and The Virtual Health Library. From the 724 articles identified through the search criteria, 28 full-text articles fulfilled all inclusion criteria. Two additional papers were identified through snowballing, resulting in a total of 30 articles reporting data from ten countries. All serological tests measured antibodies against phenolic glycolipid-I or synthetic derivatives thereof, either quantitatively (ELISA or UCP-LFA) or qualitatively (ML-flow or NDO-LID rapid test). The median seroprevalence in children in endemic areas was 14.9% and was stable over time if disease incidence remained unchanged. Importantly, seroprevalence decreased with age, indicating that children are a suitable group for sensitive assessment of recent *M. leprae* infection. However, direct comparison between areas, solely based on the data reported in these studies, was impeded by the use of different tests and variable cut-off levels. Quantitative anti-PGL-I serology in young children holds promise as a screening test to assess *M. leprae* infection and may be applied as a proxy for transmission and thereby as a means to monitor the effect of (prophylactic) interventions on the route to leprosy elimination.

## Introduction

Leprosy is a neglected tropical disease caused by *Mycobacterium leprae* (*M. leprae*) or *M. lepromatosis* with tropism for skin and peripheral nerves often leading to skin lesions, loss of sensation and nerve damage that cause lifelong physical and social disabilities [1,2]. It afflicts marginalized populations in low- and middle-income countries (LMICs) in their most productive stage of life [3–5] and despite the availability of effective treatment still poses a public health problem [6]. The main route of *M. leprae* transmission is generally considered to be from human to human via aerosol droplets spread by the respiratory route [7]. However, in general, frequent and high dose exposure to *M. leprae* is thought to be required for development of disease. Consequently, household members of untreated leprosy patients are at higher risk of contracting leprosy [8–10].

Despite decades of control efforts using multidrug therapy (MDT), the global number of new leprosy cases has remained stable for over a decade just above 200,000 annually [11]. More than 15,000 new cases are found each year among children below 15 years of age, indicating continuous and recent *M. leprae* transmission in many endemic communities. On top of that, expertise of health care professionals to recognize signs and symptoms of leprosy, particularly at early stages, has declined [12]. This often results in delayed diagnosis, which increases the risk of permanent disabilities and other consequences [13–17].

In order to achieve elimination of leprosy, strategies involving early diagnosis through active case finding and contact tracing combined with postexposure prophylactic (PEP) treatment [18–23] are essential to interrupt transmission and prevent development of leprosy in high-risk contacts [24,25].

In response to the current status of leprosy health care and the stable number of new cases, the Global Leprosy Strategy 2021–2030 has set a target of 90% reduction in rate per million of new cases with grade 2 disability (G2D) as well as 90% reduction in rate of new child cases with leprosy [26], thus combining activities promoting early detection of leprosy and reduction of transmission. Current (programme) indicators used by the WHO to monitor the progress of elimination of leprosy are based on the proportion of child cases (below 15 years of age) among total new cases detected [27]. However, as the incubation period of leprosy is long, on average 5 years, but often much longer, utilizing a time span of 15 years does not sensitively reflect recent transmission. In addition, only a small percentage (estimated 5%) of individuals infected with *M. leprae* actually develop disease [1] which renders use of new leprosy cases, as used in the current indicators for leprosy elimination [27], unsuitable for determination of *M. leprae* transmission in an area, stressing the need for an alternative indicator.

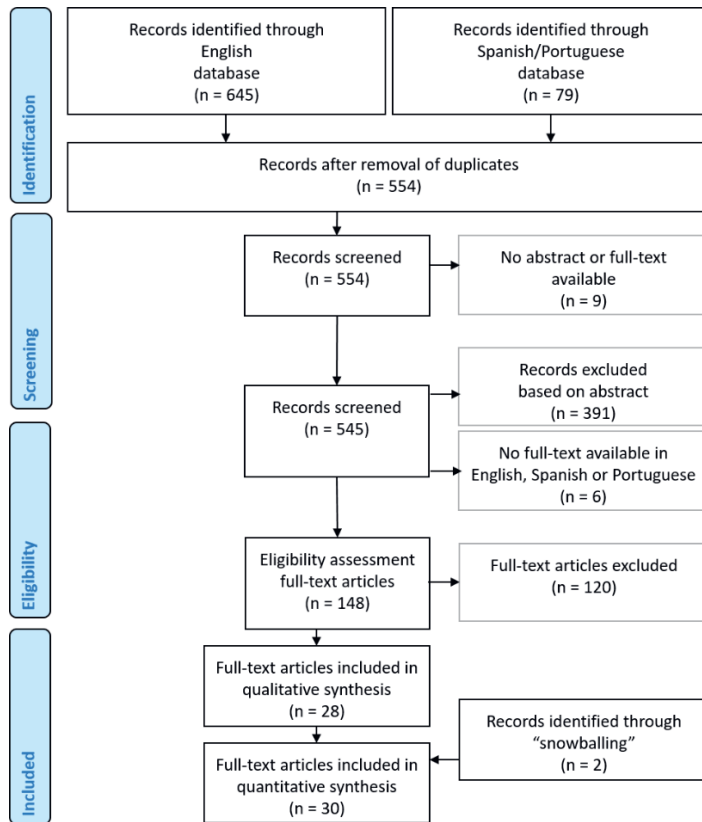
It has been amply described that levels of IgM antibodies against *M. leprae* phenolic glycolipid I (PGL-I) correlate strongly with the bacterial load within individuals [28–30] and animals [31]. Moreover, these antibodies are reduced upon efficient treatment of patients [32–34]. These findings have generated the concept of serological tests that detect antibodies against *M. leprae* as an indicator of the proportion of the population that has been infected. This includes asymptomatic infection, which is overlooked if only patients are considered [35–38]. Although antibody levels shed some light on the infection status [28,33],

their presence cannot discriminate sufficiently between recent and past infection [39]. However, finding evidence of infection in (young) children would by definition indicate recent transmission. Therefore, measuring seroprevalence among young children represents a potential tool to monitor the intensity of recent transmission, thereby informing on the elimination of transmission. Particularly when antibody levels are assessed quantitatively [28], monitoring longitudinal changes in seroprevalence measured cross-sectionally among young children of a certain age group is indicative of the status of transmission as well as the effect of control measures taken in an area [38,40].

We conducted a systematic literature review to determine what has been investigated with respect to screening of anti-*M. leprae* antibodies in children without leprosy and known contact with patients diagnosed with leprosy.

## Methods

A systematic review was conducted according to the recommendations of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) [41], targeting leprosy serology in children using PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Web of Science ([www.webofknowledge.com](http://www.webofknowledge.com)), Infolep (<https://www.leprosy-information.org/>) and The Virtual Health Library (VHL; <https://pesquisa.bvsalud.org/portal/>) as sources. The search strategy shown in the PRISMA flowchart (Fig 1) using the search strings listed in Table 1, included all available peer-reviewed publications until July 1, 2020. If no full-text was available in the databases described above, full-texts were found in one of the following online libraries: Europepmc (<https://europepmc.org/>), ILSL (<http://www.ilsil.br/>), J-STAGE (<https://www.jstage.jst.go.jp/>), Oxford Academic (<https://academic.oup.com/journals>), Researchgate (<https://www.researchgate.net/>), SAGE journals (<https://journals.sagepub.com>), Scielo (<https://scielo.org/en/>), Sciencedirect (<https://www.sciencedirect.com/>), Semantic Scholar (<https://www.semanticscholar.org/>), Taylor & Francis online (<https://www.tandfonline.com/>), the WHO website (<https://www.who.int/>), Wiley Online (<https://onlinelibrary.wiley.com/>), and obtained from the Royal Library (Koninklijke Bibliotheek) (<https://www.kb.nl/>) in The Hague, the Netherlands. Articles identified through the English databases were screened for eligibility based on the title, abstract, and finally on a full-text assessment by three authors (LP, AT and AG). The resulting lists were compared and a consensus reached on eligibility of the articles for selection. Additionally, the reference lists of the included articles were manually scrutinized for primary studies that could have been lost in the electronic search ("snowballing"). Articles in Portuguese or Spanish were screened for eligibility by two native speakers (MLN and RR). The authors were not blinded to the names of the study authors, journals or institutions.



**Figure 1. PRISMA flow chart.** Overview of the selection procedure of 30 full-text articles included in the review.

| Database                   | Hyperlink                                                                               | Search string                                                                                                                                                                                                       |
|----------------------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Pubmed                     | <a href="https://pubmed.ncbi.nlm.nih.gov/">https://pubmed.ncbi.nlm.nih.gov/</a>         | (leprosy AND schoolchildren AND detection)<br>OR (leprosy AND (children OR adolescent) AND (serum OR serology))                                                                                                     |
| Web of Science             | <a href="http://www.webofknowledge.com">www.webofknowledge.com</a>                      | ALL=(leprosy AND schoolchildren AND detection)<br>OR ALL=(leprosy AND (children OR adolescent) AND (serum OR serology))                                                                                             |
| Infolep                    | <a href="https://www.leprosy-information.org/">https://www.leprosy-information.org/</a> | 1. "leprosy" "children" "serology"<br>2. "leprosy" "children" "serum"<br>3. "leprosy" "adolescent" "serology"<br>4. "leprosy" "schoolchildren" "detection"                                                          |
| The Virtual Health Library | <a href="https://pesquisa.bvsalud.org/portal/">https://pesquisa.bvsalud.org/portal/</a> | 1. (leprosy AND schoolchildren AND detection)<br>OR (leprosy AND (children OR adolescent) AND (serum OR serology))<br>2. (lepra AND escolares AND detección) OR (lepra AND (niños OR adolescentes) AND (serología)) |

**Table 1. Search strings applied per database**

This table shows the hyperlinks and search strings used for Pubmed, Web of Science, Infolep and The Virtual Health Library.

## **Inclusion and exclusion criteria**

Articles were included if they described original studies on leprosy serology in children below 15 years of age who were not affected by leprosy. Articles were excluded if they primarily discussed studies on (neglected tropical) diseases other than leprosy, reported studies on serology related only to leprosy disease or to leprosy reactions, or if they lacked critical information on the serological methods applied or the number of the children recruited. Articles were also excluded if the abstract in English fulfilled the inclusion criteria but the rest of the text was not available in English, Spanish, or Portuguese.

## **Data extraction and analysis**

After inclusion of eligible papers, information about the study design, the type of serological test used (including the (laboratory) protocol and target antigen), the year, the research location, the study population, the age or age group, gender, the sample size of children tested and the seropositivity percentage(s) reported were extracted into Microsoft Excel 2016. Data extraction was done in duplicate (LP and AT for English publications; MLN and RR for Portuguese and Spanish publications). If reported, the sampling and selection method for the inclusion of participants was recorded. Graphs and figures were created in GraphPad Prism 8.4.2 and Microsoft Powerpoint 2016.

Quantitative data was copied from the full texts. In case percentages for seropositivity in children were not available or could not be derived from the text, they were calculated or estimated from figures in the articles.

The median of the seroprevalence reported was calculated in Microsoft Excel 2016 by including all seropositivity percentages reported. In case multiple seropositivity percentages were reported in one article, those were included in line with how they were presented e.g., by district, by age group, or by test and depicted separately. The IQR was determined as the difference between the third and first quartile when the set of data was divided into four equal portions.

Pearson correlation method in Graphpad Prism 8 (version 8.4.2) was used for comparison of anti-PGL-I ELISA data (OD450) of 58 serum samples of children below the age of 15, which were previously assessed in two different studies [28,42] using ELISAs with distinct cut-off values. These cut-offs were either based on ODs corrected for background OD values that were higher than 0.2 [28] or OD values not corrected for background, but higher than the average plus three times the standard deviation of the test results from 14 healthy subjects from the same hyperendemic area (0.295) [42].

No formal methods for assessing risk of bias were used due to the variation of study methods. Study limitations influencing outcomes and conclusions were considered and described in the Discussion section of this review. For the PRISMA checklist we refer to S1 Table.

## Results

Applying the search strategy described above, a total of 724 articles were identified from the major electronic databases utilized (Fig 1). After removing 170 duplicates, 554 articles were screened for the inclusion criteria of this review. Nine articles were excluded as only a title was available, while the abstract and full-text were lacking. Subsequently, 391 additional articles were excluded based on the abstracts which indicated the lack of data on leprosy and/or serology and/or children without leprosy. Six English abstracts (of which one article in Bahasa, Indonesia) fulfilled the inclusion criteria, but no full-text was available in English, Spanish or Portuguese. For the remaining 148 articles, a full-text screening was performed, resulting in the exclusion of another 120 articles according to the selection criteria. Snowballing yielded an additional two articles. Therefore, the final selection included in this review consisted of 30 full-text articles; 27 in English and three in Portuguese.

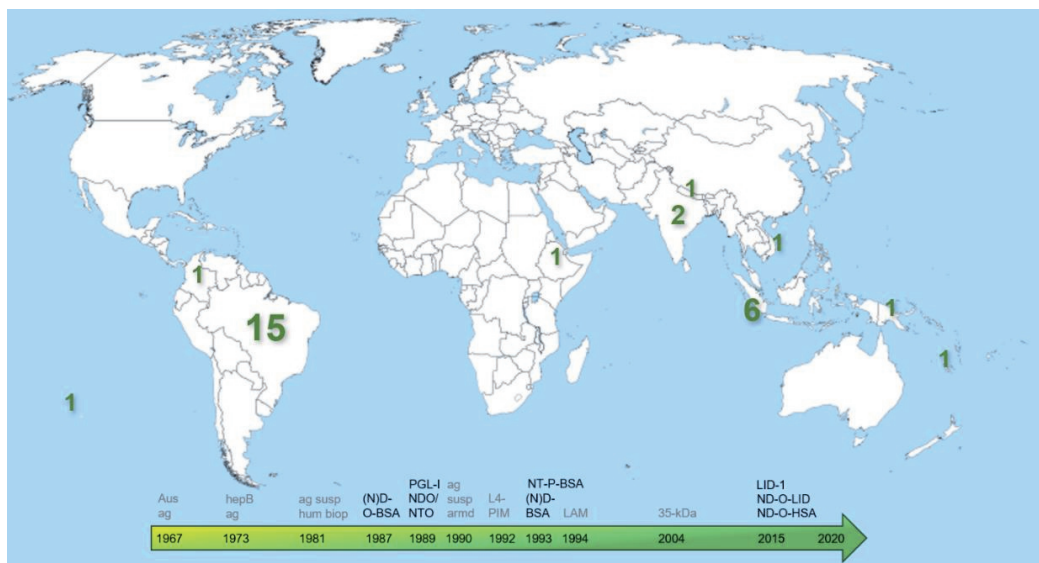
### Participant characteristics

The data presented in the selected articles covered more than 18,000 children across ten countries studied between 1987 and 2020 (Table 2 and Fig 2). Half of the studies were conducted in Brazil (n = 15). Children included household contacts of persons affected by leprosy, non-household contacts, schoolchildren and residents from regions considered high-, medium-, low- and non-endemic for leprosy, who were not known to be affected by leprosy. For most studies, sampling methods were not reported. The results on the prevalence of seropositivity for *M. leprae* in children found in the included articles are summarized in Table 3 and S2 Table and S1 Fig. For each area, results are shown separately for children with and without known contact with leprosy patients. The median seroprevalence in children in the study areas included was 14.9% (IQR = 7.3–27.7).

**Table 2. Publications on leprosy serology in children without leprosy from ten countries**

| Country          | Publications | # of children  | Publication date (range) |
|------------------|--------------|----------------|--------------------------|
| Brazil           | 15           | 10,423         | 2008 - 2019              |
| Colombia         | 1            | 49             | 2008                     |
| Ethiopia         | 1            | 40             | 1987                     |
| French Polynesia | 1            | ~20*           | 1993                     |
| India            | 2            | 3,449          | 1991 - 1994              |
| Indonesia        | 6            | ~3,880*        | 1994 - 2020              |
| Nepal            | 1            | 93             | 1994                     |
| New Caledonia    | 1            | 15             | 1989                     |
| Papua New Guinea | 1            | 62             | 1990                     |
| Vietnam          | 1            | 342            | 2018                     |
| <b>Total</b>     | <b>30</b>    | <b>~18,373</b> | <b>1987 - 2020</b>       |

This table shows the sum of the number of children that were reported in the included studies listed per country. \*Estimated from figure.



**Figure 2. Leprosy serology in children without leprosy.** Schematic representation of the countries where the selected studies in this review were located. The green numbers indicate the number of studies per country. The arrow indicates the chronological use of target antigens applied for leprosy serology. The antigens colored in grey, are those used in studies reporting leprosy serology among children without leprosy, which did not fulfil all inclusion criteria of this review. Aus ag: Australia antigen; hepB ag: hepatitis B antigen; ag susp hum biop: antigen suspension prepared from human leprosy biopsy tissue; ag susp arm: suspension of *M. leprae* purified from an infected armadillo's liver; (N)D-O-BSA: (natural) disaccharide-octyl bovine serum albumin; PGL-I: phenolic glycolipid I; NTO: natural trisaccharide-octyl; L4-PIM: L4-reactive antigen from *M. tuberculosis*; NT-P-BSA: natural trisaccharide phenylpropionyl bovine serum albumin; LAM: lipoarabinomannan; 35-kDa: 35-kilodalton protein of *M. tuberculosis*; LID-1: leprosy IDRI diagnostic-1; ND-O-LID: semi-synthetic disaccharide attached to the octyl radical, which mimics PGL-I, conjugated with two fusion proteins, ML0304 and ML0331, forming LID; ND-O-HSA: natural disaccharide-octyl human serum albumin. The template for this world map was retrieved from: <https://www.worldofmaps.net/weltkarten/weltkarten-und-weltatlas/weltkarte-blankvektorgrafik.htm>. Colored numbers and a timeline were added.

## Latin America

Pará has a high annual leprosy case detection rate of 50/100,000 persons in comparison to the lower average of 17/100,000 in Brazil (data from 2012) [43]. From 2011 to 2015, Barreto and Salgado and colleagues carried out several studies in the Brazilian Amazon region on leprosy seroprevalence including children [42–44] (Table 3 and S2 and S3 Tables). Seropositivity in their studies was determined by ELISA and defined as all values above the mean optical density (OD) plus three standard deviations (from sera from 14 healthy subjects from the same hyperendemic area). In 2011, they found proportions of 63.3% and 57.4% seropositive for antibodies against PGL-I in a cross-sectional study among random samples of 49 and 47 schoolchildren from two elementary schools in Castanhal, state of Pará, Brazil [42]. No correlation was found between age of the children and seroprevalence. Based on the high seroprevalence rates, the authors assumed that *M. leprae* is circulating actively among this population. In 2015 [43], these researchers identified a similar seroprevalence rate (77.6%) among 134 schoolchildren. Child contacts of leprosy patients (44.1% of 68 children) did not show higher seroprevalence [42].

In contrast to the high percentages above, only 6% seropositivity was reported by van Hooij *et al.*, measuring anti-PGL-I IgM levels using the up-converting phosphor lateral flow assay (UCP-LFA) [28,45] in sera of 207 schoolchildren from Pará without known contact with leprosy patients. As the variety of serological tests used in the selected studies impede direct comparison of results, we performed a direct comparison of the serology data of children from the same region in Pará, obtained by anti-PGL-I IgM ELISAs of both research groups ( $n = 58$ ) which applied a different cut-off, but showed excellent correlation ( $p < 0.0001$ ; Pearson  $r = 0.92$ ; S2A Fig). Focusing subsequently into the twenty samples that had values around the cut-off value (S2B Fig), nine of these scored low positive in the assay performed by Barreto *et al.* [42], whereas only one scored positive in the ELISA by van Hooij *et al.* [28] (S2C Fig). This emphasizes the importance of not only the type of serological test, but also the cut-off used in seroscreening for proper assessment of transmission.

In South(east) Brazil [36], 7,073 schoolchildren were assessed in three leprosy-endemic states Espírito Santo (ES), Minas Gerais (MG) and Santa Catarina (SC), with a leprosy incidence of 41, 16 and 4 per 100,000, respectively (1998) using both quantitative anti-PGL-I ELISA and qualitative ML-dipstick (Table 3 and S2 Table). In all areas, seropositivity levels ranged from 8.5% to 24%, and there was no clear correlation found between leprosy detection rates and seropositivity rates. In addition, in two other areas in Minas Gerais seroprevalence rates of 1.4% (2011;  $n = 355$ ) [46] and 10.1% (2019;  $n = 358$ ) among schoolchildren were found [47].

In the same state, seropositivity percentages (ML-flow tests) among child household contacts ranged from 12.3% to 18.6% [48–50]. In São Paulo state, 18% of child household contacts tested seropositive (ELISA) [51] whereas this was the case in 14.7% in Rio de Janeiro (ML-dipstick/ML-flow) [52].



**Table 3. Prevalence of seropositivity for *M. leprae* antibodies in children without leprosy sorted per country and test group.**

| Country (area)             | Ref. no. | Author          | Year of publication | Applied methods |         |             |              |                    |      | NCDR (per 100,000) per area | Seropositivity (%) for:         |                               |
|----------------------------|----------|-----------------|---------------------|-----------------|---------|-------------|--------------|--------------------|------|-----------------------------|---------------------------------|-------------------------------|
|                            |          |                 |                     | ELISA           | UCP-LFA | ML-dipstick | ML-flow test | NDO-LID rapid test | GPAT |                             | (endemic) controls              | contacts                      |
| Brazil (Pará)              | 42       | Barreto         | 2011                | yes             |         |             |              |                    |      |                             | ranged from 57.4 to 63.6        | 44.1                          |
| Brazil (Pará)              | 43       | Barreto         | 2015                | yes             |         |             |              |                    |      |                             | 77.6                            |                               |
| Brazil (Pará)              | 45       | van Hooij       | 2018                |                 | yes     |             |              |                    |      |                             | 6.0                             |                               |
| Brazil (South(east))       | 36       | Bührer-Sékula   | 2008                | yes             |         | yes         |              |                    |      | Governador Valadares: 112   | 18.6 / 14.2                     |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Santa Luzia: 10             | 23.0 / -                        |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Barbacena: 2                | 15.0 / 12.8                     |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Aracruz: 37                 | 24.0 / 14.4                     |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Colatina: 35                | 14.0 / 10.1                     |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Santa Teresa: 22            | 15.0 / 17.4                     |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Itajai: 9                   | 16.9 / 14.2                     |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Laguna: 5                   | 12.0 / 12.5                     |                               |
| Brazil (South(east))       | 46       | da Conceição    | 2011                | yes             |         |             |              |                    |      | Tubarão: 5                  | 12.0 / 8.5                      |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      |                             | 1.4                             |                               |
|                            |          |                 |                     | yes             |         |             |              |                    |      |                             | 10.1                            |                               |
|                            |          |                 |                     |                 |         |             | yes          |                    |      |                             |                                 | 12.3                          |
|                            |          |                 |                     |                 |         |             | yes          |                    |      |                             |                                 | 17.8                          |
|                            |          |                 |                     |                 |         |             | yes          |                    |      |                             |                                 | 18.6                          |
|                            |          |                 |                     | yes             |         |             |              |                    |      |                             |                                 | 18.0                          |
|                            |          |                 |                     |                 |         | yes         | yes          |                    |      |                             |                                 | 14.7                          |
| Brazil (Midwest)           | 53       | Limeira         | 2013                |                 |         |             | yes          |                    |      |                             |                                 | 0.0                           |
| Brazil (Midwest)           | 54       | Gomes           | 2019                |                 |         |             |              | yes                |      |                             |                                 | 6.2                           |
| Brazil (Northeast)         | 55       | Loureño         | 2017                |                 |         |             | yes          |                    |      |                             |                                 | 33.7                          |
| Brazil (Northeast)         | 56       | TiemiNagao-Dias | 2019                | yes             |         |             |              |                    |      |                             |                                 | IgM: ranged from 40.0 to 46.7 |
|                            |          |                 |                     | yes             |         |             |              |                    |      |                             |                                 | IgA: ranged from 0.0 to 4.4   |
|                            |          |                 |                     | yes             |         |             |              |                    |      |                             |                                 | IgG: ranged from 0.0 to 2.2   |
| Colombia                   | 57       | Cardona-Castro  | 2008                | yes             |         |             |              |                    |      |                             |                                 | 51.0                          |
| New Caledonia              | 58       | Desforges       | 1989                | yes             |         |             |              |                    |      |                             |                                 | NDO: 26.7                     |
| French Polynesia           | 59       | Chanteau        | 1993                | yes             |         |             |              |                    |      |                             |                                 | NTO: 13.3                     |
| Papua New Guinea           | 61       | Bagshawe        | 1990                | yes             |         |             |              |                    |      |                             |                                 | ranged from ~18.0 to 23.0*    |
| Ethiopia                   | 62       | Menzel          | 1987                | yes             |         |             |              |                    |      |                             | ranged from 21.0 to 30.6        |                               |
| Nepal                      | 63       | Soares          | 1994                | yes             |         |             |              |                    |      |                             | 35.7                            | 50.0                          |
| India (South)              | 64       | Krishnamurthy   | 1991                | yes             |         |             |              |                    |      |                             |                                 | 1.1                           |
| India                      | 65       | Shah            | 1994                | yes             |         |             |              |                    |      |                             | ranged from 5.14 to 16.74       | ranged from 6.98 to 28.13     |
|                            |          |                 |                     |                 |         |             |              |                    |      |                             | LER: 0.0                        |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      |                             | HER: 6.9                        |                               |
| Vietnam                    | 66       | Khang           | 2018                |                 |         |             |              |                    | yes  |                             |                                 | 21.4                          |
| Indonesia (South Sulawesi) | 68       | van Beers       | 1994                |                 |         |             |              |                    | yes  |                             | 2.5**                           | 17.0                          |
|                            |          |                 |                     | yes             |         |             |              |                    |      |                             | ranged from ~35.0 to 37.0*      |                               |
|                            |          |                 |                     | yes             |         |             |              |                    |      |                             | IgM: ranged from ~37.0 to 40.0* |                               |
| Indonesia (Sulawesi)       | 35       | v an Beers      | 1999                |                 |         |             |              |                    | yes  | Kauditan: 43                | 26.0                            |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Bantimurung: 41             | 28.0                            |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Bontomarannu: 34            | 26.0                            |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Lawa: 6                     | 7.0                             |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      | Tongkuno: 9                 | 7.0                             |                               |
| Indonesia (South Sulawesi) | 73       | Bakker          | 2005                | yes             |         |             |              |                    |      |                             | 14.3                            |                               |
| Indonesia (South Sulawesi) | 74       | Putri           | 2010                | yes             |         |             |              |                    |      |                             | ranged from 39.7 to 48.3        |                               |
| Indonesia (South Sulawesi) | 75       | Rachmawati      | 2013                | yes             |         |             |              |                    |      |                             | ranged from 40.0 to 57.5        |                               |
| Indonesia (East Java)      | 76       | Adriaty         | 2020                | yes             |         |             |              |                    |      |                             | non-endemic: 8.3**              |                               |
|                            |          |                 |                     |                 |         |             |              |                    |      |                             | endemic: 48.0                   |                               |

Overview of the prevalence of seropositivity for anti-*M. leprae* antibodies in children without leprosy sorted per country and test group (endemic controls or contacts of leprosy patients). For each study, the country, reference number, author, year of publication, laboratory assay and seroprevalence (%) for *M. leprae* antibodies are reported for either (endemic) controls or (household) contacts of leprosy patients. For the

numbers of children included and the method used per study, we refer to the Supplementary. The boxes in grey indicate that this data was not available. The antibody subtypes measured are indicated by the colors in the applied methods column; green: immunoglobulin M (IgM), blue: immunoglobulin A (IgA), yellow: immunoglobulin G (IgG), orange: both IgM and IgG and purple: not reported. \*Estimated from graph/figure. \*\*Reportedly leprosy non-endemic area. CP: contact population from a leprosy colony; ELISA: enzyme-linked immunosorbent assay; F: females; GPAT: gelatin particle agglutination test; HER: children from reportedly high-endemic region; LER: children from reportedly low-endemic region; M: males; NCDR: new case detection rate; NDO: natural disaccharide-octyl; NTO: natural trisaccharide-octyl; ref. no.: reference number; UCP-LFA: up-converting phosphor lateral flow assay.

Besides test type and cut-off, also the sample size needs to be taken into consideration when comparing seropositivity data as demonstrated by the differences in seropositivity identified in a very small study in Midwest Brazil ( $n = 15$ ; 0% positivity) [53], and 4 to 6.9% among 210 contacts in another study from the same area (Table 3) [54].

In contrast, in Northeast Brazil, higher seropositivity rates were found among household and neighborhood contacts under 15 years of age; 33.7% in Ceará [55] and 40% to 46.7% (IgM) in Alagoas were seropositive [56]. However, seropositivity for IgA (0 to 6.6%) and IgG (0 to 2.2%) was very low (Table 3).

In Colombia, a cross-sectional survey among household child contacts in 13 towns located in Bolívar, Córdoba and Sucre, Colombia [57] (Table 3) found that 25 out of 49 (51%) contacts had positive anti-PGL-I IgM titers. No association was found between IgM positivity and clinical diagnosis of the patients.

## The South-Pacific

In 1989, serological screening was conducted among household contacts of leprosy patients ( $n = 15$ ) in New Caledonia using ELISAs for two synthetic forms of PGL-I [58]. For ND-O-BSA, 26.7% and for NT-O-BSA, 13.3% of the contacts was positive (Table 3). Although the number of children in the study was limited, a trend was observed that seropositivity was higher in individuals below the age of 20 (compared to participants over the age of 20) and inversely decreased with age, which led the authors to conclude that the transmission rate in younger individuals is higher. Furthermore, ND-O-BSA was considered to be more sensitive, and NT-O-BSA more specific for screening for *M. leprae* infection among household contacts.

A 10-year study in French Polynesia [59] also based on anti-PGL-I ELISAs showed that of family contacts of patients diagnosed with leprosy ( $n = 20$ ; note that this sample size is very small), approximately 18% of the boys and about 23% of the girls were seropositive (Table 3). Of note is that in individuals over the age of 19, seropositivity started to decrease with age. Although some adult participants were followed up, no longitudinal results were provided for children. The authors conclude that the predictive value of positivity for anti-PGL-I is low, as most of the seropositive participants did not progress to disease during the study period. A finding which was confirmed years later in Bangladesh as well [60].

A two-year follow-up study measured serum anti-PGL-I levels in residents from Kalo village, Papua New Guinea, in 1984 and 1986, among 62 children [61] (Table 3). In 1984, 13 out of

62 (21%) children were seropositive and in 1986, this was the case for 19 out of 62 (30.6%). Similar to what was found in New Caledonia, seropositivity among participants below 20 years of age was significantly higher (20.6% among 379 individuals) compared to that among older participants (7%;  $p < 0.001$ ).

## Africa

In 1987 in Ethiopia, Menzel *et al.* studied child household contacts of leprosy patients while also taking into account the index cases' leprosy type [62]. Six out of 12 (50%) children with 1 or more years of exposure to a lepromatous patient were positive in anti-PGL-I ELISAs versus 10 out of 28 (35.7%) amongst endemic controls (Table 3).

## Asia

In Nepal, the use of anti-PGL-I serology by ELISA for early diagnosis among close child contacts from a reportedly low-endemic area was evaluated in 1994 [63]. Only one out of 93 children (1.1%) tested positive, and none of the contacts developed clinical disease after 6 months.

Our literature search resulted in two studies from India. The first study in 1991 in an endemic population from South India [64] included 179 child contacts and 1,564 non-contacts using cluster sampling. Contacts included children who had stayed for at least half a year with the index case. Forty-eight contacts aged 0 to 4, 75 contacts aged 5 to 9 and 56 contacts aged 10 to 14 were included. The non-contact population consisted of 467 children aged 0 to 4, 586 children aged 5 to 9 and 511 children aged 10 to 14. Seroprevalence among contacts was higher in girls (28.1%; boys: 12.5%), whereas in non-contacts boys and girls showed similar seroprevalence (S2 Table). In 1994 [65], using ELISAs children living in a reportedly low-endemic region (LER; 71 children), a high-endemic region (HER; 1495 children) – Mumbai – and a contact population (CP; 140 children) comprising household contacts of leprosy patients living in a leprosy colony were assessed. Seropositivity among these children ranged from 0% in the low endemic region, 6.9% in the high endemic area to 21.4% in the contact population (Table 3).

A divergent leprosy-specific serological test, the gelatin particle agglutination test (GPAT), has been applied by Khang *et al.* in Vietnam from 1989 to 2016 to screen 78 healthy child controls (<15) without known contact with leprosy patients living in reportedly leprosy-exempt areas and 264 household child contacts [66]. This test, based on semi-synthetic trisaccharides antigen (NT-P-BSA), was positive in 2.5% of controls and 17% of contacts (Table 3). Interestingly, there was no significant difference in seroprevalence between children and adults.

Most of the studies reporting on serum anti-PGL-I antibodies in children from Indonesia took place in Sulawesi, which in the 90s was an area with one of the highest prevalences of leprosy in Indonesia [67]. In 1994, van Beers *et al.* investigated household child contacts of leprosy cases and non-contacts in the villages Bantimala and Tondongkura for their serum anti-PGL-I antibody levels as well as the presence of *M. leprae* DNA in their nasal cavities [68]. Although serum anti-PGL-I IgM antibodies are studied most frequently and are in

general more often positive [40,56,69–72], van Beers *et al.* also evaluated anti-PGL-I IgG in approximately 370 children. An agglutination test (MLPA; IgM) was used as well as an ELISA (IgM and IgG) with NT-P-BSA as target antigen. As seropositivity rates did not differ between household contacts and non-contacts, results were combined. Seropositivity for MLPA and anti-PGL-I IgM was highest in the younger age groups and reduced with increasing age (test agreement of 91%). In contrast, anti-PGL-I IgG seropositivity was low in all age groups. Comparison of PCR-test results (detecting bacterial DNA) with serological test outcome did not show a good correlation. According to the authors, this is not surprising as nasal carriage does not necessarily imply infection [30].

Five years later, the same researchers conducted a study in Sulawesi [35] to assess whether seroprevalence among schoolchildren included by cluster sampling could serve as a valid marker of leprosy endemicity. In this study, they included 2,835 schoolchildren from South, North and Southeast Sulawesi, with case detection rates of 39, 18 and 9.9 per 100,000, respectively. For areas with case detection rates over 30 in 100,000, seroprevalence ranged from 26 to 28%, whereas for reportedly low-endemic areas, seroprevalence was approximately 7% using MLPA. In Bantimurung, considered high-endemic in this study, a second survey was performed three years later among 905 children yielding the same seroprevalence rate of 28%. Based on their findings the authors concluded that seropositivity is related to the incidence of leprosy thus representing a rapid, reliable and relatively inexpensive approach to estimate the leprosy burden in a certain region. Another study reporting data from South Sulawesi (the island of Kembanglemari, a high-endemic area) by Bakker *et al.* [73], measured anti-PGL-I levels of inhabitants by ELISA. In the age group from 0 to 5, one out of seven individuals (14.3%) was seropositive for anti-PGL-I IgM. Clinical disease was more prevalent in adults while seropositivity was higher in children. Longitudinal investigation (2007–2010) in schoolchildren in South Sulawesi [74] detected 39.7% seropositivity (23 out of 58) in 2007, which increased three years later to 48.3% (28 out of 58 children). None of the 23 seropositive children showed clinical signs of leprosy in 2010, and 22 of them remained seropositive. Also, mean titers of anti-PGL-I IgM significantly increased from 2007 to 2010. Another study on schoolchildren in 2013, compared anti-PGL-I IgM levels and PCR results from nasal swabs in South Sulawesi [75] for 80 schoolchildren. Twenty-three out of 40 children (57.5%) from lowland were seropositive compared to 16 out of 40 (40%) from highland. Only one child in both low- and highland tested positive by PCR (2.5%). Most of the seropositive children were not known to have any contact history with leprosy patients and the authors suggested that infection may have originated from unidentified “back-log cases”, from non-human *M. leprae* sources in the environment or from travelling to surrounding areas. A recent paper from 2020 analyzed the presence of *M. leprae* in nasal swabs and seropositivity levels among 530 elementary schoolchildren from the northern and southern coast of East Java [76]. In Pacitan, an area considered non-endemic for leprosy, 25 out of 301 children (8.3%) were seropositive and in Lamongan, an area considered endemic for leprosy, seropositivity was 48% (110 out of 229). PCR results showed 23 out of 229 nasal swabs from children to contain *M. leprae* DNA in Lamongan (10%) in contrast to Pacitan, with only six positive nasal swab samples from a total of 301 (2%). It was calculated that children living in endemic areas were approximately five times more likely to become infected than children living in non-endemic areas.

## Cross-sectional studies in time

As fluctuations in seropositivity in time can provide information on changes in transmission in a certain area, the changes in seroprevalence among children without leprosy in the ten longitudinal studies included in the selected articles were considered (S3 Table): nine of these followed the same children over time to investigate the assessment of anti-PGL-I as a predictive marker for leprosy [43,53,56,58,59,61,63,66,74]. The remaining study measured seropositivity cross-sectionally at two time points with a 3-year interval and validated the use of anti-PGL-I seroprevalence in schoolchildren in Sulawesi (n = 2,835; 10 to 12-year-olds) as an indicator for the level of leprosy endemicity [35]. In the study of van Beers *et al.*, the percentage of seropositivity (28%), as well as the leprosy case detection rate (41/100,000) in the area remained similar over three years [35].

## Discussion

### Relevance of anti-*M. leprae* antibody detection for monitoring transmission

Accurate knowledge on transmission in an area can provide insight into the effect of interventions as well as enable monitoring of the transmission level towards interruption of transmission. Monitoring transmission using disease incidence implies a delay in describing the status of transmission. This is particularly the case for leprosy due to its incubation time of typically three to five years, which can, however, also exceed ten years. For this reason, the incidence of leprosy in children under 15 – the normal definition used in leprosy statistics to date – is not very sensitive to changes in transmission as older children may have been infected ten or more years ago. In contrast, monitoring of *M. leprae* infection in young children, represents a more up-to-date view on the status of transmission in an area.

This systematic review assessed 30 scientific articles reporting on *M. leprae* serology in children under 15 years evaluating to what extent and in what context children have been assessed for the presence of anti-*M. leprae* antibodies as a proxy for infection. We aimed to investigate whether seroprevalence among children could serve as a reliable indicator for the intensity of recent transmission in a community.

### Factors influencing seroprevalence

The 30 included articles showed that seroprevalence in over 18,000 children from over ten countries varied from 0 to 77.6% with a median value of 14.9% (IQR: 7.3–27.7). Children often were only a subgroup of a larger study also including other age groups.

Importantly, six independent studies using different serological tests conducted in (South)eastern Brazil reported similar seropositivity percentages in the range of 10.1 to 18.6% [47–52]. Surprisingly, seroprevalence rates did not differ significantly between household contacts and noncontacts. This may be related to the fact that most studies were done in (highly) endemic areas in which other sources than contact cases have been reported to play a role in transmission [77].

Interestingly, multiple studies (also including adults) [58,59,61,68,73] observed higher positivity rates in the younger age groups and a decrease in seropositivity inversely related with age, a finding which was already described 30 years ago by Ulrich *et al.* in Venezuela [78]. According to Menzel *et al.*, this underlines the good sensitizing capacity of the immune system present at a young age [62]. Thus, children can be considered a suitable group for sensitive assessment of recent *M. leprae* infection. Fine *et al.* studied anti-PGL-I antibody seroprevalence among children in Northern Malawi [79], in a paper excluded during the screening process of this review as the numbers of the children included were not reported. In agreement with what was described above, this study in Malawi described that the seroprevalence rate peaks between the ages of 20 and 30 and then drops. Regardless of age, they also found consistently higher seropositivity rates among females compared to males. In contrast, Andrade *et al.* found positivity to be significantly higher among male household contacts (22.4%) than among females (17.9%) ( $p = 0.003$ ) [49], so the evidence for an effect of gender on seropositivity levels is not consistent.

### The effect of type of test on seroprevalence

In total six types of serological assays were identified, all based on detection of antibodies against the *M. leprae*-specific PGL-I or derivatives thereof. All papers reported measurement of the IgM subtype, probably because anti-PGL-I IgM seroprevalence is higher than IgG/IgA seroprevalence in endemic areas. Moreover, IgM seropositive individuals have a higher risk of developing disease [80]. However, IgM seropositivity is not predictive for disease [56,60], whereas anti-PGL-I IgG has been described to predict leprosy disease [56]. However, to assess (past) infection with *M. leprae* as a proxy for transmission, anti-PGL-I IgM is a much more sensitive biomarker than anti-PGL-I IgG as it can be detected in many individuals (previously) infected with the mycobacterium despite the absence of disease [40,71,72,80].

Three individual studies each using a single, study-specific test showed that anti-PGL-I seropositivity in children correlated with the level of leprosy endemicity [35,65,76]: in India seropositivity rates of 0%, 6.9% and 21.4% were described for a reportedly low-endemic region, a high-endemic region and a contact population, respectively [65]. In Sulawesi [35] seropositivity rates ranging from 26 to 28% were measured in three reportedly high-endemic areas (case detection rates  $> 30/100,000$ ) compared to 7% in two low-endemic areas (case detection rates of  $10/100,000$ ). Of note was that seroprevalence and incidence rates as well as their correlation remained stable over a period of three years. In 2020, an Indonesian study reported seropositivity of 8.3% in Pacitan (considered a non-endemic district) compared to 48% in Lamongan (considered an endemic district) [76]. Thus, these studies convincingly show the relation between the overall disease incidence in an area and the seroprevalence among children. On the other hand, in one study among 7,073 schoolchildren in South(east) Brazil [36], seropositivity levels (ranging from 8.5 to 24%) did not correlate completely with endemicity (ranging from 2 to  $112/100,000$  population). The authors suggest that this may have been caused by differences in immune responsiveness per population, e.g. different social economic status, which may cause differences in disease incidence whereas asymptomatic *M. leprae* infection may be common. Alternatively, case detection depends on operational factors, such as local health structure, active case finding and the local ability to diagnose leprosy cases which may lead to underdiagnosis in certain

areas. This may be an additional advantage of monitoring serology in children. Also, the authors mentioned that the study sample may not always have been representative for the total population in certain municipalities as participation was dependent on the presence of schoolchildren at school at the time of study and consent from their parents. Nevertheless, the seropositivity percentages reported seem reliable, as most of them lie in the same range as the six other studies from the same area discussed above [47–52].

Direct comparison of results between studies was difficult due to the variety of tests and/or different cut-off levels used. For instance, measurements were performed using either fingerprick blood or serum with variable dilutions, and the target antigens in the tests varied from native PGL-I to synthetic PGL-I recognized by various IgM, IgG or IgA isotypes. Importantly, cut-off values were often chosen arbitrarily. For example, in the state of Pará in Brazil, the PGL-I IgM ELISA data of Barreto *et al.* [42] and van Hooij *et al.* [28] for a selection of 58 schoolchildren from Pará correlated excellently (S2 Fig), but there was a large disparity in seropositivity rates because of differences in determination of the cut-off value for positivity. This stresses that the test cut-off used is vital and may impede comparison of seroprevalence data between studies even if quantitative levels correlate well.

The type of serological test and standardization of the cut-off for positivity used in seroscreening for assessment of transmission is thus crucial. In addition, quantitative results (being either obtained by ELISA or field-friendly lateral flow tests) can potentially assess differences in seropositivity with higher accuracy than operator-dependent, visual tests, which is beneficial for monitoring changes in transmission over time within an area and for assessing seroprevalence in very low-endemic areas. Since leprosy-endemic areas are often situated in low resource settings, field-friendly rapid tests are recommended. In this review three types of field-friendly tests were identified, in two of which data were obtained visually and scored qualitatively by operators after five to ten minutes [49,50,55], whereas the anti-PGL-I UCP-LFA [28] offers quantitative determination of antibodies. Of note is that anti-PGL-I IgM UCP-LFA provided similar sensitivity and specificity as the NDO-LID rapid-test [28] and can also be used for and/or in combination with different biomarkers (multiplexing) thereby offering added value [45,81].

## Limitations

No formal methods for assessing risk of bias (a component of PRISMA) were used due to the variation of study methods in the selected studies. The fact that we eventually have included English, Spanish and Portuguese articles only in our literature search may have slightly limited the completeness of this overview. However, these are the languages in which studies of the two most endemic countries are reported. Our strategy resulted in the exclusion of eight articles in French, three in Japanese, one in Swedish and one in Bahasa, Indonesia that were identified through Pubmed, Infoplep and Web of Science of which only the latter article [82] seemed eligible for inclusion as the other papers did not describe leprosy serology in children (based on screening of the English abstracts). Furthermore, the variety of serological tests used in the selected studies impede direct comparison of the results.

## Conclusions

On the route to leprosy elimination, an indicator for the intensity of transmission would be highly valuable. Quantitative anti-PGL-I serology in young children as a measure for *M. leprae* infection shows potential as a proxy for recent transmission and also correlates with disease incidence. Therefore, it can provide a means to monitor progress towards interruption of transmission, as well as to assess the effect of prophylactic interventions on transmission. This systematic review also underscores that, to collect definitive evidence on the utility of PGL-I serology in (young) children as a marker of transmission of *M. leprae*, further studies are needed to evaluate a standardized, quantitative test in multiple areas with different levels of leprosy endemicity. In addition, to assess transmission in an area over time, cross-sectional studies on individuals of the same age group and not on the same individuals are needed. In such studies, the target group would be children without leprosy who are not known contacts of (former) leprosy patients. Based on the findings of this literature search, primary school-age children would represent a suitable target group for sensitive detection of recent transmission, which will allow monitoring of its elimination.

## Data availability

All relevant data are within the manuscript and its Supporting information files.

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## Competing interests

The authors have declared that no competing interests exist.



## References

1. Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL. *The continuing challenges of leprosy*. Clin Microbiol Rev. 2006; 19(2):338–81. <https://doi.org/10.1128/CMR.19.2.338-381.2006> PMID: 16614253
2. Geluk A. *Correlates of immune exacerbations in leprosy*. Semin Immunol. 2018; 39:111–8. <https://doi.org/10.1016/j.smim.2018.06.003> PMID: 29950273
3. Blok DJ, De Vlas SJ, Richardus JH. *Global elimination of leprosy by 2020: are we on track?* Parasit Vectors. 2015; 8:548. <https://doi.org/10.1186/s13071-015-1143-4> PMID: 26490878
4. Alemu Belachew W, Naafs B. *Position statement: LEPROSY: Diagnosis, treatment and follow-up*. J Eur Acad Dermatol Venereol. 2019; 33(7):1205–13. <https://doi.org/10.1111/jdv.15569> PMID: 30945360
5. Polycarpou A, Walker SL, Lockwood DN. *New findings in the pathogenesis of leprosy and implications for the management of leprosy*. Curr Opin Infect Dis. 2013; 26(5):413–9. <https://doi.org/10.1097/QCO.0b013e3283638b04> PMID: 23982232
6. World Health Organization. *Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030: overview*. World Health Organization; 2020. Contract No.: WHO/UCN/NTD/2020.01.
7. Bratschi MW, Steinmann P, Wickenden A, Gillis TP. *Current knowledge on Mycobacterium leprae transmission: a systematic literature review*. Lepr Rev. 2015; 86(2):142–55. PMID: 26502685
8. Fine PE, Sterne JA, Ponnighaus JM, Bliss L, Sauji J, Chihana A, et al. *Household and dwelling contact as risk factors for leprosy in northern Malawi*. Am J Epidemiol. 1997; 146(1):91–102. <https://doi.org/10.1093/oxfordjournals.aje.a009195> PMID: 9215227
9. Moet FJ, Meima A, Oskam L, Richardus JH. *Risk factors for the development of clinical leprosy among contacts, and their relevance for targeted interventions*. Lepr Rev. 2004; 75(4):310–26. PMID: 15682969
10. Quilter EEV, Butlin CR, Singh S, Alam K, Lockwood DNJ. *Patients with skin smear positive leprosy in Bangladesh are the main risk factor for leprosy development: 21-year follow-up in the household contact study (COCO)*. PLoS Negl Trop Dis. 2020; 14(10):e0008687. <https://doi.org/10.1371/journal.pntd.0008687> PMID: 33125403
11. World Health Organization. *Global leprosy (Hansen disease) update, 2019: time to step-up prevention initiatives*. World Health Organization; 2020 Sept. Report No.: WER No 36, 2020, 95, 417–440.
12. Geluk A. *Challenges in immunodiagnostic tests for leprosy*. Expert Opin Med Diagn. 2013; 7(3):265–74. <https://doi.org/10.1517/17530059.2013.786039> PMID: 23537134
13. Bekri W, Gebre S, Mengiste A, Saunderson PR, Zewge S. *Delay in presentation and start of treatment in leprosy patients: a case-control study of disabled and non-disabled patients in three different settings in Ethiopia*. Int J Lepr Other Mycobact Dis. 1998; 66(1):1–9. PMID: 9614833
14. Nicholls PG, Wiens C, Smith WC. *Delay in presentation in the context of local knowledge and attitude towards leprosy—the results of qualitative fieldwork in Paraguay*. Int J Lepr Other Mycobact Dis. 2003; 71(3):198–209.
15. Meima A, Saunderson PR, Gebre S, Desta K, van Oortmarssen GJ, Habbema JD. *Factors associated with impairments in new leprosy patients: the AMFES cohort*. Lepr Rev. 1999; 70(2):189–203. <https://doi.org/10.5935/0305-7518.19990022> PMID: 10464437
16. Van Veen NH, Meima A, Richardus JH. *The relationship between detection delay and impairment in leprosy control: a comparison of patient cohorts from Bangladesh and Ethiopia*. Lepr Rev. 2006; 77 (4):356–65. PMID: 17343222
17. Srinivas G, Muthuvel T, Lal V, Vaikundanathan K, Schwienhorst-Stich EM, Kasang C. *Risk of disability among adult leprosy cases and determinants of delay in diagnosis in five states of India: A case-control study*. PLoS Negl Trop Dis. 2019; 13(6):e0007495. <https://doi.org/10.1371/journal.pntd.0007495> PMID: 31247040
18. Mieras LF, Taal AT, van Brakel WH, Cambau E, Saunderson PR, Smith WCS, et al. *An enhanced regimen as post-exposure chemoprophylaxis for leprosy: PEP+*. BMC Infect Dis. 2018; 18(1):506. <https://doi.org/10.1186/s12879-018-3402-4> PMID: 30290790
19. Richardus JH, Mieras L, Saunderson P, Ignotti E, Virmond M, Arif MA, et al. *Leprosy post-exposure prophylaxis risks not adequately assessed—Author’s reply*. Lancet Glob Health. 2021; 9(4):e402–e3. [https://doi.org/10.1016/S2214-109X\(21\)00047-4](https://doi.org/10.1016/S2214-109X(21)00047-4) PMID: 33740406
20. Tiwari A, Blok DJ, Arif M, Richardus JH. *Leprosy post-exposure prophylaxis in the Indian health system: A cost-effectiveness analysis*. PLoS Negl Trop Dis. 2020; 14(8):e0008521. <https://doi.org/10.1371/journal.pntd.0008521> PMID: 32750059
21. Steinmann P CA, Aerts A, Anand S, Arif M, Ay SS, et al. *The Leprosy Post-Exposure Prophylaxis (LPEP) programme: update and interim analysis*. Leprosy Rev. 2018; 89(2):102–16.
22. Richardus R, Alam K, Kundu K, Chandra Roy J, Zafar T, Chowdhury AS, et al. *Effectiveness of singledose rifampicin after BCG vaccination to prevent leprosy in close contacts of patients with newly diagnosed leprosy: A cluster randomized controlled trial*. Int J Infect Dis. 2019; 88:65–72. <https://doi.org/10.1016/j.ijid.2019.08.035> PMID: 31499206

23. Ortuno-Gutierrez N, Younoussa A, Randrianantoandro A, Braet S, Cauchoix B, Ramboarina S, et al. *Protocol, rationale and design of PEOPLE (Post ExpOsure Prophylaxis for LEprosy in the Comoros and Madagascar): a cluster randomized trial on effectiveness of different modalities of implementation of post-exposure prophylaxis of leprosy contacts*. BMC Infect Dis. 2019; 19(1):1033. <https://doi.org/10.1186/s12879-019-4649-0> PMID: 31805862
24. Roset Bahmanyar E, Smith WC, Brennan P, Cummings R, Duthie M, Richardus JH, et al. *Leprosy Diagnostic Test Development As a Prerequisite Towards Elimination: Requirements from the User's Perspective*. PLoS Negl Trop Dis. 2016; 10(2):e0004331. <https://doi.org/10.1371/journal.pntd.0004331> PMID: 26866699
25. World Health Organization. *Guidelines for the diagnosis, treatment and prevention of leprosy*. World Health Organization. Regional Office for South-East Asia; 2018.
26. World Health Organization. *Global Leprosy (Hansen's disease) Strategy 2021–2030: Towards Zero Leprosy*. World Health Organization; 2020.
27. World Health Organization. *Global Leprosy Strategy 2016–2020: Accelerating towards a leprosy-free world*. World Health Organization. Regional Office for South-East Asia; 2016.
28. van Hooij A, Tjon Kon Fat EM, van den Eeden SJF, Wilson L, Batista da Silva M, Salgado CG, et al. *Field-friendly serological tests for determination of M. leprae-specific antibodies*. Sci Rep. 2017; 7(1):8868. <https://doi.org/10.1038/s41598-017-07803-7> PMID: 28827673
29. Zenha EM, Ferreira MA, Foss NT. *Use of anti-PGL-1 antibodies to monitor therapy regimes in leprosy patients*. Braz J Med Biol Res. 2009; 42(10):968–72. <https://doi.org/10.1590/s0100-879x2009001000016> PMID: 19784481
30. Tio-Coma M, Avanzi C, Verhard EM, Pierneef L, van Hooij A, Benjak A, et al. *Genomic Characterization of Mycobacterium leprae to Explore Transmission Patterns Identifies New Subtype in Bangladesh*. Front Microbiol. 2020; 11:1220. <https://doi.org/10.3389/fmicb.2020.01220> PMID: 32612587
31. Schilling AK, van Hooij A, Corstjens P, Lurz PWW, DelPozo J, Stevenson K, et al. *Detection of humoral immunity to mycobacteria causing leprosy in Eurasian red squirrels (Sciurus vulgaris) using a quantitative rapid test*. European Journal of Wildlife Research. 2019; 65(3).
32. Roche PW, Britton WJ, Failbus SS, Neupane KD, Theuvsen WJ. *Serological monitoring of the response to chemotherapy in leprosy patients*. Int J Lepr Other Mycobact Dis. 1993; 61(1):35–43. PMID: 8326179
33. Khadge S, Banu S, Bobosha K, van der Ploeg-van Schip JJ, Goulart IM, Thapa P, et al. *Longitudinal immune profiles in type 1 leprosy reactions in Bangladesh, Brazil, Ethiopia and Nepal*. BMC Infect Dis. 2015; 15:477. <https://doi.org/10.1186/s12879-015-1128-0> PMID: 26510990
34. Drowart A, Chanteau S, Huygen K, De Cock M, Cartel JL, De Bruyn J, et al. *Effects of chemotherapy on antibody levels directed against PGL-I and 85A and 85B protein antigens in lepromatous patients*. Int J Lepr Other Mycobact Dis. 1993; 61(1):29–34. PMID: 8326178
35. van Beers S, Hatta M, Klatser PR. *Seroprevalence rates of antibodies to phenolic glycolipid-I among school children as an indicator of leprosy endemicity*. Int J Lepr Other Mycobact Dis. 1999; 67(3):243–9. PMID: 10575403
36. Buhner-Sekula S, van Beers S, Oskam L, Lecco R, Madeira ES, Dutra MA, et al. *The relation between seroprevalence of antibodies against phenolic glycolipid-I among school children and leprosy endemicity in Brazil*. Rev Soc Bras Med Trop. 2008; 41 Suppl 2:81–8. <https://doi.org/10.1590/s0037-86822008000700017> PMID: 19618082
37. Douglas JT, Cellona RV, Fajardo TT Jr, Abalos RM, Balagon MV, Klatser PR. *Prospective study of serological conversion as a risk factor for development of leprosy among household contacts*. Clin Diagn Lab Immunol. 2004; 11(5):897–900. <https://doi.org/10.1128/CDLI.11.5.897-900.2004> PMID: 15358649
38. Lyon S, Lyon AC, Da Silva RC, Grossi MA, Lyon SH, Buhner-Sekula S, et al. *A comparison of ML Flow serology and slit skin smears to assess the bacterial load in newly diagnosed leprosy patients in Brazil*. Lepr Rev. 2008; 79(2):162–70. PMID: 18711938
39. Geluk A, van Meijgaarden KE, Wilson L, Bobosha K, van der Ploeg-van Schip JJ, van den Eeden SJ, et al. *Longitudinal immune responses and gene expression profiles in type 1 leprosy reactions*. J Clin Immunol. 2014; 34(2):245–55. <https://doi.org/10.1007/s10875-013-9979-x> PMID: 24370984
40. Levis WR, Meeker HC, Schuller-Levis G, Sersen E, Schreiber B. *IgM and IgG antibodies to phenolic glycolipid I from Mycobacterium leprae in leprosy: insight into patient monitoring, erythema nodosum leprosum, and bacillary persistence*. J Invest Dermatol. 1986; 86(5):529–34. PMID: 3528312
41. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. *Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement*. Syst Rev. 2015; 4:1. <https://doi.org/10.1186/2046-4053-4-1> PMID: 25554246
42. Barreto JG, Guimaraes Lde S, Leao MR, Ferreira DV, Lima RA, Salgado CG. *Anti-PGL-I seroepidemiology in leprosy cases: household contacts and school children from a hyperendemic municipality of the Brazilian Amazon*. Lepr Rev. 2011; 82(4):358–70. PMID: 22439276

43. Barreto JG, Bisanzio D, Frade MA, Moraes TM, Gobbo AR, de Souza Guimaraes L, et al. *Spatial epidemiology and serologic cohorts increase the early detection of leprosy*. BMC Infect Dis. 2015; 15:527. <https://doi.org/10.1186/s12879-015-1254-8> PMID: 26573912
44. Barreto JG, Guimaraes Lde S, Frade MA, Rosa PS, Salgado CG. *High rates of undiagnosed leprosy and subclinical infection amongst school children in the Amazon Region*. Mem Inst Oswaldo Cruz. 2012; 107 Suppl 1:60–7. <https://doi.org/10.1590/s0074-02762012000900011> PMID: 23283455
45. van Hooij A, Tjon Kon Fat EM, Batista da Silva M, Carvalho Bouth R, Cunha Messias AC, Gobbo AR, et al. *Evaluation of Immunodiagnostic Tests for Leprosy in Brazil, China and Ethiopia*. Sci Rep. 2018; 8(1):17920. <https://doi.org/10.1038/s41598-018-36323-1> PMID: 30560920
46. da Conceição Oliveira Coelho Fabri A. *Prevalência de infecção pelo Mycobacterium leprae na população da microrregião de Almenara, Minas Gerais*: Universidade Federal de Minas Gerais; 2011.
47. Ribeiro GC. *Prevalência e distribuição da infecção pelo Mycobacterium leprae por meio de marcadores sorológicos e geoprocessamento em Diamantina, Minas Gerais*: Universidade Federal de Minas Gerais; 2019.
48. Ferreira MA, Antunes CM. *Factors associated with ML Flow test seropositivity in leprosy patients and household contacts under the age of 18*. Rev Soc Bras Med Trop. 2008; 41 Suppl 2:60–6. <https://doi.org/10.1590/s0037-86822008000700013> PMID: 19618078
49. Andrade AR, Grossi MA, Buhner-Sekula S, Antunes CM. *Seroprevalence of ML Flow test in leprosy contacts from State of Minas Gerais, Brazil*. Rev Soc Bras Med Trop. 2008; 41 Suppl 2:56–9. <https://doi.org/10.1590/s0037-86822008000700012> PMID: 19618077
50. Carvalho AP, da Conceicao Oliveira Coelho Fabri A, Correa Oliveira R, Lana FC. *Factors associated with anti-phenolic glycolipid-I seropositivity among the household contacts of leprosy cases*. BMC Infect Dis. 2015; 15:219. <https://doi.org/10.1186/s12879-015-0955-3> PMID: 26024906
51. Wambier CG, Wambier SP, Furini RB, Simao JC, Frade MA, Foss NT. *Factors associated with seropositivity for APGL-I among household contacts of leprosy patients*. Rev Soc Bras Med Trop. 2016; 49(1):83–9. <https://doi.org/10.1590/0037-8682-0325-2015> PMID: 27163568
52. Düppre NC. *Risco de desenvolver hanseníase em contatos de pacientes, segundo positividade ao teste anti PGL-I e situação vacinal (BCG)*. Escola Nacional de Saude Publica Sergio Arouca; 2008.
53. Limeira OM, Gomes CM, Morais OO, Cesetti MV, Alvarez RR. *Active search for leprosy cases in Midwestern Brazil: a serological evaluation of asymptomatic household contacts before and after prophylaxis with bacillus Calmette-Guerin*. Rev Inst Med Trop Sao Paulo. 2013; 55(3).
54. Gomes LC, Cortela D, Silva EA, Silva A, Ferreira SMB. *Leprosy: prevalence and factors associated with seropositivity for anti-NDO-LID antibodies in children under 15 years of age*. An Bras Dermatol. 2019; 94(4):405–10. <https://doi.org/10.1590/abd1806-4841.20197543> PMID: 31644611
55. Lourenco DSD, Campelo TA, Cruz GA, de Almeida PC, Goncalves HD, Pontes MAD, et al. *Detection of subclinical Mycobacterium leprae infection in children, contacts of leprosy cases, Fortaleza Ceara, Brazil*. Leprosy Rev. 2017; 88(2):184–96.
56. TiemiNagao-Dias A, Casimiro de Macedo A, Rodrigues RO, Pedroza FHC, Albuquerque AA, Moreira FA, et al. *Serum Anti-PGL-1 IgG, IgM, and IgA in a 3-Year Follow-up Study of 4-15-Year-old Leprosy Contacts*. Pediatr Infect Dis J. 2019; 38(9):e193–e8. <https://doi.org/10.1097/INF.0000000000002337> PMID: 31220042
57. Cardona-Castro N, Beltran-Alzate JC, Manrique-Hernandez R. *Survey to identify Mycobacterium leprae-infected household contacts of patients from prevalent regions of leprosy in Colombia*. Mem Inst Oswaldo Cruz. 2008; 103(4):332–6. <https://doi.org/10.1590/s0074-02762008000400003> PMID: 18660985
58. Desforges S, Bobin P, Brethes B, Huerre M, Moreau JP, Bach MA. *Specific anti-M leprae PGL-I antibodies and Mitsuda reaction in the management of household contacts in New Caledonia*. Int J Lepr Other Mycobact Dis. 1989; 57(4):794–800. PMID: 2681462
59. Chanteau S, Glaziou P, Plichart C, Luquiaud P, Plichart R, Faucher JF, et al. *Low predictive value of PGL-I serology for the early diagnosis of leprosy in family contacts: results of a 10-year prospective field study in French Polynesia*. Int J Lepr Other Mycobact Dis. 1993; 61(4):533–41. PMID: 8151183
60. Richardus RA, van der Zwet K, van Hooij A, Wilson L, Oskam L, Faber R, et al. *Longitudinal assessment of anti-PGL-I serology in contacts of leprosy patients in Bangladesh*. PLoS Negl Trop Dis. 2017; 11(12):e0006083. <https://doi.org/10.1371/journal.pntd.0006083> PMID: 29228004
61. Bagshawe AF, Garsia RJ, Baumgart K, Asbury L. *IgM serum antibodies to phenolic glycolipid-I and clinical leprosy: two years' observation in a community with hyperendemic leprosy*. Int J Lepr Other Mycobact Dis. 1990; 58(1):25–30. PMID: 2181044
62. Menzel S, Harboe M, Bergsvik H, Brennan PJ. *Antibodies to a synthetic analog of phenolic glycolipid-I of Mycobacterium leprae in healthy household contacts of patients with leprosy*. Int J Lepr Other Mycobact Dis. 1987; 55(4):617–25. PMID: 3323365
63. Soares DJ, Failbus S, Chalish Y, Kathet B. *The role of IgM antiphenolic glycolipid-1 antibodies in assessing household contacts of leprosy patients in a low endemic area*. Lepr Rev. 1994; 65(4):300–4. <https://doi.org/10.5935/0305-7518.19940031> PMID: 7861916

64. Krishnamurthy P, Rao PS, Reddy BN, Subramanian M, Dhandayudapani S, Bhatia V, et al. *Seroepidemiological study of leprosy in a highly endemic population of south India based on an ELISA using synthetic PGL-I*. Int J Lepr Other Mycobact Dis. 1991; 59(3):426–31. PMID: 1890366
65. Shah DH, Vartak RR, Naik SS, Dandekar SR, Ganapati R. *Epidemiological studies in children of a lowendemic region, a high-endemic region, and dwellers of a leprosy colony: evaluation of anti-ND-BSA antibodies and lepromin response*. Int J Lepr Other Mycobact Dis. 1994; 62(4):539–46. PMID: 7868951
66. Khang TH, Thanh LT, Lanh PH. *Predictive value of gelatin particle agglutination test (GPAT) in leprosy detection*. Indian J Lepr. 2018; 90:61–7.
67. Day R, Lever P, Asri M. *Leprosy control in 7 districts of south Sulawesi, Indonesia, 1986–91*. Lepr Rev. 1992; 63(3):247–54. <https://doi.org/10.5935/0305-7518.19920029> PMID: 1406019
68. van Beers SM, Izumi S, Madjid B, Maeda Y, Day R, Klatser PR. *An epidemiological study of leprosy infection by serology and polymerase chain reaction*. Int J Lepr Other Mycobact Dis. 1994; 62(1):1–9. PMID: 8189074
69. Brito e Cabral P, Junior JE, de Macedo AC, Alves AR, Goncalves TB, Brito e Cabral TC, et al. *Anti-PGL1 salivary IgA/IgM, serum IgG/IgM, and nasal Mycobacterium leprae DNA in individuals with household contact with leprosy*. Int J Infect Dis. 2013; 17(11):e1005–10. <https://doi.org/10.1016/j.ijid.2013.05.011> PMID: 23871279
70. Gormus BJ, Baskin GB, Xu KY, Ratterree MS, Martin LN, Mack PA, et al. *Antileprosy protective vaccination of sooty mangabey monkeys with BCG, or BCG plus heat-killed Mycobacterium leprae: Immunologic observations*. Int J Leprosy. 2000; 68(4):434–43.
71. Cho SN, Yanagihara DL, Hunter SW, Gelber RH, Brennan PJ. *Serological Specificity of Phenolic Glycolipid-I from Mycobacterium-Lepae and Use of Serodiagnosis in Leprosy*. Int J Leprosy. 1983; 51(4):659–60. <https://doi.org/10.1128/iai.41.3.1077-1083.1983> PMID: 6193065
72. Bach MA, Wallach D, Flageul B, Hoffenbach A, Cottenot F. *Antibodies to Phenolic Glycolipid-1 and to Whole Mycobacterium-Lepae in Leprosy Patients—Evolution during Therapy*. Int J Leprosy. 1986; 54(2):256–67. PMID: 3522769
73. Bakker MI, May L, Hatta M, Kwenang A, Klatser PR, Oskam L, et al. *Genetic, household and spatial clustering of leprosy on an island in Indonesia: a population-based study*. BMC Med Genet. 2005; 6:40. <https://doi.org/10.1186/1471-2350-6-40> PMID: 16307680
74. Putri RD, Amiruddin MD, Tabri F, Adriaty D, Wahyuni R, Iswahyudi, et al. *Evaluation of anti-PCL-1 antibody titer in a group of healthy school children who live in leprosy endemic area from 2007–2010*. IJTID. 2010; 1(3):110–3.
75. Rachmawati, Mataallo TT, Adam S, Adam AM, Amin S, Tabri F, et al. *Comparative study on the intensity of Mycobacterium leprae exposure to children who live in low and high altitude in low leprosy endemic area of South Sulawesi*. IJTID. 2013; 4(4):1–4.
76. Adriaty D, Rosita C, Iswahyudi, Wahyuni R, Agusni I, Izumi S. *Leprosy transmission in endemic and non-endemic areas based on the profile of antibody response of PGL-1 and PCR detection of Mycobacterium leprae DNA from nasal swab among healthy children of East Java, Indonesia*. Infect Dis Rep. 2020; 12(8748):109–12. <https://doi.org/10.4081/idr.2020.8748> PMID: 32874474
77. Ortuno-Gutierrez N, Baco A, Braet S, Younoussa A, Mzembaba A, Salim Z, et al. *Clustering of leprosy beyond the household level in a highly endemic setting on the Comoros, an observational study*. BMC Infect Dis. 2019; 19(1):501. <https://doi.org/10.1186/s12879-019-4116-y> PMID: 31174481
78. Ulrich M, Smith PG, Sampson C, Zuniga M, Centeno M, Garcia V, et al. *IgM antibodies to native phenolic glycolipid-I in contacts of leprosy patients in Venezuela: epidemiological observations and a prospective study of the risk of leprosy*. Int J Lepr Other Mycobact Dis. 1991; 59(3):405–15. PMID: 1890364
79. Fine PE, Ponnighaus JM, Burgess P, Clarkson JA, Draper CC. *Seroepidemiological studies of leprosy in northern Malawi based on an enzyme-linked immunosorbent assay using synthetic glycoconjugate antigen*. Int J Lepr Other Mycobact Dis. 1988; 56(2):243–54. PMID: 3045222
80. Gormus BJ, Baskin GB, Xu K, Ratterree MS, Martin LN, Mack PA, et al. *Antileprosy protective vaccination of rhesus monkeys with BCG or BCG plus heat-killed Mycobacterium leprae: immunologic observations*. Int J Lepr Other Mycobact Dis. 2000; 68(1):27–39. PMID: 10834067
81. Corstjens PL, de Dood CJ, van der Ploeg-van Schip JJ, Wiesmeijer KC, Riuttamaki T, van Meijgaarden KE, et al. *Lateral flow assay for simultaneous detection of cellular- and humoral immune responses*. Clin Biochem. 2011; 44(14–15):1241–6. <https://doi.org/10.1016/j.clinbiochem.2011.06.983> PMID: 21763300
82. Sujagat A, Astuti FD, Saputri EM, Sani A, Prasetya AD. *Case Finding of Subclinical Leprosy Infection among Children through Detection of Antibodies (IgM) Anti PGL-1 Level*. Kesmas. 2015; 10(2):74–9.

## Supporting information

**S1 Table. Research PRISMA Checklist–Detection of anti-*M. leprae* antibodies in children in leprosy-endemic areas: A systematic review.**

<https://doi.org/10.1371/journal.pntd.0009667.s001>

**S2 Table. Seroprevalence results for *M. leprae* antibodies in children without leprosy sorted per country.**

<https://doi.org/10.1371/journal.pntd.0009667.s002>

**S3 Table. Test methods applied for detection of antibodies against *M. leprae*-specific antigens.**

<https://doi.org/10.1371/journal.pntd.0009667.s003>

**S4 Table. Longitudinal studies included in the review.**

<https://doi.org/10.1371/journal.pntd.0009667.s004>

**S1 Fig. Overview of seropositivity in different study areas.**

<https://doi.org/10.1371/journal.pntd.0009667.s005>

**S2 Fig. Correlation analysis of ELISA data from different studies**

<https://doi.org/10.1371/journal.pntd.0009667.s006>





# 3

## **Rapid test for *Mycobacterium leprae* infection: a practical tool for leprosy**

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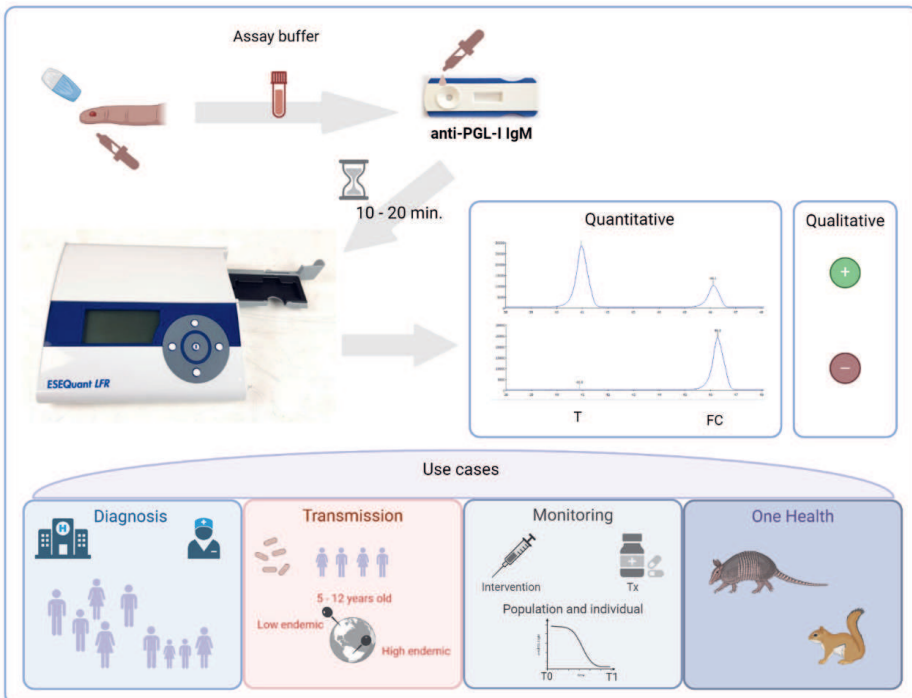
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## Abstract

Detection of infection with *Mycobacterium leprae* allows timely prophylactic treatment, thereby reducing transmission as well as the risk of permanent, leprosy-associated nerve damage. However, since there is no worldwide-implemented standard test for *M. leprae* infection, detection of infection in asymptomatic individuals remains a major challenge for control programs in endemic areas. In previous studies, we developed and field-tested a lateral flow assay (LFA) quantitatively detecting human IgM against *M. leprae*-specific phenolic glycolipid I (anti-PGLI), a marker for both active and past infection. This rapid test utilizes luminescent, background-free, up-converting reporter particles (UCP) and immunochromatography (i.e. the UCP-LF test platform) for accurate quantitation of anti-PGL-I IgM without operator bias. The aim of this study was to evaluate the final version of this quantitative UCP-based rapid test (i.e. PGL-I QURapid), using serum and fingerstick blood (FSB). The test comprises a lateral flow strip, in a standard plastic or biodegradable cassette. It can be provided with a humanized, recombinant control to monitor test performance and calculate accurate anti-PGL-I IgM levels. The performance of this QUR-test was assessed using serum and FSB from patients with leprosy ( $n = 214$ ), tuberculosis ( $n = 20$ ), buruli ulcer ( $n = 19$ ), leishmaniasis ( $n = 14$ ), non-tuberculous mycobacterial ( $n = 35$ ) infections, as well as healthy Dutch individuals ( $n = 710$ ) and humanized, recombinant anti-PGL-I IgM antibodies. Plot receiver operating characteristic curves were created and sensitivity (Sn), specificity (Sp) and the area under the curve were calculated to evaluate test performance. Test results classified multibacillary leprosy patients with 95.0% Sn and 100% Sp using serum and 91.5% Sn and 99.8% Sp using FSB. Qualitative test results could be read after 2 min flow time, with accurate quantitation from 10 min onwards. The new anti-PGL-I IgM control supports production of batches with predetermined seropositivity thresholds and monitoring of the PGL-I QUR-test in various settings. The operational version of the PGL-I QURapid with point-of-care applicability, meets the WHO target product profile criteria. Thus, this QUR-test is ready for public health implementations.



## Introduction

Leprosy is a neglected tropical disease (NTD) caused by *Mycobacterium leprae* or *M. lepromatosis* affecting the skin and the peripheral nerves (1-3). Despite the available multidrug therapy (MDT) – which can effectively kill *M. leprae* when treatment is started in time – leprosy remains a significant health problem in endemic areas where about 200,000 new cases are reported annually from over 120 countries (4, 5). The World Health Organization (WHO)'s Global Leprosy Strategy 2021-2030 aims to significantly reduce the number of new cases with grade 2 disability and new child cases by focusing on early detection of disease and interruption of transmission (6). To achieve the latter, it is vital to identify and prophylactically treat *M. leprae* infected individuals (to prevent progression to leprosy disease) as well as therapeutically treat leprosy patients (7, 8).

Leprosy diagnosis is still dependent on the identification of clinical symptoms and studies have shown that health practitioners lacking sufficient experience in clinical leprosy, may not recognize disease symptoms (5, 9). In addition, detecting *M. leprae* infection in individuals without clinical symptoms remains a major challenge for control programs in endemic areas. In this respect, the use of different tests to detect infection, in particular variable analyses, assays, or qualitative measurements only, furthermore impedes comparison of *M. leprae* infection rates globally (10). The most severe form of leprosy (multibacillary) is characterized by numerous antibodies (11), in particular immunoglobulin M (IgM), whereas IgG and IgA are detected mostly in lower levels and in patients only (12). Blood levels of IgM against *M. leprae*-specific phenolic glycolipid I (anti-PGL-I), correspond to an individual's bacterial load (11), thereby allowing detection of *M. leprae* infection as well as treatment monitoring of MB leprosy patients (11, 13, 14). However, most PB patients are able to kill *M. leprae* efficiently and therefore do not mount antibody responses directed against PGL-I (1, 11, 15-17). Furthermore, besides detecting infection with *M. leprae*, anti-PGL-I antibodies were also detected in leprosy patients and red squirrels infected with *M. lepromatosis* (unpublished data and (18)).

Since *M. leprae* infection in young children is recent by definition, anti-PGL-I seroprevalence in healthy young children could be a proxy of transmission in a population (10, 19). Therefore, the WHO-Task Force on definitions, criteria and indicators for interruption of transmission and elimination of leprosy recommends to consider using anti-PGL-I seroprevalence in children as a criterium for monitoring (interruption of) transmission in areas aiming at elimination of leprosy (20). Additionally, seroprevalence has potential to assess the effect of interventions like post-exposure prophylaxis at population as well as individual level. For all applications (quantitative) assessment of anti-PGL-I-serology is essential. Thus, availability of a collectively acceptable user-friendly test would facilitate strategies to uniformly measure infection.

Given the fact that leprosy particularly affects poor communities in low-resource settings, a rapid, low-complexity, field-friendly diagnostic test is needed to identify infected individuals. Other tests developed so far, are either qualitative or not rapid (Supplementary table 1) (11, 21-25). Therefore, we have developed a robust, user-friendly test to quantitatively detect anti-PGL-I IgM using up-converting phosphor (UCP) technology in a low-cost LFA format

(11, 16, 17, 26, 27). Previously, the anti-*M. leprae* PGL-I UCP-LFA (PGL-I UCP-LFA) has been used in a serosurvey among 1857 children in India (19) where it was well-accepted for field work. Currently, this quantitative UCP-based rapid test (i.e. PGL-I QURapid) is applied in a clinical trial in Bangladesh (NCT06222372: 03/01/2024; <https://clinicaltrials.gov/study/NCT06222372>) and evaluated in ongoing field studies in Brazil, Bolivia, Indonesia, Nepal, and Madagascar. The basis of the UCP-LFA is formed by ultrasensitive reporter particles (28), which, upon excitation by IR light, up-convert the energy to give a visible 550-nm green emission. Since no biological specimen in nature up-converts low-energy IR light, UCP applications are unaffected by specimen background and display excellent signal-to-noise ratios (29). This QUR-test does not require sophisticated analytical laboratory equipment or elaborate staff training and portable battery-operated readers provide full instrument-assisted analysis. In view of field and point-of-care/point-of-contact (POC) applications, the assay utilizes a minimally invasive fingerstick blood (FSB) sample. The PGL-I QURapid allows convenient storage at ambient temperature and worldwide shipping without the need for a cold chain.

Here, we analyze serum and FSB samples from patients with leprosy. For specificity purposes, sera from patients with other mycobacterial infections, such as tuberculosis (TB) or buruli ulcer (BU), as well as non-tuberculous mycobacterial (NTM) infections were similarly assessed. Also, as leishmaniasis is included in the differential diagnosis of leprosy (30) and as both diseases overlap geographically, sera of leishmaniasis patients were included.

For the optimization of the performance of any quantitative test, the determination of the threshold for positivity is essential. Therefore, the optimally required sensitivity (Sn)/specificity (Sp) as defined in a target product profiles (TPP) published by the WHO in 2023 were used as initial targets for this study (ideal:  $\geq 94\%$  Sn;  $\geq 99.9\%$  Sp) (31). Since a robust quality control is vital for monitoring test performance, humanized, recombinant anti-PGL-I IgM was developed, to allow normalization of threshold values between batches of the PGL-I QURapid.

We present an operational QUR-test for the detection of *M. leprae* infection which can be made available for research- and largescale population studies and clinical trials. This study aims to describe the performance of this new QUR-test in detail, regarding its optimal flow time, reproducibility, robustness, sensitivity and specificity. Importantly, the PGL-I QURapid can be used for different applications and populations, providing a significant tool for multiple use cases in the leprosy field worldwide.

## Methods

**Samples.** Biobanked samples were derived from the following study groups (Table 1):

**Leprosy patients.** Serum/plasma and FSB samples from newly diagnosed leprosy patients recruited between June 2013 and May 2022 in leprosy endemic areas in the Northwest of Bangladesh (13, 17, 32, 33), were tested. Patients were diagnosed according to the National Leprosy Control Program (32). Patients with five or fewer skin lesions were grouped as paucibacillary (PB), whereas patients with more than five skin lesions were grouped as

multibacillary (MB) leprosy. In this study, PB patients with a bacterial index (BI) of 0 ( $n = 76$ ) and MB patients with a positive BI (1-6;  $n = 133$ ) were included. From leprosy patients recruited in the Netherlands ( $n = 5$ ), FSB samples were collected on a voluntary basis between January 2020 and February 2023 at the Department of Dermatology, Erasmus MC, University Medical Center (EMC), Rotterdam, the Netherlands (26). Leprosy in the Netherlands was diagnosed based on clinical examination, histopathology of skin biopsies and PCR of skin biopsies, using anti-PGL-I serology and microbiological testing as adjunct diagnostic tools. Additionally, leprosy histology of biopsies was applied for classification according to Ridley and Jopling (34).

**TB patients.** Serum samples from TB patients ( $n = 20$ ) during or after treatment, recruited between January 2002 and January 2003 at the Leiden University Medical Center (LUMC), Leiden, the Netherlands (35, 36) were included.

**Leishmaniasis patients.** Serum samples of leishmaniasis patients were either sent to the Clinical Microbiological Laboratory of the Department of Medical Microbiology, LUMC, Leiden, the Netherlands, for routine leishmaniasis diagnostic testing ( $n = 7$ ) (37) or collected from PCR- and/or microscope-confirmed leishmaniasis patients at the Department of Medical Microbiology and Infectious Diseases, EMC, Rotterdam, the Netherlands ( $n = 7$ ) (38).

**Buruli ulcer (BU) patients.** Serum samples from BU patients ( $n = 19$ ) were obtained from the Swiss Tropical and Public Health Institute, Allschwil, Switzerland, and collected from villages within the Obom subdistrict of the Ga-South district of Ghana (39). *IS2404* PCR was used to confirm infection with *Mycobacterium ulcerans*. Lesions were classified following the WHO classification (40).

**Patients infected with non-tuberculous mycobacteria (NTM).** Sera from individuals with various NTM infections ( $n = 35$ ) were collected between March 2000 and March 2003 at the Department of Infectious Diseases, LUMC, Leiden, the Netherlands (41-44).

**Healthy controls.** FSB ( $n = 500$ ) and serum ( $n = 115$ ) samples of health care workers from three hospitals in the Netherlands (LUMC, Radboud University Medical Center and University Medical Center Utrecht) who participated on a voluntary basis in a BCG-vaccination trial during the COVID-19 pandemic (45), were selected as non-endemic controls (NEC). This included an even division of samples derived from either BCG- or placebo-vaccinated health care workers 12 weeks after vaccination. In addition, serum samples from Dutch healthy blood bank donors ( $n = 95$ ) were tested.

**Table 1. Characteristics of participants' samples**

| Group            | Country of sampling | <i>n</i> | Age, years, mean (range) | Sex (% female) | Refs.            |
|------------------|---------------------|----------|--------------------------|----------------|------------------|
| Leprosy patients | Bangladesh          | 209      | 38 (8–83)                | 20             | [13, 17, 32, 33] |
|                  | The Netherlands     | 5        | 46 (24–64)               | 60             | [26]             |
| TB patients      | The Netherlands     | 20       | 44 (15–84)               | 20             | [35, 36]         |
| Leish patients   | The Netherlands     | 7        | 59 (24–82)               | 29             | [37]             |
|                  | The Netherlands     | 7        | na                       | na             | [38]             |
| BU patients      | Ghana               | 19       | 13*                      | 59             | [39]             |
| NTM patients     | The Netherlands     | 35       | 30 (1–72)                | 56             | [41–44]          |
| Healthy controls | The Netherlands     | 710      | 42 (18–67)               | 72             | [45]             |

Overview of the different samples tested including country of sampling, number of individuals, age mean (range), and sex (% female). BU: buruli ulcer; Leish: leishmaniasis; na: not available; NTM: non-tuberculous mycobacterial infection; TB: tuberculosis. \*median

**Quality control samples.** As part of the quality control for the PGL-I QURapid, sera of clinically-diagnosed leprosy patients were selected based on their anti-PGL-I IgM levels in standard anti-PGL-I IgM ELISAs (11, 21): anti-PGL-I IgM highly seropositive (High;  $n = 2$ ), medium (Med; with an OD around the cut-off for seropositivity in ELISAs;  $n = 1$ ). Seronegative (Neg;  $n = 2$ ) samples included were from healthy Dutch blood bank donors without travel history to leprosy endemic areas. These control samples were also used to assess intra- and inter-operator variability of the assay. “Inter-operator variation” was here defined as the amount of variation between the results obtained by three operators testing the same sample using the PGL-I QURapid (each preparing their own dilutions). “Intra-operator variation” was referred to as the amount of variation in the test results when one operator tested the same samples more than once (e.g. replicates in the same experiment and over multiple days). Anti-PGL-I IgM highly seropositive, medium and seronegative control sera and FSB samples were analyzed at 2, 5, 10, 20, 60 minutes and 24 hours after sample addition to determine the effect of time after start of sample flow until measurement for the PGL-I QURapid. As a control, humanized, recombinant anti-PGL-I IgM produced by hybridoma technology (Eurogentec, Seraing, Belgium; stock concentration: 1 mg/ml) was used.

**PGL-I QURapid.** Individually packaged UCP-LFA cassettes for detection of human anti-PGL-I IgM antibodies were produced by MaximBio (Rockville, MD, USA) as described previously (19). The air-tight pouches with test cassettes contained silica dry packs allowing extended shelf life and protection against humidity. The Test (T) line on the LF strip (nitrocellulose membrane; Sartorius UniSart CN95) comprised 100 ng of synthetic PGL-I, phenolic trisaccharide functionalized with a hexanoic acid linker for conjugation to BSA (NPT1-H-BSA; Leiden, the Netherlands (21)). The flow-control (FC) line comprised 100 ng rabbit anti-goat IgG (G4018; Sigma-Aldrich, Inc., St. Louis, MO, USA). Goat IgG specific for anti-human IgM (I0759; Sigma-Aldrich, Inc., St. Louis, MO, USA) was conjugated to polyacrylic acid functionalized UCPs [200 nm, NaYF<sub>4</sub>:Yb<sup>3+</sup>, Er<sup>3+</sup>; Intelligent Material Solutions Inc. (IMS); Princeton, NJ, USA MS] according to previously described protocols at

a concentration of 50 µg antibody per mg UCP (16). Stock solutions were kept at 4 °C until use. To dry the UCPs onto the glass fiber conjugate-release pad, the material was diluted in a buffer containing 100 mmol/L Tris pH 8.0, 270 mmol/L NaCl, 10% (w/v) sucrose, 1% (w/v) BSA, 0.5% Tween-20, and striped at a density of 100 ng/mm. Components were mounted on plastic backing cards which were cut into LF strips of 4.8 mm width by 6 cm length, added to an appropriate cassette, and individually sealed in a pouch together with a silica dry pack.

**UCP-LFA.** 50 µl of the 50-fold diluted FSB or serum/plasma sample or diluted recombinant anti-PGL-I IgM stock was added to the test to initiate LF. QUR-tests were analyzed using a battery operated UCP-adapted portable lightweight standalone reader (ESEQuant LFR adapted for UCP; DIALUNOX, Stockach, Germany). Results were calculated as the ratio value (R) between test line (T) and flow control line (FC) signal based on relative fluorescence units (RFUs) measured at the respective lines.

**Statistical analysis.** The statistical software GraphPad Prism version 9.0.1 for Windows (GraphPad Software, San Diego, CA, USA) was used to perform statistical analysis. Mann-Whitney U and Kruskal-Wallis tests were performed to determine the statistical significance between two and three independent groups, respectively. Plot receiver operating characteristic (ROC) curves were created and Sn, Sp and the area under the curve (AUC) were calculated to evaluate test performance. According to analyses described in our previous research on UCP-LFAs (16, 17, 33, 46), the Youden's index (47) was used to assess cut-offs for anti-PGL-I IgM seropositivity in serum/plasma and FSB samples for this QUR-test batch. However, the WHO TPP published in 2023 (31) was leading in terms of required minimum Sn/Sp for establishing a cut-off. An indecisive range was determined by calculating a lower-specificity cut-off (average of a set of negative controls + 2 × SD) and a high-specificity cut-off (the highest of a set of negative controls + 2 × SD) using R-values below the 99<sup>th</sup> percentile (48).

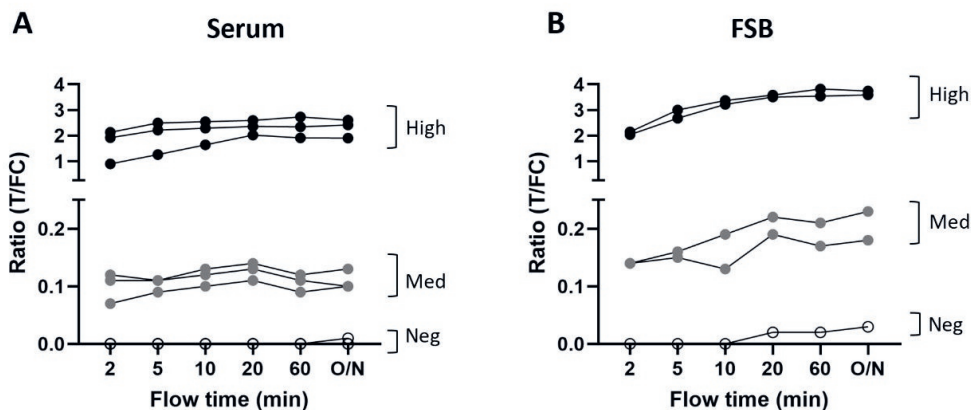
**Ethics.** Ethical permission for leprosy patient samples was received from the national Research Ethics Committee (Bangladesh Medical Research Council) in Bangladesh (Ref no. BMRC/NREC/2010-2013/1534) (15) and local ethical boards in the Netherlands (MEC-2012-589). Anonymized use of residual serum samples of leishmaniasis patients for scientific purposes was granted by the Medical Ethics Review Board of the EMC, Rotterdam, the Netherlands (MEC 2012-047 and MEC-2015-306) and institutional review board (IRB) of the LUMC, Leiden, the Netherlands (B21.048). The biomaterial and associated clinical data of donors collected in the LUMC healthy voluntary donor service (LuVDS) are released for research purposes only, after being approved by the IRB. The LuVDS Biobank is stored in, and direct use coordinated by, the central biobanking facility at the LUMC, Leiden, the Netherlands. The LUMC Biobank facility was implemented by the LUMC Executive Board as part of the university research infrastructure and acts as a separate entity, servicing all departments of the university medical center. Use of residual serum samples of TB, NTM and BU patients for scientific purposes was approved by the Medical Ethics Committee and IRB of the LUMC (METC project nr: P07.048 & P207/99; TB and NTM patients), and the

institutional review board of the Noguchi Memorial Institute for Medical Research (Federalwide Assurance number FWA00001824; BU patients) in Ghana, respectively. The BCG-CORONA trial was registered at [clinicaltrials.gov](https://clinicaltrials.gov) (identifier: NCT04328441) and the Dutch Trial Registry ([trialregister.nl](https://trialregister.nl), identifier Trial NL8477) and the study protocol was approved by the IRB of the LUMC (NL73249.041.20). All donors gave broad consent.

## Results

### Determining the optimal flow time

Since most leprosy cases occur in remote and/or resource-limited areas, a diagnostic tool for detection of *M. leprae* infection, should be of low-complexity nature such that it is easily implementable in field settings. The here described PGL-I QURapid can (in addition to serum/plasma) be performed with FSB, an easily obtained, low-invasive bio-sample. Since the WHO TPP for an optimal diagnostic test for *M. leprae* infection (31) requires a sample-to-result time of maximum 30 minutes, we determined the effect of time after start of sample flow until measurement for the PGL-I QURapid using either serum or FSB. Already after 10–20 minutes, sufficiently stable R-values were observed for both serum (Figure 1A) and FSB (Figure 1B) quality control samples that did not vary significantly from values obtained the next day (for T and FC lines see Supplementary figure 1). Thus, 10–20 minutes would be the preferred minimum time after sample addition until scanning of the PGL-I QURapid. Of note is that the strips are stable at ambient temperature and therefore, QUR-tests can be scanned at any later point in time which is convenient for checking data at a central facility.



**Figure 1. Scanning of PGL-I QURapid by Quant LFR over sample flow time.**

Anti-PGL-I IgM highly seropositive (High; black dots), medium (Med; grey dots) and seronegative (Neg; open dots) control sera (Figure 1A) and FSB samples (Figure 1B) were assessed using the PGL-I QURapid. Samples were measured at 2, 5, 10, 20, 60 minutes and 24 hours (O/N) after sample addition. Ratio (R)-values (Y-axis) were calculated by dividing the peak area of the test line (T) by the peak area of the flow control line (FC).



### Determining test performance

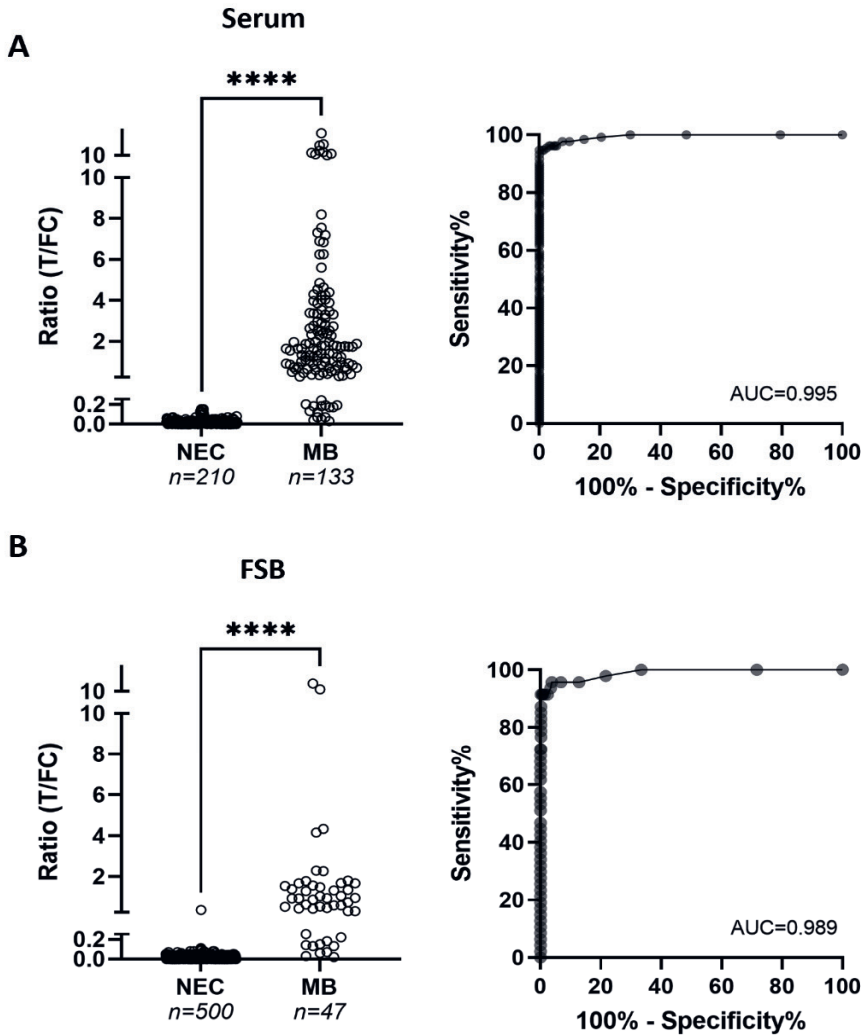
To determine test performance, results in sera from MB leprosy patients from Bangladesh ( $n = 133$ ) and healthy adults from the Netherlands (NEC;  $n = 210$ ) were compared. As (previous) infection with *M. leprae* in endemic areas can never completely be excluded, we selected Dutch individuals as control group, since leprosy is an import disease in the Netherlands and there have not been any autochthonous cases for centuries. Test sensitivity was determined based on the requirement to detect clinically diagnosed, BI-positive leprosy patients. R-values, the quantitative test outcome of UCP-LFAs, were significantly higher for MB leprosy patients compared to NEC (Figure 2A;  $P < 0.0001$ ; AUC: 0.995). Using the Youden's index, the cut-off value to discriminate clinically diagnosed MB leprosy patients from NEC, was  $R \geq 0.160$  with a corresponding Sn of 95% (95% CI: 89.5-97.4) and Sp of 100% (95% CI: 98.2-100) (Supplementary table 2), meeting the WHO TPP for a test for *M. leprae* infection (ideal:  $\geq 94\%$  Sn;  $\geq 99.9\%$  Sp). When using the lower-specificity cut-off ( $R = 0.08$ ; Sn: 96%; Sp: 95%) and the high-specificity cut-off ( $R = 0.19$ ; Sn: 92%; Sp: 100%), an indecisive range was determined as:  $R\text{-values between } 0.08 \leq R \leq 0.19$ . As the cut-off determined by the Youden's index,  $R \geq 0.16$ , fell inside this range and also met the WHO TPP criteria, this was considered appropriate for use as the cut-off for seropositivity in serum.

For FSB, test performance for this batch of the PGL-I QURapid was determined by comparing samples collected from 500 NEC and 47 untreated MB leprosy patients (Figure 2B). Similar to results obtained with sera, R-values were significantly increased for MB leprosy patients compared to NEC ( $P < 0.0001$ ; AUC: 0.989). The cut-off best corresponding to the optimal WHO TPP ( $\geq 94\%$  Sn;  $\geq 99.9\%$  Sp),  $R \geq 0.12$ , resulted in a Sn of 91.5% (95% CI: 80.1-96.6) and Sp of 99.8% (95% CI: 98.9-100; Supplementary table 3). When using the lower-specificity cut-off ( $R = 0.05$ ; Sn: 96%; Sp: 96%) and the high-specificity cut-off ( $R = 0.12$ ; Sn: 91%; Sp: 100%), an indecisive range was determined for R-values between 0.05 and 0.12. Placing Sp at 100%,  $R \geq 0.12$  was concluded as appropriate cut-off for seropositivity in FSB.

### Inter- and intra-operator variation

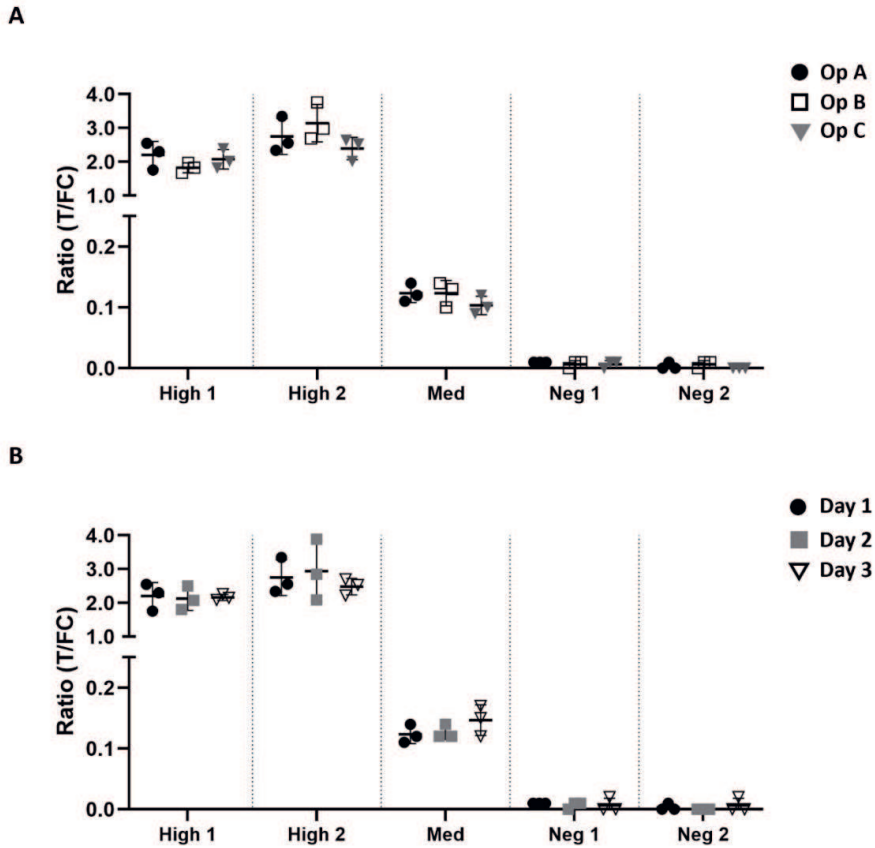
To evaluate assay robustness, two anti-PGL-I IgM highly seropositive, one medium and two seronegative control serum samples were tested in triplicate by three different operators. This multi-operator comparison showed that inter-operator differences were not detectable or very low (Figure 3A; Supplementary table 4). In addition, reproducibility (intra-operator variation) was evaluated by testing the same five control serum samples on three QUR-tests by each operator on three different days (Figure 3B; Supplementary table 4). Day-to-day as well as triplicate differences on the same day were minor (Supplementary table 4), confirming excellent assay reproducibility.





**Figure 2. Anti-PGL-I IgM in NEC and MB leprosy patients.**

Serum samples (A) of NEC ( $n = 210$ ) and MB leprosy patients ( $n = 133$ ) and FSB samples (B) of NEC ( $n = 500$ ) and MB leprosy patients ( $n = 47$ ) were examined using the PGL-I QURapid. Left panels: R-values for anti-PGL-I IgM in NEC and MB leprosy patients; right panels: corresponding ROC curves. R-values (Y-axis) were calculated by dividing the peak area of the test line (T) by the peak area of the flow control line (FC). A Mann-Whitney U test was performed to determine the statistical significance between the two groups (\*\*\*\* $P \leq 0.0001$ ). AUC: Area Under the Curve; NEC: non-endemic controls; MB: multibacillary; ROC: receiver operating characteristic.

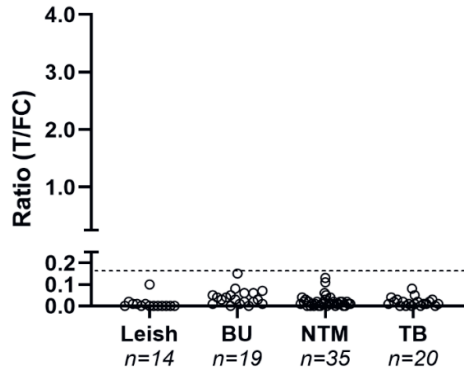


**Figure 3. Inter- and intra-operator differences for the PGL-I QURapid.**

Two anti-PGL-I IgM highly seropositive (High 1-2), one medium (Med) and two seronegative (Neg 1-2) serum samples were examined by the PGL-I QURapid by three different operators (A) on three different days (B). Each sample was tested in triplicate. Ratio (R-)values (Y-axis) were calculated by dividing the peak area of the test line (T) by the peak area of the flow control line (FC). Mean and SD are shown. Two example figures are shown here. See Supplementary information for complete data. A: black dots: Operator A (Op A); open squares: Operator B (Op B); grey triangles: Operator C (Op C). B: Black dots: Test day 1; grey squares: Test day 2; open triangles: Test day 3.

### Specificity for *M. leprae* infection in the context of other (mycobacterial) infections

To evaluate assay specificity, serum samples of leishmaniasis (Leish;  $n = 14$ ), BU ( $n = 19$ ), NTM infection ( $n = 35$ ), and TB ( $n = 20$ ) patients from a European area non-endemic for leprosy were tested. Applying a cut-off value of  $R \geq 0.16$ , all leishmaniasis, BU, NTM and TB patients were seronegative for anti-PGL-I IgM (Figure 4).

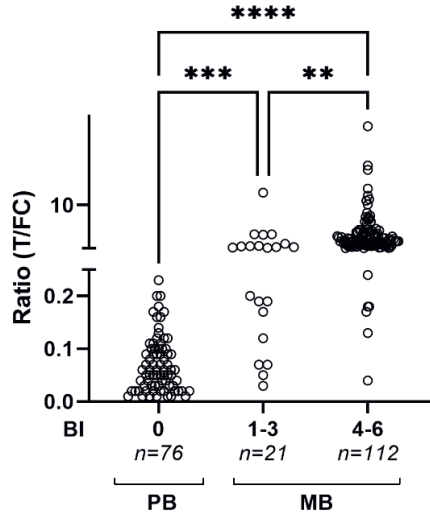


**Figure 4. Anti-PGL-I IgM in individuals with leishmaniasis, BU, NTM infections and TB.**

Serum samples of leishmaniasis (Leish;  $n = 14$ ), buruli ulcer (BU;  $n = 19$ ), non-tuberculous mycobacterial infection (NTM;  $n = 35$ ), and tuberculosis (TB;  $n = 20$ ) patients were examined using the PGL-I QURapid. Ratio (R-)values (Y-axis) were calculated by dividing the peak area of the test line (T) by the peak area of the flow control line (FC). The dotted line represents the cut-off for seropositivity ( $R \geq 0.16$ ) in serum.

### Bacterial load

PGL-I QURapid sensitivity was then assessed for 209 sera collected from leprosy patients with varying bacterial loads in Bangladesh. In total, 134 out of 209 individuals (64.1%) tested seropositive for anti-PGL-I IgM (cut-off  $R \geq 0.16$ ); 8 out of 76 PB patients (10.5%) and 126 out of 133 MB patients (94.7%) (Figure 5). Patients with a BI of 1-3 and BI 4-6 had significantly increased anti-PGL-I IgM R-values compared to those with BI 0, with only 7 testing seronegative (Kruskal-Wallis test;  $P \leq 0.001$  and  $P \leq 0.0001$ , respectively). Anti-PGL-I IgM R-values were also significantly higher in MB patients with BI 4-6 compared to those with a BI of 1-3 (Kruskal-Wallis test;  $P \leq 0.01$ ).

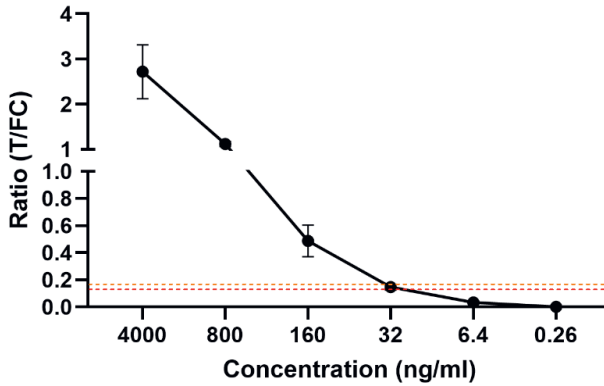


**Figure 5. Anti-PGL-I IgM in PB and MB leprosy patients.**

Sera from PB ( $n = 76$ ) and MB ( $n = 133$ ) leprosy patients were examined by PGL-I QURapid. Anti-PGL-I IgM was measured by the PGL-I QURapid for PB and MB patients. Leprosy patients were stratified for bacterial index (BI 0:  $n = 76$ ; BI 13:  $n = 21$ ; BI 4-6:  $n = 112$ ). Ratio (R-)values (Y-axis) were calculated by dividing the peak area of the test line (T) by the peak area of the flow control line (FC). A Kruskal-Wallis test with Dunn's correction for multiple testing was performed to determine the statistical significance between three groups ( $P$ -values:  $**P \leq 0.01$ ,  $***P \leq 0.001$ ,  $****P \leq 0.0001$ ).

### Anti-PGL-I IgM standard

In view of quality assessment for the PGL-I QURapid (e.g. batch-to-batch comparison or monitor different study sites using the same batch), humanized, recombinant anti-*M. leprae* PGL-I-specific IgM antibodies were custom-made. A standard dilution series of this recombinant anti-PGL-I IgM in buffer was applied to QUR-tests showing good performance (Figure 6). The above determined cut-offs for seropositivity in serum ( $R \geq 0.16$ ) and FSB ( $R \geq 0.12$ ) corresponded to an antibody concentration of approximately 32 ng/ml.



**Figure 6. Recombinant anti-PGL-I IgM dilution series in buffer using PGL-I QURapid.**

A dilution series of the custom-made anti-PGL-I IgM was applied to the PGL-I QURapid. Each dilution was tested in triplicate. Ratio (R)-values (Y-axis) were calculated by dividing the peak area of the test line (T) by the peak area of the flow control line (FC). Mean values and standard deviations are shown. The orange and red dotted lines represent the cut-off for seropositivity ( $R \geq 0.16$ ) in serum and FSB ( $R \geq 0.12$ ), respectively.

## Discussion

Here, we present the PGL-I QURapid, which is an operational, quantitative rapid test for measurement of anti-PGL-I IgM levels in blood, permitting detection of past or present infection with *M. leprae*. This QUR-test shows excellent performance: its outcome is highly associated with bacillary load providing a 94.7% Sn for MB leprosy, while samples from patients with leishmaniasis or other mycobacterial diseases were all seronegative, indicating excellent specificity. Moreover, vaccination of healthy Dutch health care workers with live *M. bovis* BCG, did not affect the lack of anti-PGL-I seropositivity in this group.

### MB versus PB

These findings are in line with previous studies (11, 13, 14), and support the use of the PGL-I QURapid next to clinical assessment as an (adjunct) diagnostic test for MB leprosy and monitoring the effect of treatment (49, 50). Inherent to PB leprosy is the very low number of bacteria in these patients that are only detectable by invasive biopsies. Thus, since the quantitative result of the PGL-I QURapid is associated with the number of bacteria, the here described low-invasive QUR-test could function as a discriminatory tool, applicable to distinguish MB from PB in settings where biopsies and their analysis are not easily performed. In this study, the QUR-test showed 10.5% Sn for PB patients. Therefore, for diagnosis of this patient group, detection of additional host biomarkers should be included. Of note is that this can also be accommodated on the UCP-LF platform, as was demonstrated for patients in Bangladesh, Brazil, China and Ethiopia (16, 17, 33). In this respect, the development of multi-biomarker tests (MBTs) (33) is promising.

### Field-friendly, low-invasive and rapid test

The PGL-I QURapid is very well-suited for field settings as it only requires 20 µl of blood obtained by a finger prick. Furthermore, test results are visible within minutes after addition of sample to the QUR-test, and accurate quantitative values are available from 10 minutes onwards for both serum and FSB. This test-to-result time is well within the required optimal time window prescribed by the WHO TPP (31). After use, the QUR-test can be stored indefinitely as a hard copy and rescanned when required. Inter- and intra-operator variability assessment demonstrated that results were highly reproducible thereby permitting utilization in field settings after limited training of local staff. In view of environmental considerations, the QUR-test was also tested successfully using plant-based biodegradable cassettes as a green solution for LFAs (Okos Diagnostics, the Netherlands, <https://www.okosdiagnostics.com>; data not shown).

### Test applications

A major benefit of a quantitative compared to a qualitative rapid test, is that one QUR-test can serve multiple use cases. Besides detection of infection on an individual level, another use case of the PGL-I QURapid is monitoring of recent *M. leprae* transmission in a population by serosurveys in healthy young children (10, 51). This concept was piloted successfully with this format of the PGL-I QURapid in a serosurvey in a leprosy endemic area in India and was well accepted by health care staff as well as the targeted population (19). In this respect, it is of note that assessment of sera of young children ( $n = 70$ ) from a non-endemic area (the Netherlands) all resulted in R-values of zero ((19) and unpublished data), underlining the specificity of this QUR-test. Thus, based on the rationale that infection in young children reflects recent transmission in a population, this QUR-test can be applied to compare pediatric seroprevalence between different areas, as well as seroprevalence in a community before and after introduction of postexposure prophylactic interventions.

A skin disease that is endemic in areas where leprosy occurs, is leishmaniasis caused by *L. donovani* (52). All leishmaniasis patients tested here were anti-PGL-I seronegative, emphasizing the high specificity of the PGL-I QURapid for *M. leprae* infection. Since leishmaniasis is included in the differential diagnosis of leprosy (30), developing a combined rapid test for the detection of infection with *M. leprae* and *L. donovani* could be both cost- and time-saving (52).

### Detecting infection in animals

Besides in humans, this QUR-test can also be used to detect *M. leprae* infection in different species as well as infection with *M. lepromatosis*. Previous research from our group has shown that the PGL-I QURapid can be applied to nine-banded armadillos and Eurasian red squirrels (18, 27, 53). Also, we have recently applied the QUR-test to earprick samples of ten experimentally infected live armadillos showing high correlation with infection (in collaboration with the National Hansen's Disease Program, USA; data not shown). Moreover, in agreement with what has been reported for ELISAs by Avanzi et al. (54), our

QUR-test was able to detect anti-PGL-I antibodies in samples from *M. lepromatosis* infected squirrels (18) as well as a human case with this infection (data not shown).

### **Standardization and quality control**

The humanized, recombinant anti-PGL-I IgM control introduced in this study as part of the QUR-test kit, provides an essential tool to indicate thresholds for specific applications (sample type and use case). It offers high flexibility for manufacturing as test thresholds can be evaluated with a control sample of known IgM concentration. Production lots can be provided with predetermined cut-off R-values and UCP-LF strip readers eventually could be provided with standard curves calculating actual concentrations rather than R-values. This will allow improved comparison of data obtained with the PGL-I QURapid at different field sites or at different points in time.

### **Limitation of the study**

A limitation of this study was the low numbers available of serum samples from patients with TB, BU, leishmaniasis and NTMs from areas that are non-endemic for leprosy and from patients infected with *M. lepromatosis*, as well as FSB samples from leprosy patients. Future studies testing higher sample numbers with this QUR-test should be performed to validate our findings.

### **Conclusions**

The PGL-I QURapid for detection of *M. leprae* infection is operational and applicable for widescale use in leprosy research. The reported findings support production and distribution of this QUR-test with predetermined thresholds for seropositivity, set according to use case and sample type. Test results are operator-independent and performance over time can be monitored using humanized, recombinant anti-PGL-I IgM. The test protocol is straightforward, requires zero infrastructure, allowing application in POC settings although a UCP compatible reader is required. As the test provides a quantitative result that can also be translated into a qualitative result, the PGL-I QURapid can be employed for various use cases in leprosy research and control programs.

### **Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Competing interests

The authors declare that they have no competing interests.

## Ethics approval and consent to participate

Ethical permission was received from national and local ethical boards: leprosy patient samples (Bangladesh Medical Research Council; Ref no. BMRC/NREC/2010-2013/1534 and MEC-2012-589, the Netherlands), leishmaniasis (MEC 2012-047 & MEC-2015-306 & B21.048, the Netherlands), TB (METC P07.048, the Netherlands), NTM (METC P207/99, the Netherlands) and BU samples (FWA00001824; Ghana). The BCG-CORONA trial was registered at clinicaltrials.gov (identifier: NCT04328441) and the Dutch Trial Registry (trialregister.nl, identifier Trial NL8477) and the study protocol was approved by the IRB of the LUMC (NL73249.041.20). The studies were conducted in accordance with local legislation and institutional requirements. All participants gave broad consent.

## References

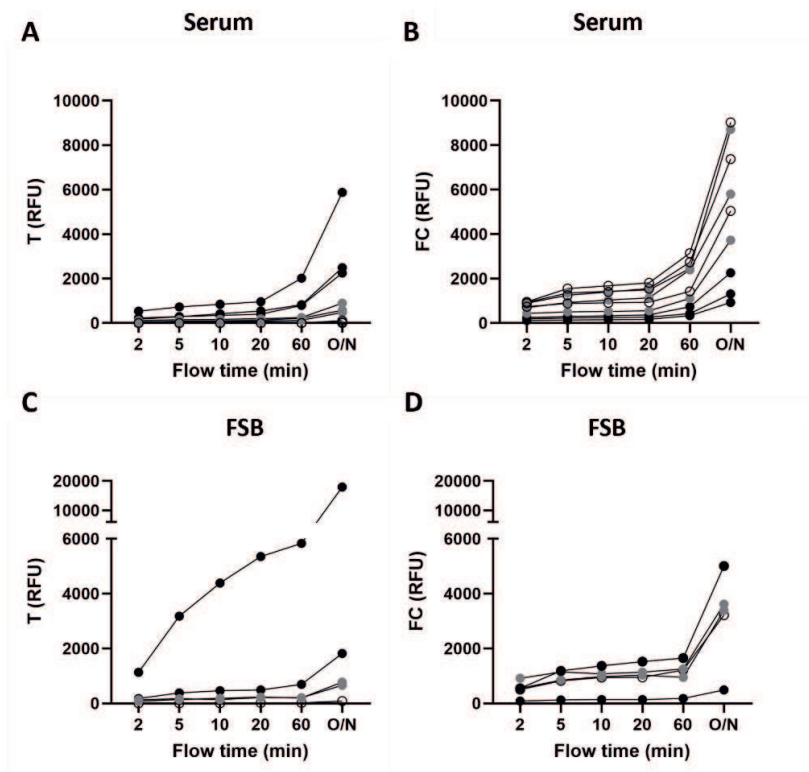
1. Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL. The continuing challenges of leprosy. *Clin Microbiol Rev.* 2006;19(2):338–81.
2. Dets P, Collin SM. *Mycobacterium lepromatosis* as a second agent of Hansen's disease. *Front Microbiol.* 2021;12: 698588.
3. Geluk A. Correlates of immune exacerbations in leprosy. *Semin Immunol.* 2018;39:111–8.
4. Blok DJ, De Vlas SJ, Richardus JH. Global elimination of leprosy by 2020: are we on track? *Parasit Vectors.* 2015;8:548.
5. Chen KH, Lin CY, Su SB, Chen KT. Leprosy: a review of epidemiology, clinical diagnosis, and management. *J Trop Med.* 2022;2022:8652062.
6. Towards Zero Leprosy. Global Leprosy (Hansen's disease) Strategy 2021–2030. New Delhi: World Health Organization, Regional Office for South-East Asia; 2017.
7. Smith WCS, Aerts A. Role of contact tracing and prevention strategies in the interruption of leprosy transmission. *Leprosy Rev.* 2014;85(1):2–17.
8. Sales AM, de Leon AP, Duppre NC, Hacker MA, Nery JAC, Sarno EN, et al. Leprosy among patient contacts: a multilevel study of risk factors. *Plos Neglect Trop D.* 2011;5(3): e1013.
9. Belachew WA, Naafs B. Position statement: LEPROSY: diagnosis, treatment and follow-up. *J Eur Acad Dermatol.* 2019;33(7):1205–13.
10. Pierneef L, van Hooij A, Taal A, Rumbaut R, Nobre ML, van Brakel W, et al. Detection of anti-*M. leprae* antibodies in children in leprosy-endemic areas: a systematic review. *PLoS Negl Trop Dis.* 2021;15(8): e0009667.
11. van Hooij A, Tjon Kon Fat EM, van den Eeden SJF, Wilson L, Batista da Silva M, Salgado CG, et al. Field-friendly serological tests for determination of *M. leprae*-specific antibodies. *Sci Rep.* 2017;7(1):8868.



12. TiemiNagao-Dias A, Casimiro de Macedo A, Rodrigues RO, Pedroza FHC, Albuquerque AA, Moreira FA, et al. Serum anti-PGL-1 IgG, IgM, and IgA in a 3-year follow-up study of 4–15-year-old leprosy contacts. *Pediatr Infect Dis J*. 2019;38(9):e193–8.
13. Tio-Coma M, Avanzi C, Verhard EM, Pierneef L, van Hooij A, Benjak A, et al. Genomic characterization of *Mycobacterium leprae* to explore transmission patterns identifies new subtype in Bangladesh. *Front Microbiol*. 2020;11:1220.
14. Zenha EM, Ferreira MA, Foss NT. Use of anti-PGL-1 antibodies to monitor therapy regimes in leprosy patients. *Braz J Med Biol Res*. 2009;42(10):968–72.
15. van Hooij A, Tjon Kon Fat EM, Richardus R, van den Eeden SJ, Wilson L, de Dood CJ, et al. Quantitative lateral flow strip assays as user-friendly tools to detect biomarker profiles for leprosy. *Sci Rep*. 2016;6:34260.
16. van Hooij A, Fat EMTK, da Silva MB, Bouth RC, Messias ACC, Gobbo AR, et al. Evaluation of immunodiagnostic tests for leprosy in Brazil, China and Ethiopia. *Sci Rep*. 2018;8: 17920.
17. van Hooij A, van den Eeden S, Richardus R, Fat ETK, Wilson L, Franken KLMC, et al. Application of new host biomarker profiles in quantitative point-of-care tests facilitates leprosy diagnosis in the field. *EBioMedicine*. 2019;47:301–8.
18. Zhou Z, van Hooij A, Wassenaar GN, Seed E, Verhard-Seymonsbergen EM, Corstjens P, et al. Molecular and serological surveillance for *Mycobacterium leprae* and *Mycobacterium lepromatosis* in Wild Red Squirrels (*Sciurus vulgaris*) from Scotland and Northern England. *Animals (Basel)*. 2024;14(13):2005.
19. Pierneef L, Malaviya P, van Hooij A, Sundar S, Singh AK, Kumar R, et al. Field-friendly anti-PGL-I serosurvey in children to monitor transmission in Bihar, India. *Front Med*. 2023;10:1260375.
20. Interruption of transmission and elimination of leprosy disease—technical guidance. New Delhi: World Health Organization, Regional Office for South-East Asia; 2023.
21. van Dijk JHM, van Hooij A, Groot LM, Geboers J, Moretti R, Verhard-Seymonsbergen E, et al. Synthetic phenolic glycolipids for application in diagnostic tests for leprosy. *ChemBioChem*. 2021;22(8):1487–93.
22. Duthie MS, Balagon MF, Maghanoy A, Orcullo FM, Cang M, Dias RF, et al. Rapid quantitative serological test for detection of infection with *Mycobacterium leprae*, the causative agent of leprosy. *J Clin Microbiol*. 2014;52(2):613–9.
23. Duthie MS, Orcullo FM, Abbelana J, Maghanoy A, Balagon MF. Comparative evaluation of antibody detection tests to facilitate the diagnosis of multibacillary leprosy. *Appl Microbiol Biotechnol*. 2016;100(7):3267–75.
24. Buhrer SS, Smits HL, Gussenhoven GC, van Ingen CW, Klatser PR. A simple dipstick assay for the detection of antibodies to phenolic glycolipid-I of *Mycobacterium leprae*. *Am J Trop Med Hyg*. 1998;58(2):133–6.
25. Buhrer-Sekula S, Smits HL, Gussenhoven GC, van Leeuwen J, Amador S, Fujiwara T, et al. Simple and fast lateral flow test for classification of leprosy patients and identification of contacts with high risk of developing leprosy. *J Clin Microbiol*. 2003;41(5):1991–5.
26. Corstjens P, van Hooij A, Tjon Kon Fat EM, Alam K, Vrolijk LB, Dlamini S, et al. Fingerstick test quantifying humoral and cellular biomarkers indicative for *M. leprae* infection. *Clin Biochem*. 2019;66:76–82.
27. Zhou Z, Pena M, van Hooij A, Pierneef L, de Jong D, Stevenson R, et al. Detection and monitoring of *Mycobacterium leprae* infection in nine banded armadillos (*Dasypus novemcinctus*) using a quantitative rapid test. *Front Microbiol*. 2021;12: 763289.
28. Niedbala RS, Feindt H, Kardos K, Vail T, Burton J, Bielska B, et al. Detection of analytes by immunoassay using up-converting phosphor technology. *Anal Biochem*. 2001;293(1):22–30.
29. Corstjens PL, Li S, Zuiderwijk M, Kardos K, Abrams WR, Niedbala RS, et al. Infrared up-converting phosphors for bioassays. *IEE Proc Nanobiotechnol*. 2005;152(2):64–72.
30. Trindade MA, Silva LL, Braz LM, Amato VS, Naafs B, Sotto MN. Post-kalaazar dermal leishmaniasis and leprosy: case report and literature review. *Bmc Infect Dis*. 2015;15:543.
31. World Health Organization. Target product profile for a diagnostic test to detect *Mycobacterium leprae* infection among asymptomatic household and familial contacts of leprosy patients. World Health Organization; 2023.
32. Richardus RA, Alam K, Pahan D, Feenstra SG, Geluk A, Richardus JH. The combined effect of chemoprophylaxis with single dose rifampicin and immunoprophylaxis with BCG to prevent leprosy in contacts of newly diagnosed leprosy cases: a cluster randomized controlled trial (MALTALEP study). *Bmc Infect Dis*. 2013;13:1–8.
33. van Hooij A, Fat EMTK, de Jong D, Khatun M, Soren S, Chowdhury A, et al. Prototype multi-biomarker test for point-of-care leprosy diagnostics. *Iscience*. 2021;24(1): 102006.
34. Ridley DS, Jopling WH. Classification of leprosy according to immunity. A five-group system. *Int J Lepr Other Mycobact Dis*. 1966;34(3):255–73.
35. Lin MY, Geluk A, Smith SG, Stewart AL, Friggen AH, Franken KL, et al. Lack of immune responses to *Mycobacterium tuberculosis* DosR regulon proteins following *Mycobacterium bovis* BCG vaccination. *Infect Immun*. 2007;75(7):3523–30.

36. Leyten EMS, Lin MY, Franken KLMC, Friggen AH, Prins C, van Meijgaarden KE, et al. Human T-cell responses to 25 novel antigens encoded by genes of the dormancy regulon of *Mycobacterium tuberculosis*. *Microbes Infect.* 2006;8(8):2052–60.
37. Wortmann G, Sweeney C, Hough HS, Aronson N, Stiteler J, Jackson J, et al. Rapid diagnosis of leishmaniasis by fluorogenic polymerase chain reaction. *Am J Trop Med Hyg.* 2001;65(5):583–7.
38. Kuilder JS, Wismans PJ, Baerveldt EM, van Hellemond JJ, Melo MD, van Genderen PJJ. Cutaneous leishmaniasis in 3 travelers returning from Israel to the Netherlands. *Emerg Infect Dis.* 2016;22(11):2022–4.
39. Bibert S, Bratschi MW, Aboagye SY, Collinet E, Scherr N, Yeboah-Manu D, et al. Susceptibility to *Mycobacterium ulcerans* disease (Buruli ulcer) is associated with *IFNG* and *iNOS* gene polymorphisms. *Front Microbiol.* 2017;8:1903.
40. World Health Organization. Treatment of *Mycobacterium ulcerans* disease (Buruli Ulcer): guidance for health workers. Geneva: World Health Organization; 2012.
41. Haverkamp MH, van Wengen A, de Visser AW, van Kralingen KW, van Dissel JT, van de Vosse E. Pulmonary: a canary in the cystic fibrosis coalmine. *J Infect.* 2012;64(6):609–12.
42. Kilic SS, van Wengen A, de Paus RA, Celebi S, Meziane B, Hafizoglu D, et al. Severe disseminated mycobacterial infection in a boy with a novel mutation leading to IFN- $\gamma$  R2 deficiency. *J Infect.* 2012;65(6):568–72.
43. Potjewijd J, de Paus RA, van Wengen A, Damoiseaux J, Verbon A, van de Vosse E. Disseminated infection in a patient with a novel partial interleukin-12/23 receptor  $\beta$ 1 deficiency. *Clin Immunol.* 2012;144(2):83–6.
44. Arend SM, de Palou EC, de Haas P, Janssen R, Hoeve MA, Verhard EM, et al. Pneumonia caused by in a series of patients without recognised immune defect. *Clin Microbiol Infect.* 2004;10(8):738–48.
45. Ten Doesschate T, van der Vaart TW, Debisarun PA, Taks E, Moorlag S, Paternotte N, et al. Bacillus Calmette-Guerin vaccine to reduce healthcare worker absenteeism in COVID-19 pandemic, a randomized controlled trial. *Clin Microbiol Infect.* 2022;28(9):1278–85.
46. Pierneef L, van Hooij A, de Jong D, Tjon Kon Fat EM, van Meijgaarden KE, Petruccioli E, et al. Host biomarker-based quantitative rapid tests for detection and treatment monitoring of tuberculosis and COVID-19. *iScience.* 2023;26(1): 105873.
47. Fluss R, Faraggi D, Reiser B. Estimation of the Youden index and its associated cutoff point. *Biometrical J.* 2005;47(4):458–72.
48. Mokkaipati VK, Sam Niedbala R, Kardos K, Perez RJ, Guo M, Tanke HJ, et al. Evaluation of UPLink-RSV: prototype rapid antigen test for detection of respiratory syncytial virus infection. *Ann N Y Acad Sci.* 2007;1098:476–85.
49. Corstjens P, van Hooij A, Tjon Kon EM, van den Eeden SJF, Wilson L, Geluk A. Field-friendly test for monitoring multiple immune response markers during onset and treatment of exacerbated immunity in leprosy. *Clin Vaccine Immunol.* 2016;23(6):515–9.
50. Khadge S, Banu S, Bobosha K, van der Ploeg-van Schip JJ, Goulart IM, Thapa P, et al. Longitudinal immune profiles in type 1 leprosy reactions in Bangladesh, Brazil, Ethiopia and Nepal. *Bmc Infect Dis.* 2015;15:477.
51. Zhou Z, Pierneef L, van Hooij A, Geluk A. Detection of anti-*M. leprae* antibodies in healthy children in China: a systematic review of Chinese literature. *Front Trop Dis.* 2022;3: 963674.
52. Cloots K, Uranw S, Ostyn B, Bhattarai NR, Le Rutte E, Khanal B, et al. Impact of the visceral leishmaniasis elimination initiative on *Leishmania donovani* transmission in Nepal: a 10-year repeat survey. *Lancet Glob Health.* 2020;8(2):e237–43.
53. Schilling AK, McCurdy K, Fish A, Lurz PWW, Geluk A, Van Hooij A, et al. Diagnosing and categorizing leprosy in live Eurasian red squirrels (*Sciurus vulgaris*) for management, surveillance, and translocation purposes. *J Zoo Wildl Med.* 2021;52(2):648–59.
54. Avanzi C, Del-Pozo J, Benjak A, Stevenson K, Simpson VR, Busso P, et al. Red squirrels in the British Isles are infected with leprosy bacilli. *Science.* 2016;354(6313):744–7.

Supplemental information



**Supplementary figure 1. Effect of flow time on T and FC signals obtained with the PGL-I QURapid.**

Anti-PGL-I IgM highly seropositive, medium and seronegative control sera (A-B) and FSB samples (C-D) were examined by the PGL-I QURapid. Samples were measured at 2, 5, 10, 20, 60 minutes and 24 hours (O/N) after sample addition. Black dots: anti-PGL-I IgM highly seropositive serum samples; grey dots: anti-PGL-I IgM medium serum samples; green dots: anti-PGL-I IgM seronegative serum samples.

A: T line values (Y-axis) in relative fluorescence units (RFU) as a function of serum sample flow time in minutes. B: FC line values (Y-axis) in RFU as a function of serum sample flow time in minutes. C: T line values (Y-axis) in RFU as a function of FSB sample flow time in minutes. D: FC line values (Y-axis) in RFU as a function of FSB sample flow time in minutes.

**Supplementary Table 1. Overview of tests developed for the measurement of anti-PGL-I antibodies.**

| Method                           | ref. no. | target             | Ab          | quantitative/<br>qualitative | rapid<br>(yes/no) |
|----------------------------------|----------|--------------------|-------------|------------------------------|-------------------|
| ELISA                            | 11, 21   | ND-O-HSA/NPT-H-BSA | IgM and IgG | quantitative                 | no                |
| OrangeLife NDO-LID RDT®          | 22       | NDO-LID            | IgM and IgG | qualitative                  | yes               |
| CTK OnSite Leprosy Ab Rapid Test | 11, 23   | PGL-I and LID-1    | IgM and IgG | qualitative                  | yes               |
| ML-dipstick                      | 24       | ND-O-BSA           | IgM         | qualitative                  | yes               |
| ML-flow                          | 25       | NT-P-BSA           | IgM         | qualitative                  | yes               |

Test method, reference in which the test is described, test target, and antibody measured are listed. In addition, it is indicated whether tests are quantitative or qualitative and rapid or not. Ab: antibody; ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G; IgM: immunoglobulin M; LID-1: leprosy IDRI diagnostic-1; ND-O-BSA: natural disaccharide-octyl bovine serum albumin; ND-O-HSA: natural disaccharide-octyl human serum albumin; NDO-LID: natural disaccharide-octyl leprosy IDRI diagnostic; NPT-H-BSA: phenolic trisaccharide functionalized with a hexanoic acid linker for conjugation to bovine serum albumin; NT-P-BSA: natural trisaccharide phenyl bovine serum albumin; PGL-I: phenolic glycolipid I; RDT: rapid diagnostic test.

**Supplementary Table 2. Cut-off determination according to Youden's index and WHO TPP; based on MB leprosy vs NEC serum samples.**

| Cut-off   | Sensitivity | 95% CI           | Specificity | 95% CI           | Youden's index |
|-----------|-------------|------------------|-------------|------------------|----------------|
| > 0.02500 | 1           | 0.9719 to 1.000  | 0.7         | 0.6349 to 0.7579 | 0.70           |
| > 0.03500 | 0.9925      | 0.9586 to 0.9996 | 0.7952      | 0.7356 to 0.8443 | 0.79           |
| > 0.04500 | 0.985       | 0.9468 to 0.9973 | 0.8524      | 0.7981 to 0.8940 | 0.84           |
| > 0.05500 | 0.9774      | 0.9358 to 0.9939 | 0.9         | 0.8520 to 0.9337 | 0.88           |
| > 0.06500 | 0.9774      | 0.9358 to 0.9939 | 0.9238      | 0.8798 to 0.9526 | 0.90           |
| > 0.07500 | 0.9624      | 0.9150 to 0.9838 | 0.9429      | 0.9028 to 0.9670 | 0.91           |
| > 0.08500 | 0.9624      | 0.9150 to 0.9838 | 0.9476      | 0.9087 to 0.9705 | 0.91           |
| > 0.09500 | 0.9624      | 0.9150 to 0.9838 | 0.9524      | 0.9146 to 0.9739 | 0.91           |
| > 0.1050  | 0.9624      | 0.9150 to 0.9838 | 0.9619      | 0.9266 to 0.9806 | 0.92           |
| > 0.1150  | 0.9624      | 0.9150 to 0.9838 | 0.9667      | 0.9328 to 0.9838 | 0.93           |
| > 0.1250  | 0.9549      | 0.9051 to 0.9792 | 0.9762      | 0.9455 to 0.9898 | 0.93           |
| > 0.1350  | 0.9474      | 0.8953 to 0.9743 | 0.9857      | 0.9588 to 0.9961 | 0.93           |
| > 0.1450  | 0.9474      | 0.8953 to 0.9743 | 0.9905      | 0.9659 to 0.9983 | 0.94           |
| > 0.1600  | 0.9474      | 0.8953 to 0.9743 | 1           | 0.9820 to 1.000  | 0.95           |
| > 0.1750  | 0.9323      | 0.8764 to 0.9640 | 1           | 0.9820 to 1.000  | 0.93           |
| > 0.1850  | 0.9173      | 0.8580 to 0.9532 | 1           | 0.9820 to 1.000  | 0.92           |
| > 0.1950  | 0.9023      | 0.8399 to 0.9420 | 1           | 0.9820 to 1.000  | 0.90           |
| > 0.2200  | 0.8947      | 0.8311 to 0.9363 | 1           | 0.9820 to 1.000  | 0.89           |
| > 0.2600  | 0.8872      | 0.8222 to 0.9305 | 1           | 0.9820 to 1.000  | 0.89           |
| > 0.2900  | 0.8797      | 0.8135 to 0.9246 | 1           | 0.9820 to 1.000  | 0.88           |
| > 0.3150  | 0.8722      | 0.8048 to 0.9186 | 1           | 0.9820 to 1.000  | 0.87           |
| > 0.3400  | 0.8647      | 0.7962 to 0.9126 | 1           | 0.9820 to 1.000  | 0.86           |
| > 0.3600  | 0.8571      | 0.7876 to 0.9066 | 1           | 0.9820 to 1.000  | 0.86           |
| > 0.3850  | 0.8496      | 0.7791 to 0.9005 | 1           | 0.9820 to 1.000  | 0.85           |
| > 0.4050  | 0.8421      | 0.7707 to 0.8943 | 1           | 0.9820 to 1.000  | 0.84           |
| > 0.4350  | 0.8346      | 0.7622 to 0.8882 | 1           | 0.9820 to 1.000  | 0.83           |
| > 0.4650  | 0.8271      | 0.7539 to 0.8819 | 1           | 0.9820 to 1.000  | 0.83           |
| > 0.4900  | 0.8195      | 0.7455 to 0.8756 | 1           | 0.9820 to 1.000  | 0.82           |
| > 0.5200  | 0.812       | 0.7372 to 0.8693 | 1           | 0.9820 to 1.000  | 0.81           |
| > 0.5550  | 0.8045      | 0.7290 to 0.8630 | 1           | 0.9820 to 1.000  | 0.80           |
| > 0.5900  | 0.797       | 0.7207 to 0.8566 | 1           | 0.9820 to 1.000  | 0.80           |

|          |        |                  |   |                 |      |
|----------|--------|------------------|---|-----------------|------|
| > 0.6150 | 0.7895 | 0.7126 to 0.8501 | 1 | 0.9820 to 1.000 | 0.79 |
| > 0.6350 | 0.7744 | 0.6963 to 0.8372 | 1 | 0.9820 to 1.000 | 0.77 |
| > 0.6700 | 0.7669 | 0.6882 to 0.8307 | 1 | 0.9820 to 1.000 | 0.77 |
| > 0.7050 | 0.7594 | 0.6801 to 0.8241 | 1 | 0.9820 to 1.000 | 0.76 |
| > 0.7200 | 0.7519 | 0.6721 to 0.8175 | 1 | 0.9820 to 1.000 | 0.75 |
| > 0.7350 | 0.7444 | 0.6641 to 0.8109 | 1 | 0.9820 to 1.000 | 0.74 |
| > 0.7750 | 0.7293 | 0.6482 to 0.7976 | 1 | 0.9820 to 1.000 | 0.73 |
| > 0.8200 | 0.7218 | 0.6402 to 0.7909 | 1 | 0.9820 to 1.000 | 0.72 |
| > 0.8350 | 0.7143 | 0.6323 to 0.7842 | 1 | 0.9820 to 1.000 | 0.71 |
| > 0.8500 | 0.7068 | 0.6245 to 0.7775 | 1 | 0.9820 to 1.000 | 0.71 |

Overview of the results from calculating a cut-off for anti-PGL-I IgM seropositivity in sera from healthy controls vs. MB leprosy patients, by using the Youden's index and WHO TPP, including corresponding sensitivity and specificity (in italic red font). Note: Sn/Sp values are depicted as fractions. Only Sn/Sp values between 0.7 and 1.0 are shown.

**Supplementary Table 3. Cut-off determination according to Youden's index and WHO TPP; based on MB leprosy vs NEC FSB samples.**

| Cut-off   | Sensitivity | 95% CI           | Specificity | 95% CI           | Youden's index |
|-----------|-------------|------------------|-------------|------------------|----------------|
| > 0.02500 | 0.9787      | 0.8889 to 0.9989 | 0.784       | 0.7458 to 0.8178 | 0.7627         |
| > 0.03500 | 0.9574      | 0.8575 to 0.9924 | 0.872       | 0.8399 to 0.8985 | 0.8294         |
| > 0.04500 | 0.9574      | 0.8575 to 0.9924 | 0.932       | 0.9065 to 0.9509 | 0.8894         |
| > 0.05500 | 0.9574      | 0.8575 to 0.9924 | 0.962       | 0.9414 to 0.9755 | 0.9194         |
| > 0.06500 | 0.9362      | 0.8284 to 0.9781 | 0.966       | 0.9462 to 0.9787 | 0.9022         |
| > 0.07500 | 0.9149      | 0.8007 to 0.9664 | 0.976       | 0.9585 to 0.9862 | 0.8909         |
| > 0.08500 | 0.9149      | 0.8007 to 0.9664 | 0.986       | 0.9714 to 0.9932 | 0.9009         |
| > 0.09500 | 0.9149      | 0.8007 to 0.9664 | 0.992       | 0.9796 to 0.9969 | 0.9069         |
| > 0.1050  | 0.9149      | 0.8007 to 0.9664 | 0.994       | 0.9825 to 0.9984 | 0.9089         |
| > 0.1200  | 0.9149      | 0.8007 to 0.9664 | 0.998       | 0.9888 to 0.9999 | 0.9129         |
| > 0.1350  | 0.8723      | 0.7483 to 0.9402 | 0.998       | 0.9888 to 0.9999 | 0.8703         |
| > 0.1450  | 0.8511      | 0.7231 to 0.9259 | 0.998       | 0.9888 to 0.9999 | 0.8491         |
| > 0.1650  | 0.8298      | 0.6986 to 0.9111 | 0.998       | 0.9888 to 0.9999 | 0.8278         |
| > 0.2000  | 0.8085      | 0.6746 to 0.8958 | 0.998       | 0.9888 to 0.9999 | 0.8065         |
| > 0.2350  | 0.7872      | 0.6510 to 0.8801 | 0.998       | 0.9888 to 0.9999 | 0.7852         |
| > 0.2750  | 0.766       | 0.6278 to 0.8640 | 0.998       | 0.9888 to 0.9999 | 0.764          |
| > 0.3300  | 0.7234      | 0.5824 to 0.8306 | 0.998       | 0.9888 to 0.9999 | 0.7214         |
| > 0.3900  | 0.7234      | 0.5824 to 0.8306 | 1           | 0.9924 to 1.000  | 0.7234         |
| > 0.4300  | 0.7021      | 0.5602 to 0.8135 | 1           | 0.9924 to 1.000  | 0.7021         |

Overview of the results from calculating a cut-off for anti-PGL-I IgM seropositivity in FSB samples from healthy controls vs. MB leprosy patients, by using the Youden's index (in italic red font) and the WHO TPP (in bold), including corresponding sensitivity and specificity. Note: Sn/Sp values are depicted as fractions. Only Sn/Sp values between 0.7 and 1.0 are shown.

**Supplementary Table 4. PGL-I QURapid inter- and intra-operator variability.**

| Control sample       | High 1 |      |      | High 2 |      |      | Med  |      |      | Neg 1 |      |      | Neg 2 |      |      |
|----------------------|--------|------|------|--------|------|------|------|------|------|-------|------|------|-------|------|------|
|                      | Ratio  |      |      |        |      |      |      |      |      |       |      |      |       |      |      |
| Operator             | A      | B    | C    | A      | B    | C    | A    | B    | C    | A     | B    | C    | A     | B    | C    |
| Day 1                | 1.76   | 1.97 | 1.82 | 2.34   | 3.76 | 2.53 | 0.14 | 0.13 | 0.12 | 0.01  | 0.01 | 0.01 | 0.00  | 0.00 | 0.00 |
|                      | 2.30   | 1.68 | 2.39 | 3.35   | 2.98 | 2.02 | 0.11 | 0.14 | 0.10 | 0.01  | 0.01 | 0.00 | 0.01  | 0.01 | 0.00 |
|                      | 2.55   | 1.83 | 2.02 | 2.56   | 2.69 | 2.64 | 0.12 | 0.10 | 0.09 | 0.01  | 0.00 | 0.01 | 0.00  | 0.01 | 0.00 |
| Mean of triplicate   | 2.20   | 1.83 | 2.08 | 2.75   | 3.14 | 2.40 | 0.12 | 0.12 | 0.10 | 0.01  | 0.01 | 0.01 | 0.00  | 0.01 | 0.00 |
| SD of triplicate     | 0.40   | 0.15 | 0.29 | 0.53   | 0.55 | 0.33 | 0.02 | 0.02 | 0.02 | 0.00  | 0.01 | 0.01 | 0.01  | 0.01 | 0.00 |
| SD between operators | 0.19   |      |      | 0.37   |      |      | 0.01 |      |      | 0.00  |      |      | 0.00  |      |      |
| Day 2                | 1.81   | 1.94 | 2.34 | 3.89   | 2.75 | 2.38 | 0.14 | 0.14 | 0.19 | 0.00  | 0.00 | 0.01 | 0.00  | 0.01 | 0.00 |
|                      | 2.08   | 2.16 | 1.82 | 2.85   | 2.51 | 3.19 | 0.12 | 0.13 | 0.15 | 0.01  | 0.00 | 0.01 | 0.00  | 0.01 | 0.00 |
|                      | 2.50   | 1.86 | 1.96 | 2.09   | 3.33 | 2.37 | 0.12 | 0.14 | 0.13 | 0.01  | 0.01 | 0.01 | 0.00  | 0.01 | 0.01 |
| Mean of triplicate   | 2.13   | 1.99 | 2.04 | 2.94   | 2.86 | 2.65 | 0.13 | 0.14 | 0.16 | 0.01  | 0.00 | 0.01 | 0.00  | 0.01 | 0.00 |
| SD of triplicate     | 0.35   | 0.16 | 0.27 | 0.90   | 0.42 | 0.47 | 0.01 | 0.01 | 0.03 | 0.01  | 0.01 | 0.00 | 0.00  | 0.00 | 0.01 |
| SD between operators | 0.07   |      |      | 0.15   |      |      | 0.02 |      |      | 0.00  |      |      | 0.01  |      |      |
| Day 3                | 2.09   | 2.00 | 2.04 | 2.22   | 3.48 | 2.60 | 0.17 | 0.11 | 0.10 | 0.00  | 0.01 | 0.01 | 0.02  | 0.01 | 0.00 |
|                      | 2.26   | 2.32 | 2.10 | 2.53   | 4.24 | 2.42 | 0.12 | 0.14 | 0.13 | 0.02  | 0.00 | 0.01 | 0.00  | 0.00 | 0.00 |
|                      | 2.15   | 2.26 | 1.53 | 2.70   | 2.50 | 1.90 | 0.15 | 0.17 | 0.10 | 0.00  | 0.01 | 0.00 | 0.00  | 0.00 | 0.01 |
| Mean of triplicate   | 2.17   | 2.19 | 1.89 | 2.48   | 3.41 | 2.31 | 0.15 | 0.14 | 0.11 | 0.01  | 0.01 | 0.01 | 0.01  | 0.00 | 0.00 |
| SD of triplicate     | 0.09   | 0.17 | 0.31 | 0.24   | 0.87 | 0.36 | 0.03 | 0.03 | 0.02 | 0.01  | 0.01 | 0.01 | 0.01  | 0.01 | 0.01 |
| SD between operators | 0.17   |      |      | 0.59   |      |      | 0.02 |      |      | 0.00  |      |      | 0.00  |      |      |
| Mean of all days     | 2.17   | 2.00 | 2.00 | 2.73   | 3.14 | 2.45 | 0.13 | 0.13 | 0.12 | 0.01  | 0.01 | 0.01 | 0.00  | 0.01 | 0.00 |
| SD of all days       | 0.04   | 0.18 | 0.10 | 0.23   | 0.27 | 0.18 | 0.01 | 0.01 | 0.03 | 0.00  | 0.00 | 0.00 | 0.00  | 0.00 | 0.00 |

Overview of the results from testing the PGL-I QURapid robustness and reproducibility using anti-PGL-I IgM highly seropositive (High 1-2), medium (Med) and seronegative (Neg 1-2) control serum samples by three operators on three different days. Results are shown per operator (A-C), day (1-3) and control sample. Mean and standard deviation (SD) are shown.





# 4

## **Field-friendly anti-PGL-I serosurvey in children to monitor *Mycobacterium leprae* transmission in Bihar, India**

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## Abstract

It has been amply described that levels of IgM antibodies against *Mycobacterium leprae* (*M. leprae*) phenolic glycolipid I (PGL-I) correlate strongly with the bacterial load in an infected individual. These findings have generated the concept of using seropositivity for antibodies against *M. leprae* PGL-I as an indicator of the proportion of the population that has been infected. Although anti-PGL-I IgM levels provide information on whether an individual has ever been infected, their presence cannot discriminate between recent and past infections. Since infection in (young) children by definition indicates recent transmission, we piloted the feasibility of assessment of anti-PGL-I IgM seroprevalence among children in a leprosy endemic area in India as a proxy for recent *M. leprae* transmission. A serosurvey for anti-PGL-I IgM antibodies among children in highly leprosy endemic villages in Bihar, India, was performed, applying the quantitative anti-PGL-I UCP-LFA cassette combined with low-invasive, small-volume fingerstick blood (FSB). Local staff obtained FSB of 1,857 children (age 3–11 years) living in 12 leprosy endemic villages in Bihar; of these, 215 children (11.58%) were seropositive for anti-PGL-I IgM. The anti-PGL-I seroprevalence level of 11.58% among children corresponds with the seroprevalence levels described in studies in other leprosy endemic areas over the past decades where no prophylactic interventions have taken place. The anti-PGL-I UCP-LFA was found to be a low-complexity tool that could be practically combined with serosurveys and was well-accepted by both healthcare staff and the population. On route to leprosy elimination, quantitative anti-PGL-I serology in young children holds promise as a strategy to monitor recent *M. leprae* transmission in an area.

## Introduction

Leprosy, caused by *Mycobacterium leprae* (*M. leprae*) or *M. lepromatosis*, is a debilitating neglected tropical disease (NTD) still predominantly forming a health threat for poor and marginalized populations from over 120 countries (1–3). It is a chronic infectious disease that can cause long-term nerve damage and often results in both physical and social disabilities (4, 5). *M. leprae* is believed to be transmitted via aerosol droplets from the respiratory system, during repeated and close contact with untreated patients (6). Only approximately 5% of persons infected with *M. leprae* develop disease symptoms (4). However, it is assumed that infected, asymptomatic individuals carrying sufficient amounts of the mycobacterium contribute to its transmission (7,8).

Multidrug therapy (MDT) effectively kills *M. leprae* and provides an effective cure if treatment is initiated timely. Following MDT's introduction in 1981, leprosy prevalence has significantly dropped. Yet, transmission of *M. leprae* remains, reflected by over 9,000 new child cases detected in 2022 worldwide (9). It is also believed that large numbers go undetected as a result of the drop in leprosy-focused healthcare following the declaration of leprosy elimination on the global level (10).

The WHO's Global Leprosy Strategy 2021–2030 aims to significantly reduce the number of new cases with grade 2 disability and new child cases by focusing on early detection of disease and interruption of transmission. To achieve the latter, it is vital to identify and treat sources of infection, e.g., multibacillary (MB) cases with high bacillary loads that are highly likely to transmit bacteria (11, 12). Detecting *M. leprae* infected individuals lacking clinical symptoms who can transmit the bacterium or develop leprosy themselves remains a major challenge for dedicated control programs. Monitoring leprosy elimination is currently conducted by evaluating the proportion of new child cases (below age 15) among the total number of new cases detected (9, 13, 14). As only a small percentage of *M. leprae*-infected individuals progress to disease and it can take many years before symptoms of leprosy manifest (15), using new cases to monitor elimination does not provide sufficiently accurate and up-to-date information with respect to (elimination of) transmission.

Household contacts of MB leprosy cases have been reported to be most vulnerable to contracting disease (16, 17). Levels of IgM antibodies against phenolic glycolipid I (PGL-I), a specific cell wall component of *M. leprae*, correlate strongly with the bacterial index (BI) of *M. leprae*-infected individuals (8, 18, 19). Moreover, based on a literature review covering reports on serology for leprosy from 1987 to 2020 worldwide, we showed that quantitative anti-PGL-I serology in young children as a measure for *M. leprae* infection provides the potential for assessing recent transmission rates in a community (20, 21). These findings, on top of the observation that MB cases are more likely to transmit bacteria, provide a rationale for identifying seropositive individuals to study transmission. Although the presence of antibodies cannot discriminate between recent and past infection, infection in young children by definition indicates recent transmission. Therefore, the assessment of seropositivity in children is recommended by the WHO "Task Force on definitions, criteria and indicators for interruption of transmission and elimination of leprosy" as an indicator to

monitor elimination (9) and offers a tool to study the effects of interventions including post-exposure prophylaxis (PEP).

Over the past years, we have developed a robust, user-friendly test that detects anti-PGL-I IgM antibodies using the unique upconverting reporter particle (UCP) technology in a low-cost lateral flow-based assay (LFA) format [(18, 22–26) and Pierneef et al. (manuscript in preparation)]. The anti-PGL-I UCP-LFA offers a sensitive, low-complexity rapid test to quantitatively determine anti-PGL-I IgM levels in capillary and venous blood (24, 27).

In India, the new leprosy child case detection rate was approximately 10 per 1,000,000 child population in 2020 (28). Of the new cases of all ages registered in India, 15 to 20% (16,000–20,000 individuals each year) are located in Bihar (29), a socioeconomically poor state in the eastern part of the country with an estimated population of over 100 million people (30). In this study, we report for the first time the application of the fully integrated anti-PGL-I UCP-LFA cassette in a larger serosurvey using fingerstick blood (FSB). We aimed to assess the use of the anti-PGL-I UCP-LFA as a tool for measuring *M. leprae* infection among children as a proxy for transmission in Bihar, India.

## Methods

### Study participants

Study participants were consenting individuals living in a Health and Demographic Surveillance System (HDSS) site in Muzaffarpur district, Bihar, India.

### Children cohort

As part of the Tropical Medicine Research Center (TMRC) study on leishmaniasis in the already-existing Muzaffarpur HDSS, a door-to-door screening was performed by the staff of the Banaras Hindu University (BHU; Varanasi, India) (31, 32). The screening started in August 2020 and was completed in January 2021. FSB from children ( $n = 1,857$ ; 987 male/870 female) between 3 and 11 years of age living in Bihar was collected (Table 1). The inclusion criteria were all children without any signs of leprosy or other diseases residing permanently in one of the following villages in the old HDSS area (32): Singar Phulkahan, Madhopur

**Table 1. Age and gender of the 1,857 children per village of residence.**

| Village | Village name       | # Children | Age mean (range) | Gender (% female) |
|---------|--------------------|------------|------------------|-------------------|
| A       | Singar Phulkahan   | 114        | 7 (3–9)          | 43.86             |
| B       | Madhopur Chhapra   | 126        | 7 (5–9)          | 45.24             |
| C       | Godai Phulkahan    | 216        | 7 (5–9)          | 50.46             |
| D       | Godai Jamal        | 110        | 7 (5–9)          | 45.45             |
| E       | Vishwanathpur      | 89         | 7 (5–9)          | 49.44             |
| F       | Raksha North       | 164        | 7 (5–11)         | 49.39             |
| G       | Raksha North Chauk | 204        | 7 (5–9)          | 48.04             |
| H       | Raksha South West  | 97         | 7 (5–9)          | 50.52             |
| I       | Raksha South       | 411        | 7 (5–9)          | 47.69             |
| J       | Raksha Deah        | 250        | 7 (3–9)          | 40.80             |
| K       | Nariyar Nawada     | 70         | 7 (5–9)          | 48.57             |
| L       | Arizpur Kothi      | 6          | 7 (5–9)          | 0                 |
|         | Total              | 1,857      | 7 (3–9)          | 46.85             |

The number (#) of children, mean age (range), and percentage of females per village of residence are provided.

Chhapra, Godai Phulkahan, Godai Jamal, Vishwanathpur, Raksha North, Raksha North Chauk, Raksha South West, Raksha South, Raksha Deah, Nariyar Nawada, and Arizpur Kothi. Excluded were children below 2 years of age. BCG status was not recorded in this study but has been above 84% and almost uniform across India since the time of the study (2020) (33).

## Leishmaniasis

In addition, the following biobank samples collected during the TMRC study in Bihar on leishmaniasis were included.

Serum samples from leishmaniasis patients from the same area were collected, including visceral leishmaniasis (VL; age 12–52; n = 20) and post-kala-azar dermal leishmaniasis (PKDL; age 12–62; n = 20). VL patients were rK39 antibody/splenic aspirate positive and displayed clinical symptoms of active VL. PKDL patients were rK39 antibody or skin slit smear/PCR positive and had a history of VL (34).

### **Asymptomatic individuals**

Serum samples from asymptomatic individuals (ASY; age 10–70) were collected. ASY were individuals without any clinical symptoms of leishmaniasis, who were seropositive for rK39 antibodies in the Direct Agglutination Test (DAT; cutoff titer for positivity  $\geq 1:1,600$ ) (34).

### **Endemic controls**

Serum samples from endemic controls (ECs; age 8–50;  $n = 20$ ) were collected as part of the NIH-TMRC project. ECs were individuals who tested seronegative for rK39 antibodies/DAT (34).

### **Fingerstick blood collection**

FSB was collected using disposable 20  $\mu$ l Minivette collection tubes (Heparin coated; Sarstedt) and directly mixed with 980  $\mu$ l high salt finger stick (HSFS) buffer: 100mM Tris pH 8.0, 270mM NaCl, 1% (v/v) Triton X-100, and 1% (w/v) BSA. FSB was applied to the anti-PGL-I UCP-LFA cassette on the spot immediately after collection.

### **Serum collection**

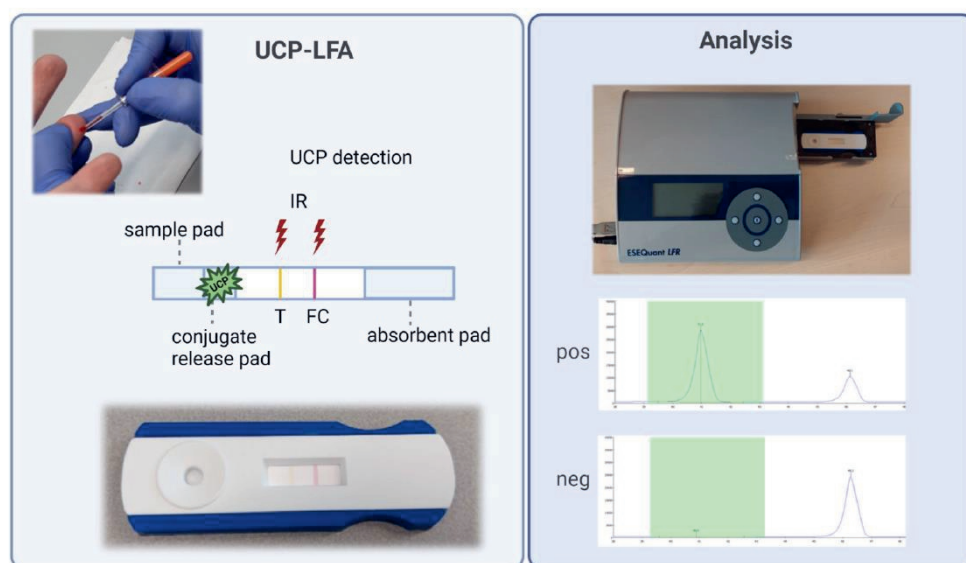
For samples that were used as positive and negative controls throughout the study, venous blood samples were collected, in 4 ml plain BD vacutainer serum tubes (BD, Franklin Lakes, NJ, USA). Tubes were centrifuged at an RCF of 500  $\times g$  for 10 min and sera were subsequently aliquoted and frozen ( $-80^{\circ}\text{C}$ ) until use.

### **Anti-PGL-I UCP-LFA cassette**

Fully integrated and individually packaged UCP-LFA cassettes for the detection of human anti-PGL-I IgM antibodies were produced by MaximBio (Rockville, MD, USA; schematic overview shown in Figure 1). The air-tight pouches with test cassettes contained silica dry packs allowing extended shelf life and protection against humidity. The Test (T) line on the LF strip comprised 100 ng of synthetic PGL-I, phenolic trisaccharide functionalized with a hexanoic acid linker for conjugation to BSA [NPT1-H-BSA; Leiden, the Netherlands (35)]. The Flow- Control (FC) line comprised 100 ng rabbit anti-goat IgG [G4018; Sigma-Aldrich, Inc., St. Louis, MO, USA]. Goat IgG specific for anti-human IgM (I0759; Sigma-Aldrich, Inc., St. Louis, MO, USA) was conjugated to polyacrylic acid functionalized UCPs [200 nm, NaYF<sub>4</sub>:Yb<sup>3+</sup>, Er<sup>3+</sup>; Intelligent Material Solutions Inc. (IMS); Princeton, NJ, USA MS] according to previously described protocols at a concentration of 50  $\mu$ g antibody per mg UCP (23). Stock solutions were kept at  $4^{\circ}\text{C}$  until use. To dry the UCPs onto the glass fiber conjugate-release pad, the material was diluted in a buffer containing 100mM Tris pH 8.0, 270mM NaCl, 10% (w/v) sucrose, 1% (w/v) BSA, 0.5% Tween-20, and striped at a density of 100 ng/mm. Components were mounted on plastic backing cards which were cut into LF strips of 4.8mm width by 6 cm length, added to an appropriate cassette, and individually sealed in a pouch together with a silica dry pack.

## UCP-LFA

To initiate LF, 50  $\mu$ l of the 50-fold diluted FSB or serum sample was added to the test cassette. Cassettes were analyzed using a UCP adapted portable reader (LFR; Qiagen, Hilden, Germany; Figure 1). The results were calculated as the ratio value (R) between T and FC signals based on relative fluorescence units (RFUs) measured at the respective lines. The cutoff for positivity ( $R \geq 0.12$ ) for the UCP-LFA batch used in this study was based on the median of a sextuple test of a standard control serum sample (+) performed in India plus the standard deviation (SD). Measurements in India yielded an identical cutoff level as in Leiden using aliquots of the same control serum sample (+). In addition, one highly anti-PGL-I IgM-positive serum sample (++) and one negative serum sample (–) were tested as control samples in sextuple to monitor the reproducibility of the test.



**Figure 1. Fully integrated anti-PGL-I UCP-LFA cassette and analysis.** Left: FSB is collected with a minivette (20  $\mu$ l). To initiate LF, a diluted FSB sample is added to the cassette onto the sample pad. Hydration of the anti-IgM UCP-conjugate will allow the binding of anti-PGL-I IgM antibodies from the sample to the conjugate and sequential binding to the T line with synthetic PGL-I. The FC line can bind UCP conjugate not bound to the T line. Color-coded T and FC lines visible by the eye disappear upon flow. Right: UCP signals are detected with a reader upon excitation with 980 IR light generating 540 nm emission. Results for positive and negative samples are shown. Results are calculated as R-value, with the T signal divided by the FC signal. This figure was created with BioRender.com. FC, flow control line; FSB, fingerstick blood; IgM, immunoglobulin M; IR, infrared excitation; PGL-I, phenolic glycolipid I; R, ratio value, result of the UCP-LFA; T, test line; UCP-LFA, upconverting reporter particle lateral flow assay.

## Ethics

This study was performed in accordance with the Helsinki Declaration (7th revision, 64th Meeting, 2013, Fortaleza). This study was a component of Muzaffarpur NIH-TMRC HDSS



for which ethics approval was received from the institutional review boards of the Institute of Medical Sciences, Banaras Hindu University (BHU; reference number Dean/2017/EC/185; Dated 24/10/2017), Varanasi, India, and the review committee of the U.S. National Institutes of Health (NIH). The institutional review board of BHU has received accreditation from the National Institutes of Health in the United States. Ethics approval was also obtained by the institutional review board of the Institute of Tropical Medicine, Antwerp (reference number 1305/19), and the Ethics Committee of the University Hospital of Antwerp (reference number 19/28/342), Belgium. All data were anonymized. Participants were informed about the study objectives, sampling protocol, and their right to refuse to take part or withdraw from the study without consequences for their treatment at any point in time. The refusal rate was lower than 5%. Written informed consent was obtained from a parent, guardian, or village head before enrollment. Consent forms were kept in a secure file cabinet at Kala-azar Medical Research Center (KAMRC), Muzaffarpur, Bihar, India.

### Statistical analysis

GraphPad Prism version 9.0.1 for Windows (GraphPad Software, San Diego, CA, USA) and RStudio version 4.2.1 (Boston, MA, USA) were used to perform statistical analysis. The distribution of anti-PGL-I data was checked for normality by plotting a histogram. Data were then log-transformed based on the natural logarithm of the anti-PGL-I R-value plus 1. Mann–Whitney U-tests and Kruskal–Wallis tests were performed to determine the statistical significance between two and three independent groups, respectively. A logistic regression analysis was performed to assess the association between age and anti-PGL-I positivity.

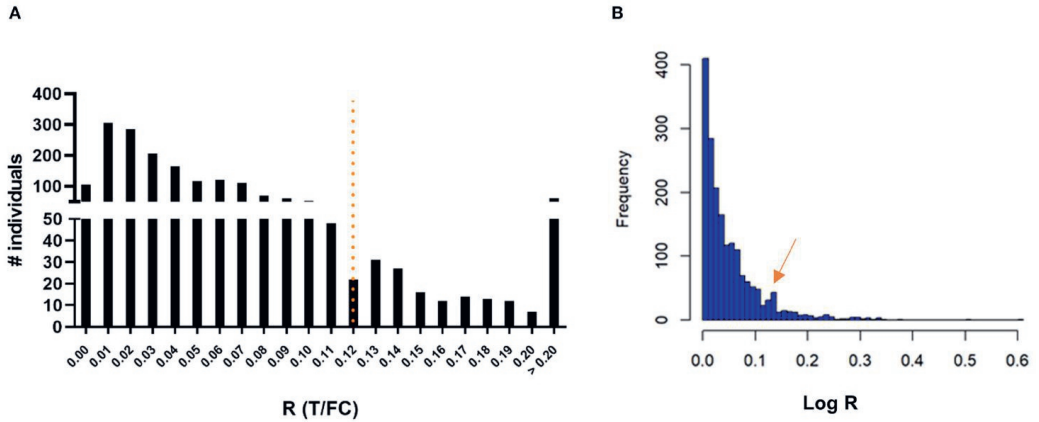
### Results

This was the first time applying the anti-PGL-I UCP-LFA cassette format at a larger scale in a field setting. A quality control protocol (as described in the Methods) was performed to monitor reproducibility (data not shown). The anti-PGL-I UCP-LFA was considered feasible and accepted by both healthcare staff and the population.

### Serosurvey for anti-PGL-I IgM among children in India

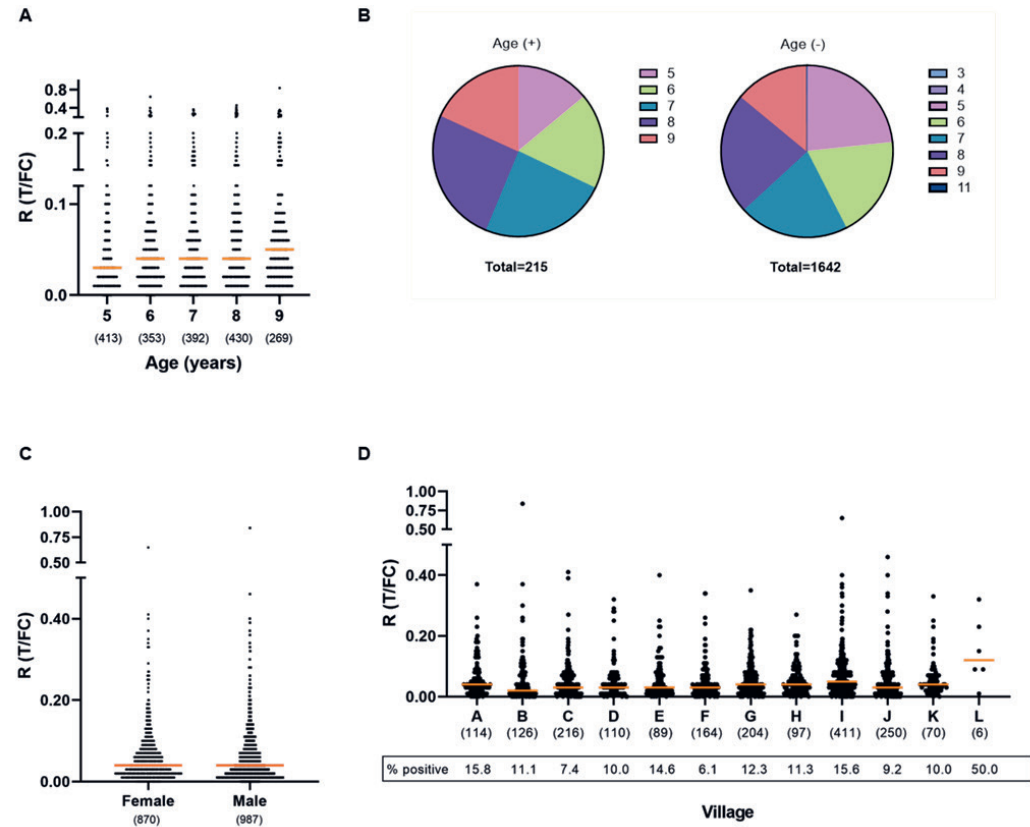
To assess seroprevalence for anti-PGL-I IgM in a leprosy endemic area, FSB samples of 1,857 children living in the state of Bihar, India, were obtained during a field serosurvey and screened using the anti-PGL-I UCP-LFA cassette. Among these children, 215 (11.58%) tested positive for anti-PGL-I IgM (Figure 2A; Supplementary Table 1). Anti-PGL-I R values ranged from 0 to 0.84 with a median of 0.04 (IQR 0.02–0.07). The distribution appeared unimodal with a mode of 0.01 and strongly skewed to the right. Upon log transformation (based on the natural logarithm of the anti-PGL-I R-value plus 1), the distribution was still very skewed with a small dip around 0.11, followed by a much lower second mode. The log-transformed value of 0.11 corresponds with 0.12 on the original scale, and this confirms the cutoff value of  $R \geq 0.12$  used, as shown by the second peak in the histogram (Figure 2B). From the seropositive children with R-values  $>0.2$  (median of seropositive children plus SD of seronegative children;  $n = 61$ ), 51 could be followed up in October 2021 and examined

for signs and symptoms of leprosy. One child (male, 9 years old) was diagnosed at follow-up at the primary healthcare center as a new paucibacillary (PB) leprosy case 1 year after the serosurvey (test performed in October 2020; R-value 0.21), showing a lesion on his left upper arm.



**Figure 2. Presence of anti-PGL-I IgM antibodies in FSB of 1,857 children 3–11 years old in Bihar, India.** UCP-LFA cassettes were used to obtain a quantitative R-value (T/FC) indicating the presence of anti-PGL-I IgM antibodies using a UCP reader. (A) Histogram with a mode of 0.01 for R: the highest R-value detected was 0.84 ( $n = 1$ ). The cutoff for positivity ( $R \geq 0.12$ ) for the UCP-LFA batch used in this study is indicated by the dotted line and was based on the median of a sextuple test performed in India of a standard control serum sample (+) plus its standard deviation (SD). (B) Histogram of log-transformed R-values. FC, flow control line; IgM, immunoglobulin M; PGL-I, phenolic glycolipid I; R, ratio value, result of the UCP-LFA; T, test line.

For all participants, information on age, gender, and place of origin was recorded at the time of sampling FSB. The ages of the children in this cohort ranged from 3 to 11, with the majority of the children being 5 ( $n = 410$ ), 6 ( $n = 353$ ), 7 ( $n = 392$ ), 8 ( $n = 430$ ), or 9 ( $n = 268$ ) years of age. For children aged 5–9 years, the percentage of seropositive children showed an increasing trend with age: from 7.32% at age 5 to 14.55% in age group 9 (Figures 3A, B; Supplementary Table 2). The odds of testing positive for anti-PGL-I IgM in this age range (5–9) increased by 18% per year (logistic regression;  $p = 0.002$ ).



**Figure 3. Presence of anti-PGL-I IgM antibodies in FSB of children in relation to age, gender, and place of residence.** UCP-LFA cassettes were used to obtain a quantitative ratio ( $R$ ) value ( $T/FC$ ) indicating the presence of anti-PGL-I IgM using a UCP reader. Median values for each group are indicated by horizontal bars. The cutoff for positivity ( $R \geq 0.12$ ) for the UCP-LFA batch used in this study was based on the median of a sextuple test performed in India of a standard control serum sample (+) plus its standard deviation (SD). The numbers of individuals per group are given in parentheses. (A) Anti-PGL-I IgM  $R$  values per age group. Two children aged 3 years and one child aged 4 years were included in the 5-year-old age group and one child aged 11 years was included in the 9-year-old age group. (B) Venn diagrams displaying the proportion of children per age category for anti-PGL-I IgM-positive (+) and -negative (-) children. Colors indicate the age groups. (C) Anti-PGL-I IgM  $R$  values per gender. (D) Anti-PGL-I IgM  $R$  values per village. A: Singar Phulkahan, B: Madhopur Chhapra, C: Godai Phulkahan, D: Godai Jamal, E: Vishwanathpur, F: Raksha North, G: Raksha North Chauk, H: Raksha South West, I: Raksha South, J: Raksha Deah, K: Nariyar Nawada, L: Arizpur Kothi. FC, flow control line; IgM, immunoglobulin M; PGL-I, phenolic glycolipid I;  $R$ , ratio value, result of the UCP-LFA; T, test line.

Ages were represented equally among the 987 male and 870 female participants (Table 2). No correlation was found between gender and the presence of anti-PGL-I IgM antibodies (Mann–Whitney U-test;  $p = 0.2314$ ; Figure 3C). For female and male participants, almost identical seropositivity percentages of 11.61 and 11.55% were found, respectively.

**Table 2. Age distribution among female and male children aged 3–11 in Bihar, India.**

| Gender | Age | # Children | Cumulative # children | Percentage (%) | Cumulative percentage (%) |
|--------|-----|------------|-----------------------|----------------|---------------------------|
| Female | 3   | 2          | 2                     | 0.23           | 0.23                      |
|        | 4   | 0          | 2                     | 0              | 0.23                      |
|        | 5   | 192        | 194                   | 22.07          | 22.30                     |
|        | 6   | 148        | 342                   | 17.01          | 39.31                     |
|        | 7   | 206        | 548                   | 23.68          | 62.99                     |
|        | 8   | 203        | 751                   | 23.33          | 86.32                     |
|        | 9   | 119        | 870                   | 13.68          | 100                       |
|        | 11  | 0          | 870                   | 0              | 100                       |
| Male   | 3   | 0          | 0                     | 0              | 0                         |
|        | 4   | 1          | 1                     | 0.10           | 0.10                      |
|        | 5   | 218        | 219                   | 22.09          | 22.19                     |
|        | 6   | 205        | 424                   | 20.77          | 42.96                     |
|        | 7   | 186        | 610                   | 18.84          | 61.80                     |
|        | 8   | 227        | 837                   | 23.00          | 84.80                     |
|        | 9   | 149        | 986                   | 15.10          | 99.90                     |
|        | 11  | 1          | 987                   | 0.10           | 100                       |

The number (#) of children per age and gender are provided with the corresponding percentage of that group in relation to total male/female participants.

Children from 12 different villages were included (Supplementary Figure 1). The majority of the children were resident in Raksha South ( $n = 411$ ). In general, seropositivity rates among the villages were similar (Figure 3D; Supplementary Table 3). However, a slight increase in levels of antibodies (R-values) among Raksha South with villages Madhopur Chhapra, Godai Phulkahan, Godai Jamal, Raksha North, and Raksha Deah was observed (Kruskal–Wallis;  $p = 0.0003$  to  $p = 0.0154$ ).

### Screening sera of leishmaniasis patients and controls

In addition to being endemic for leprosy, the area of Bihar is also endemic for leishmaniasis (32, 36). As leishmaniasis is a differential diagnosis of leprosy (37), although not the main purpose of this study, biobanked serum samples from individuals from exactly the same area with VL ( $n = 20$ ), PKDL ( $n = 20$ ), asymptomatic *Leishmania donovani* (*L. donovani*) infection ( $n = 20$ ), and endemic controls ( $n = 20$ ) were tested with the anti-PGL-I UCP-LFA cassette as well. Two of the 60 individuals with confirmed *L. donovani* infection tested positive (3.33%) for anti-PGL-I IgM (Table 3): one ( $R = 0.12$ ) PKDL case and one person with an asymptomatic *L. donovani* infection ( $R = 0.26$ ). None of the endemic controls (age 8–50) from the same area tested positive for anti-PGL-I IgM (Table 4). Moreover, plasma samples of young children (age 1–6;  $n = 37$ ) from a non-endemic area (the Netherlands) all tested negative using the anti-PGL-I UCP-LFA cassette (Pierneef et al., unpublished data).

**Table 3. Ratio values for anti-PGL-I IgM measured in individuals infected with *L. donovani* in Bihar, India.**

| R    | # Individuals | Cumulative # individuals | Percentage (%) | Cumulative percentage (%) |
|------|---------------|--------------------------|----------------|---------------------------|
| 0.00 | 15            | 15                       | 25.00          | 25.00                     |
| 0.01 | 16            | 31                       | 26.67          | 51.67                     |
| 0.02 | 12            | 43                       | 20.00          | 71.67                     |
| 0.03 | 7             | 50                       | 11.67          | 83.33                     |
| 0.04 | 2             | 52                       | 3.33           | 86.67                     |
| 0.05 | 2             | 54                       | 3.33           | 90.00                     |
| 0.06 | 2             | 56                       | 3.33           | 93.33                     |
| 0.07 | 1             | 57                       | 1.67           | 95.00                     |
| 0.09 | 1             | 58                       | 1.67           | 96.67                     |
| 0.12 | 1             | 59                       | 1.67           | 98.33                     |
| 0.26 | 1             | 60                       | 1.67           | 100                       |

The number (#) of individuals per ratio (R) value is provided with the corresponding percentage of that group in relation to the whole cohort. The cutoff for positivity ( $R \geq 0.12$ ) for the UCP-LFA batch used in this study was based on the median of a sextuple test performed in India of a standard control serum sample (+) plus its standard deviation (SD).

**Table 4. Ratio values for anti-PGL-I IgM measured in endemic controls in Bihar, India.**

| R    | # Individuals | Cumulative # individuals | Percentage (%) | Cumulative percentage (%) |
|------|---------------|--------------------------|----------------|---------------------------|
| 0.00 | 2             | 2                        | 10.00          | 10.00                     |
| 0.01 | 3             | 5                        | 15.00          | 25.00                     |
| 0.02 | 8             | 13                       | 40.00          | 65.00                     |
| 0.03 | 2             | 15                       | 10.00          | 75.00                     |
| 0.04 | 1             | 16                       | 5.00           | 80.00                     |
| 0.05 | 2             | 18                       | 10.00          | 90.00                     |
| 0.09 | 1             | 19                       | 5.00           | 95.00                     |
| 0.10 | 1             | 20                       | 5.00           | 100                       |

The number (#) of individuals per ratio (R) value is provided with the corresponding percentage of that group in relation to the whole cohort. The cutoff for positivity ( $R \geq 0.12$ ) for the UCP-LFA batch used in this study was based on the median of a sextuple test performed in India of a standard control serum sample (+) plus its standard deviation (SD).

## Discussion

Measuring seroprevalence among young children represents a potential tool to monitor the intensity of recent transmission, particularly when antibody levels are assessed quantitatively. Changes in seroprevalence measured in repeated cross-sectional surveys among young children of a certain age group in an area can indicate the rate of transmission as well as the effect of control measures or interventions in an area. On route to leprosy

elimination, an indicator for the intensity of transmission would be highly valuable, as the proportion of child cases, which is currently applied by the WHO, does not provide information swiftly as it can take years for an infected individual to develop leprosy. Moreover, it is not an accurate representation of infection as only a minority of the infected individuals develop the disease (9).

In this study, using the anti-PGL-I UCP-LFA, we found seropositivity of 11.58% among children in Bihar, a leprosy endemic state in India, with a prevalence rate of 17.1 per 10,000 population in 2019 (31), which is above the elimination threshold of 1 per 10,000 population defined by the WHO (38). In agreement with previous research, this percentage falls in line with the seroprevalence (median 14.9%) found in studies from endemic areas – mostly Brazil, India, and Indonesia (20). Seropositivity slightly increased with age (between 5 and 9 years of age). Previous reports describe a similar increase with age, followed by a decrease in antibodies after the age of 20 years (20, 39). Children of school-going age would thus be a suitable target group for sensitive assessment of recent *M. leprae* infection.

The slightly higher R-values (individual anti-PGL-I IgM levels) found in Raksha South compared to five other villages could not be explained by differences in the age and/or gender of the children as compared to the other villages. However, group sizes were not equal and Raksha South had a notably higher number of participants (n = 411), possibly affecting the analysis.

Previous studies reported that seroprevalence was stable over time if leprosy incidence in an area remained unchanged (20). A significant limitation hindering direct comparison of previous seroprevalence results from endemic areas (mostly from India, Brazil, and Indonesia) was the use of different assays measuring anti-*M. leprae*-specific antibodies. Measurements were performed using either FSB or serum with variable dilutions, and target antigens varied from native to synthetic PGL-I recognized by various IgM, IgG, or IgA isotypes. In addition, cutoff values were often chosen arbitrarily. However, analysis of data available from China showed that if the same method was used, a decrease in disease prevalence or new case detection rate corresponded to a decrease in anti-*M. leprae* antibody seroprevalence in children (21), adding the potential of measuring antibodies in children as a tool for recent transmission.

Timely diagnosis and treatment can prevent disabilities from developing and the mycobacterium from spreading (40). Active case-finding approaches by healthcare workers are a proven method to identify cases at early stages (41). In addition to the use of serology to monitor transmission, large-scale screenings have the potential to early identify new cases. Although the presence of anti-PGL-I IgM does not predict disease, seropositive individuals are at an increased risk of developing leprosy (42). In this study, 51 children were followed up and screened for clinical symptoms. One boy aged nine (anti-PGL-I IgM R-value 0.21) was diagnosed at follow-up with PB leprosy indicating the additional benefit of community seroscreening in children even for PB leprosy. As we took a cost-efficient approach, it was outside the scope of this project to follow-up all seropositive children for clinical examination which limits the additional impact of the study. In future studies, follow-up of all seropositive children as well as a subgroup of randomly selected seronegative

children should be included besides additional screening of contacts of seropositive children to identify the source of transmission.

Single-dose rifampicin (SDR) PEP is a preventive treatment for leprosy which can decrease the risk of developing disease among (household) contacts of leprosy cases (43). Large-scale, international research proves that SDR-PEP is safe and the WHO recommends its use in the combat against leprosy (44). As many activities including PEP administration are ongoing worldwide and over 175,000 individuals have already received this regimen (44), it is of interest to study the effect over time on *M. leprae* transmission (measured by seroprevalence rates in children) of such prophylactic interventions and to compare the effect of PEP on transmission between countries. Crucial would be the use of one standardized, quantitative assay, which is preferentially field-friendly and easy to use at a large scale. Therefore, the low-complexity UCP-LFA cassette – which is more robust and easier to perform than the ELISA – quantitatively detecting anti-PGL-I IgM in FSB that was field-tested in this study offers a particularly suitable format for this purpose.

Since PKDL is a differential diagnosis of leprosy (37), although not the main aim of this study, we assessed the potential of the anti-PGL-I UCP-LFA to discriminate between the two infections. We found two out of 60 (3.33%) individuals infected with *L. donovani* to test positive for anti-PGL-I IgM. However, while those individuals are living in an area where both diseases are endemic, previous exposure to *M. leprae* cannot be excluded. Furthermore, in an area not endemic for leprosy, young children all tested negative in the anti-PGL-I UCP-LFA cassette, arguing for the specificity of the test. The HDSS team is experienced in conducting studies for leishmaniasis in the field, and this provides opportunities for combining leishmaniasis with leprosy research (32). Performing serosurveillance to monitor the transmission of multiple NTDs simultaneously by pooling expertise into one joint operation could save cost as well as time. To this end, the design of a (combined) rapid test for the detection of antibodies against *L. donovani* (and *M. leprae*) could also be valuable and is considered for future research (45).

## Conclusion

Screening for quantitative assessment of anti-PGL-I IgM levels in children identified 11.58% seropositivity in 12 villages in Bihar, India. These data are in line with seroprevalence data reported (20, 21) for other endemic areas without PEP in the past decades. There was a slight, significant increase with age, but no difference in seropositivity between genders was observed. Anti-PGL-I IgM antibodies in young children are a useful indicator for *M. leprae* infection, thereby serving as a proxy for recent transmission in an area and thus can be used as a tool for monitoring the reduction of transmission when new cases are scarce. A follow-up study 5 to 10 years later, again assessing the anti-PGL-I IgM antibodies in these villages in Bihar in the same age groups, would provide insight into the changes in transmission in this area. In addition, similar studies should be conducted in areas where leprosy is less endemic or not endemic anymore to further validate this tool for monitoring the elimination of transmission.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Acknowledgements

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

## Ethics statement

The studies involving humans were approved by Institutional Review Boards of the Institute of Medical Sciences, Banaras Hindu University (BHU; reference number Dean/2017/EC/185; Dated 24/10/2017), Varanasi, India, and the review committee of the U.S. National Institutes of Health (NIH). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.



## References

1. Blok DJ, de Vlas SJ, Richardus JH. Global elimination of leprosy by 2020: are we on track? *Trop Med Int Health.* (2015) 20:40. doi: 10.1186/s13071-015-1143-4
2. World Health Organization. Leprosy. Available online at: <https://www.who.int/news-room/fact-sheets/detail/leprosy> (accessed August 18, 2023).
3. Deps P, Collin SM. Mycobacterium lepromatosis as a second agent of Hansen's disease. *Front Microbiol.* (2021) 12:698588. doi: 10.3389/fmicb.2021.698588
4. Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL. The continuing challenges of leprosy. *Clin Microbiol Rev.* (2006) 19:338. doi: 10.1128/CMR.19.2.338-381.2006
5. Geluk A. Correlates of immune exacerbations in leprosy. *Semin Immunol.* (2018) 39:111–8. doi: 10.1016/j.smim.2018.06.003
6. Fine PEM, Sterne JAC, Ponnighaus JM, Bliss L, Saul J, Chihana A, et al. Household and dwelling contact as risk factors for leprosy in northern Malawi. *Am J Epidemiol.* (1997) 146:91–102. doi: 10.1093/oxfordjournals.aje.a009195
7. Araujo S, Freitas LO, Goulart LR, Goulart IM. Molecular evidence for the aerial route of infection of Mycobacterium leprae and the role of asymptomatic carriers in the persistence of leprosy. *Clin Infect Dis.* (2016) 63:1412–20. doi: 10.1093/cid/ciw570
8. Tio-Coma M, Avanzi C, Verhard EM, Pierneef L, van Hooij A, Benjak A, et al. Genomic characterization of mycobacterium leprae to explore transmission patterns identifies new subtype in Bangladesh. *Front Microbiol.* (2020) 11:1220. doi: 10.3389/fmicb.2020.01220
9. World Health Organization. Global leprosy (Hansen disease) update, 2021: moving towards interruption of transmission. (2022).
10. Smith WC, van BW, Gillis T, Saunderson P, Richardus JH. The missing millions: a threat to the elimination of leprosy. *PLoS Negl Trop Dis.* (2015) 9:e0003658. doi: 10.1371/journal.pntd.0003658
11. Sales AM, Ponce de Leon A, Duppre NC, Hacker MA, Nery JA, Sarno EN, et al. Leprosy among patient contacts: a multilevel study of risk factors. *PLoS Negl Trop Dis.* (2011) 5:e1013. doi: 10.1371/journal.pntd.0001013
12. Smith WCS, Aerts A. Role of contact tracing and prevention strategies in the interruption of leprosy transmission. *Leprosy Rev.* (2014) 85:2–17. doi: 10.47276/lr.85.1.2
13. World Health Organization. Leprosy Elimination Monitoring (LEM): Guidelines for monitors. (2000).
14. Ogunsumi DOO, Lal V, Puchner KP, van Brakel W, Schwienhorst-Stich EM, Kasang C, et al. Measuring endemicity and burden of leprosy across countries and regions: A systematic review and Delphi survey. *Plos Neglect Trop D.* (2021) 15:e0009769. doi: 10.1371/journal.pntd.0009769
15. Taggart M, Kelly A, Steil R, Chu E. Multibacillary leprosy with an incubation period exceeding 50 years. *Bmj Case Rep.* (2022) 15:e250835. doi: 10.1136/bcr-2022-250835
16. Moet FJ, Meima A, Oskam L, Richardus JH. Risk factors for the development of clinical leprosy among contacts, and their relevance for targeted interventions. *Leprosy Rev.* (2004) 75:310–26. doi: 10.47276/lr.75.4.310
17. Quilter EEV, Butlin CR, Singh S, Alam K, Lockwood DNJ. Patients with skin smear positive leprosy in Bangladesh are the main risk factor for leprosy development: 21-year follow-up in the household contact study (COCO). *Plos Neglect Trop D.* (2020) 14:e0008687. doi: 10.1371/journal.pntd.0008687
18. van Hooij A, Fat EMTK, van den Eeden SJF, Wilson L, da Silva MB, Salgado CG, et al. Field-friendly serological tests for determination of M. leprae-specific antibodies. *Sci Rep-Uk.* (2017) 7:8868. doi: 10.1038/s41598-017-07803-7
19. Zenha EMR, Ferreira MAN, Foss NT. Use of anti-PGL-1 antibodies to monitor therapy regimes in leprosy patients. *Braz J Med Biol Res.* (2009) 42:968–72. doi: 10.1590/S0100-879X2009001000016
20. Pierneef L, van Hooij A, Taal A, Rumbaut R, Nobre ML, van Brakel W, et al. Detection of anti-M. leprae antibodies in children in leprosy-endemic areas: A systematic review. *Plos Neglect Trop D.* (2021) 15:e0009667. doi: 10.1371/journal.pntd.0009667
21. Zhou Z, Pierneef L, van Hooij A, Geluk A. Detection of anti-M. leprae antibodies in healthy children in China: A systematic review of Chinese literature. *Front Trop Dis.* (2022) 3:963674. doi: 10.3389/ftd.2022.963674
22. Corstjens PLAM, van Hooij A, Fat EMTK, Alam K, Vrolijk LB, Dlamini S, et al. Fingerstick test quantifying humoral and cellular biomarkers indicative for M-leprae infection. *Clin Biochem.* (2019) 66:76–82. doi: 10.1016/j.clinbiochem.2019.01.007
23. van Hooij A, Fat EMTK, da Silva MB, Bouth RC, Messias ACC, Gobbo AR, et al. Evaluation of Immunodiagnostic Tests for Leprosy in Brazil, China and Ethiopia. *Sci Rep.* (2018) 8:17920. doi: 10.1038/s41598-018-36323-1
24. van Hooij A, Fat EMTK, de Jong D, Khatun M, Soren S, Chowdhury A, et al. Prototype multi-biomarker test for point-of-care leprosy diagnostics. *Iscience.* (2021) 24:102006. doi: 10.1016/j.isci.2020.102006

25. van Hooij A, van den Eeden S, Richardus R, Fat ETK, Wilson L, Franken KLMC, et al. Application of new host biomarker profiles in quantitative point-of-care tests facilitates leprosy diagnosis in the field. *Ebiomedicine*. (2019) 47:301–8. doi: 10.1016/j.ebiom.2019.08.009
26. Zhou ZJ, Pena M, van Hooij A, Pierneef L, de Jong D, Stevenson R, et al. Detection and monitoring of *Mycobacterium leprae* infection in nine banded armadillos (*Dasypus novemcinctus*) using a quantitative rapid test. *Front Microbiol*. (2021) 12:763289. doi: 10.3389/fmicb.2021.763289
27. Corstjens PLAM, Zuidervijk M, Tanke HJ, Schip JJVV, Ottenhoff THM, Geluk A, et al. User-friendly, highly sensitive assay to detect the IFN-gamma secretion by T cells. *Clin Biochem*. (2008) 41:440–4. doi: 10.1016/j.clinbiochem.2007.12.015
28. World Health Organization. The Global Health Observatory. Leprosy – New leprosy child case detection rate (less than 15 years of age) per 1,000,000 child population (2022).
29. Wagh AN, Mugudalabeta S, Gutierrez NO, Padebettu K, Pandey AK, Pandey BK, et al. Does appreciative inquiry decrease false positive diagnosis during leprosy case detection campaigns in Bihar, India? An operational research study. *Plos Neglect Trop D*. (2018) 12:7004. doi: 10.1371/journal.pntd.0007004
30. Technical Group on Population Projections. Census of India 2011: Population Projections for India and States 2011–2036. New Delhi: Ministry of Health & Family Welfare (2019).
31. Hasker E, Malaviya P, Scholar VK, de Koning P, Singh OP, Kansal S, et al. Post kala azar dermal leishmaniasis and leprosy prevalence and distribution in the Muzaffarpur health and demographic surveillance site. *Plos Neglect Trop D*. (2019) 13:e0007798. doi: 10.1371/journal.pntd.0007798
32. Malaviya P, Picado A, Hasker E, Ostyn B, Kansal S, Singh RP, et al. Health & demographic surveillance system profile: the Muzaffarpur-TMRC health and demographic surveillance system. *Int J Epidemiol*. (2014) 43:1450–7. doi: 10.1093/ije/dyu178
33. World Health Organization. Bacillus Calmette-Guérin (BCG) vaccination coverage (2023).
34. Singh OP, Tiwary P, Kushwaha AK, Singh SK, Singh DK, Lawyer P, et al. Xenodiagnosis to evaluate the infectiousness of humans to sandflies in an area endemic for visceral leishmaniasis in Bihar, India: a transmission-dynamics study. *Lancet Microbe*. (2021) 2:E23–31. doi: 10.1016/S2666-5247(20)30166-X
35. van Dijk JHM, van Hooij A, Groot LM, Geboers J, Moretti R, Verhard-Seymonsbergen E, et al. Synthetic phenolic glycolipids for application in diagnostic tests for leprosy. *Chembiochem*. (2021) 22:1487–93. doi: 10.1002/cbic.202000810
36. Kumar V, Mandal R, Das S, Kesari S, Dinesh DS, Pandey K, et al. Kala-azar elimination in a highly-endemic district of Bihar, India: A success story. *Plos Neglect Trop D*. (2020) 14:e0008254. doi: 10.1371/journal.pntd.0008254
37. Trindade MAB, Silva LLD, Braz LMA, Amato VS, Naafs B, Sotto MN. Post-kalaazar dermal leishmaniasis and leprosy: case report and literature review. *BMC Infect Dis*. (2015) 15:543. doi: 10.1186/s12879-015-1260-x
38. World Health Organization. Leprosy Resolution WHA 44.9: Forty-fourth World Health Assembly (1991).
39. Ulrich M, Smith PG, Sampson C, Zuniga M, Centeno M, Garcia V, et al. IgM antibodies to native phenolic glycolipid-I in contacts of leprosy patients in Venezuela: epidemiological observations and a prospective study of the risk of leprosy. *Int J Lepr Other Mycobact Dis*. (1991) 59:405–15.
40. Chen KH, Lin CY, Su SB, Chen KT. Leprosy: a review of epidemiology, clinical diagnosis, and management. *J Trop Med-U.S.* (2022) 2022:8652062. doi: 10.1155/2022/8652062
41. Saunderson P. Improving early case detection in leprosy: Reports from recent workshops. *Leprosy Rev*. (2022) 93:292–7. doi: 10.47276/lr.93.4.292
42. Richardus RA, van der Zwet K, van Hooij A, Wilson L, Oskam L, Faber R, et al. Longitudinal assessment of anti-PGL-I serology in contacts of leprosy patients in Bangladesh. *Plos Neglect Trop D*. (2017) 11:e0006083. doi: 10.1371/journal.pntd.0006083
43. Mieras LF, Taal AT, van Brakel WH, Cambau E, Saunderson PR, Smith WCS, et al. An enhanced regimen as post-exposure chemoprophylaxis for leprosy: PEP plus. *BMC Infect Dis*. (2018) 18:506. doi: 10.1186/s12879-018-3402-4
44. Global Partnership for Zero Leprosy I ITC. Factsheet on SDR-PEP: The latest research on single-dose rifampicin post-exposure prophylaxis (2022).
45. Cloots K, Uranw S, Ostyn B, Bhattarai NR, Le Rutte E, Khanal B, et al. Impact of the visceral leishmaniasis elimination initiative on *Leishmania donovani* transmission in Nepal: a 10-year repeat survey. *Lancet Glob Health*. (2020) 8:e237–e43. doi: 10.1016/S2214-109X(19)30536-4

## Supplementary material

**Supplementary table 1. Ratio values for anti-PGL-I IgM measured in children aged 3 – 11 in Bihar, India**

| R      | # children | Cumulative # children | Percentage (%) | Cumulative percentage (%) |
|--------|------------|-----------------------|----------------|---------------------------|
| 0.00   | 105        | 105                   | 5.65           | 5.65                      |
| 0.01   | 305        | 410                   | 16.42          | 22.08                     |
| 0.02   | 284        | 694                   | 15.29          | 37.37                     |
| 0.03   | 207        | 901                   | 11.15          | 48.52                     |
| 0.04   | 165        | 1,066                 | 8.89           | 57.40                     |
| 0.05   | 117        | 1,183                 | 6.30           | 63.70                     |
| 0.06   | 120        | 1,303                 | 6.46           | 70.17                     |
| 0.07   | 110        | 1,413                 | 5.92           | 76.09                     |
| 0.08   | 69         | 1,482                 | 3.72           | 79.81                     |
| 0.09   | 60         | 1,542                 | 3.23           | 83.04                     |
| 0.10   | 52         | 1,594                 | 2.80           | 85.84                     |
| 0.11   | 48         | 1,642                 | 2.58           | 88.42                     |
| 0.12   | 22         | 1,664                 | 1.18           | 89.61                     |
| 0.13   | 31         | 1,695                 | 1.67           | 91.28                     |
| 0.14   | 27         | 1,722                 | 1.45           | 92.73                     |
| 0.15   | 16         | 1,738                 | 0.86           | 93.59                     |
| 0.16   | 12         | 1,750                 | 0.65           | 94.24                     |
| 0.17   | 14         | 1,764                 | 0.75           | 94.99                     |
| 0.18   | 13         | 1,777                 | 0.70           | 95.69                     |
| 0.19   | 12         | 1,789                 | 0.65           | 96.34                     |
| 0.20   | 7          | 1,796                 | 0.38           | 96.72                     |
| > 0.20 | 61         | 1,857                 | 3.28           | 100                       |

The number (#) of children per ratio (R) value is provided with the corresponding percentage of that group in relation to the whole cohort. The cut-off for positivity ( $R \geq 0.12$ ) for the UCP-LFA batch used in this study was based on the median of a sextuple test performed in India of a standard control serum sample (+) plus its standard deviation (SD). Anti-PGL-I: anti-phenolic glycolipid I; IgM: immunoglobulin M; R: ratio value, result of the UCP-LFA.

**Supplementary table 2. Anti-PGL-I IgM positivity in children aged 5 – 9 in Bihar**

| Age | # children | # with R $\geq 0.12$ (%) |
|-----|------------|--------------------------|
| 5   | 410        | 30 (7.32)                |
| 6   | 353        | 39 (11.05)               |
| 7   | 392        | 52 (13.27)               |
| 8   | 430        | 55 (12.79)               |
| 9   | 268        | 39 (14.55)               |

For each age group the total number (#) of children, the number (percentage) testing positive for anti-PGL-I IgM are indicated. The cut-off for positivity ( $R \geq 0.12$ ) for the UCP-LFA batch used in this study was based on the median of a sextuple test performed in India of a standard control serum sample (+) plus its standard deviation (SD). Anti-PGL-I: anti-phenolic glycolipid I; IgM: immunoglobulin M; R: ratio value, result of the UCP-LFA.

**Supplementary table 3. Place of residence of the children aged 3 – 11 in Bihar and corresponding percentage of anti-PGL-I IgM positive children**

| Village | Village name       | # children | # with R $\geq 0.12$ | Percentage positive per village (%) | p-value* |
|---------|--------------------|------------|----------------------|-------------------------------------|----------|
| A       | Singar Phulkahan   | 114        | 18                   | 15.79                               | >0.9999  |
| B       | Madhopur Chhapra   | 126        | 14                   | 11.11                               | 0.0003   |
| C       | Godai Phulkahan    | 216        | 16                   | 7.41                                | 0.0154   |
| D       | Godai Jamal        | 110        | 11                   | 10.00                               | 0.0368   |
| E       | Vishwanathpur      | 89         | 13                   | 14.61                               | 0.6589   |
| F       | Raksha North       | 164        | 10                   | 6.10                                | 0.0011   |
| G       | Raksha North Chauk | 204        | 25                   | 12.25                               | >0.9999  |
| H       | Raksha South West  | 97         | 11                   | 11.34                               | >0.9999  |
| I       | Raksha South       | 411        | 64                   | 15.57                               | -        |
| J       | Raksha Deah        | 250        | 23                   | 9.20                                | 0.0022   |
| K       | Nariyar Nawada     | 70         | 7                    | 10.00                               | >0.9999  |
| L       | Arizpur Kothi      | 6          | 3                    | 50.00                               | >0.9999  |

For each village the total number (#) of children, the number (percentage) testing positive for anti-PGL-I IgM are indicated. The cut-off for positivity ( $R \geq 0.12$ ) for the UCP-LFA batch used in this study was based on the median of a sextuple test performed in India of a standard control serum sample (+) plus its standard deviation (SD). A Kruskal-Wallis test was performed to determine the statistical significance in anti-PGL-I IgM ratio (R) values between villages. The p-value\* indicates the difference between the corresponding village in comparison to village Raksha South (I). Anti-PGL-I: anti-phenolic glycolipid I; IgM: immunoglobulin M; R: ratio value, result of the UCP-LFA.





# 5

## **Seroprevalence of anti-*Mycobacterium leprae* PGL-I antibodies among school children in rural and urban Indonesia: a cross-sectional study (2023)**

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## Abstract

Leprosy remains a significant public health issue in endemic regions, including areas in Indonesia. Transmission of *Mycobacterium leprae* (*M. leprae*) continues as clear from the almost stable annual number of new leprosy cases amongst which around 5% are children. To interrupt transmission of *M. leprae*, it is essential to identify and treat infection sources. This study aimed to assess anti-*M. leprae* phenolic glycolipid-I (PGL-I) IgM seroprevalence among school-aged children in rural and urban Indonesia, as a proxy for recent transmission. Additionally, associations between seropositivity and gender, socioeconomic status (SES), and helminth infection were investigated. We conducted a cross-sectional serosurvey among 637 children (ages 6-15) from schools in urban and rural areas of Sulawesi, Java, and Sumba, Indonesia. The PGL-I QURapid, a field-friendly and quantitative lateral flow assay, was used to detect anti-PGL-I IgM in fingerstick blood and plasma samples. Socioeconomic and health data, including helminth infection status and body-mass index z-scores (z-BMI), were also collected. The overall seroprevalence of anti-PGL-I IgM in children was 12.2% with significantly higher seroprevalence in rural (Southwest Sumba: 31.0%, Pangkajene: 11.2%) compared to urban areas (North Jakarta: 3.8%, Makassar city: 3.1%). Moreover, seroprevalence amongst children from low-SES schools was significantly higher (13.7%) than among those from high-SES schools (4.1%,  $P = 0.007$ ). The presence of helminth infections were associated with significantly higher anti-PGL-I IgM levels ( $P < 0.0001$ ), with 22.4% of helminth-infected children testing seropositive compared to 8.8% of non-infected children ( $P < 0.001$ ). Our findings demonstrate a high anti-PGL-I IgM seroprevalence among rural, low-SES, and helminth-infected children in Indonesia, indicating elevated levels of *M. leprae* infection within these groups which again represents increased transmission rates in the population. These results underscore the need for integrated disease control strategies addressing both leprosy and helminthiasis, particularly in rural and economically disadvantaged areas. The PGL-I QURapid demonstrated to be an effective tool in serosurveys, able to aid the identification of areas with high *M. leprae* transmission rates (such as Sumba) and thereby support the implementation of targeted interventions in endemic regions.

## Introduction

Leprosy, caused by *Mycobacterium leprae* (*M. leprae*) or *Mycobacterium lepromatosis* (*M. lepromatosis*), is a neglected tropical disease that primarily affects the skin, peripheral nerves, upper respiratory tract, and eyes [1]. Although curable with multidrug therapy (MDT), leprosy remains a significant public health concern, particularly in low-income countries where healthcare access is limited, such as Indonesia [2]. Despite ongoing control efforts, many individuals continue to suffer from the long-term consequences of leprosy, including physical disability and social stigma [3].

The majority of exposed individuals does not develop clinical disease as they are adequately protected by their immune system [4], but asymptomatic carriers can still harbor the bacteria in the nasopharyngeal cavity and could contribute to transmission [5], complicating control strategies. Notably, the incubation period can generally span 2-10 years, but even over 50 years has been reported [6, 7]. Close and prolonged contact with untreated leprosy patients remains a primary risk factor, placing household contacts at the highest risk of developing the disease [8-11]. Other factors reported to be associated with increased risk of leprosy are low socioeconomic status (SES), malnutrition, and substandard living conditions [12, 13]. Moreover, in leprosy endemic areas, coinfection with soil-transmitted helminths (STH) is common [14]. Pre-existing helminth infections may enhance susceptibility to *M. leprae* infection or exacerbate disease severity through modulation of host immune responses [14, 15]. Given the high burden of helminths in leprosy endemic regions, integrated disease control strategies are vital [16].

To successfully interrupt transmission of *M. leprae*, it is essential to identify and treat infection sources early [17]. Currently, leprosy elimination efforts are monitored by assessing the proportion of new cases in children under 15 years of age among the total number of newly detected cases. However, since only a small fraction of individuals infected with *M. leprae* develop clinical disease - and because symptoms may take years to appear - this indicator does not provide timely or sufficiently accurate insight into ongoing transmission. Instead, monitoring infection rates could offer a more informative and responsive measure to guide intervention strategies, assess transmission trends, and track progress toward elimination [18-21]. Due to its usefulness in detecting infection among asymptomatic individuals [19, 22, 23], the WHO recommends using *M. leprae*-specific anti-phenolic glycolipid-I (PGL-I) serology in children as a tool for assessing ongoing *M. leprae* transmission. PGL-I is a glycolipid found exclusively in the cell wall of *M. leprae* and *M. lepromatosis*, highlighting its specificity for the leprosy causing pathogens. IgM levels against this antigen correlate strongly with the bacterial load in infected individuals [24], making it a useful marker for the identification of those most likely to contribute to transmission. Anti-PGL-I IgM can be present in individuals with either past or present *M. leprae* infection. As infection in (young) children typically indicates recent transmission (since it cannot exceed their age), the detection of antibodies in this group likely indicates recent or ongoing infection, making them a preferred group to monitor seroprevalence and thus transmission.

The type of serological test and standardisation of the cut-off for positivity used in seroscreening for assessment of transmission is crucial [19]. To facilitate field-based

surveillance, we have developed the PGL-I QURapid; a low-cost, user-friendly lateral flow assay employing upconverting reporter particle (UCP) technology for the quantitative detection of anti-PGL-I IgM in capillary or venous blood [25]. This test provides a practical and scalable tool for resource-limited settings, offering application in multiple use cases globally.

In 2000, the WHO announced that Indonesia had effectively eliminated leprosy [26], meaning that prevalence had fallen below the WHO-defined epidemiological threshold, which is distinct from complete elimination of the disease from the population. This led to significant downscaling of the national leprosy control program, especially affecting areas with limited medical services [27, 28]. Still, Indonesia ranks as the third-highest contributor to the global leprosy burden, with approximately 12,000-17,000 new cases reported annually [3].

In this study, we assessed the seroprevalence of anti-PGL-I IgM among school-aged children from rural and urban areas across Indonesia using the PGL-I QURapid. By exploring associations between seropositivity and factors such as gender, SES, and helminth infection, we sought to identify environmental and host-related determinants of *M. leprae* infection. The resulting insights may inform public health strategies for leprosy control and support the implementation of targeted interventions by identifying hotspots for *M. leprae* transmission.

## Methods

**Ethics.** This study used samples collected during two population studies conducted previously [29][Ali S., et al., manuscript under review]. The use of these samples in the current serosurvey was approved by the Ethical Committee of the School of Medicine and Health Sciences (SMHS), AJCUI (Ref. No.: 0004F/III/PPPR.PM.10.05/04/2024). The study in Sulawesi was approved by the Hasanuddin University Ethical Committee (Ref. No.: UH23080619.01/11/2023). Parents were informed about the study and written parental consent was obtained for each child to participate in the study prior to sample collection.

**Study participants and locations.** Study participants were schoolchildren (n = 637; age 6-15 years) from different socio-economic backgrounds living in rural and urban parts of three areas in Indonesia (Supplementary figure 1):

Sulawesi: 321 children from two elementary schools located in the urban centre of Makassar (Makassar city District), as well as two elementary schools situated on the rural island of Karanrang (Pangkajene dan Kepulauan District). Sample and data collection took place between September and November 2023. Schools differed in socio-economic status (SES); three schools were designated “low-SES” and one school “high-SES” [30]. The high-SES school was located in the city center and is considered of high-SES status, with a majority of the parents working as high-skilled workers or professionals with higher education. Meanwhile, the low-SES schools were located near landfill and port areas, where most of the parents are low-educated and work in low-skilled labor jobs.

**Java:** 158 children from (low-SES) public schools in the Kamal Muara (KM) sub-district, located within the Penjaringan District of North Jakarta, an urban-slum area in Java.

**Sumba:** 158 children from the rural (low-SES) Hameli-Ate sub-district within the North Kodi District of Southwest Sumba, located in eastern Indonesia.

Within each district, schools were purposively selected based on existing collaborations, prior contact with the research team and approval by the headmasters, as part of a bigger study. Schools were chosen to represent both urban and rural settings and, where possible, different socio-economic backgrounds. Children from all classes in the selected schools who were willing to participate and for whom parental informed consent was obtained were invited to take part. The study sites on Sumba and Java aimed for approximately similar numbers of participants at each site (around 160 children). For the other sites (South Sulawesi), numbers were dependent on the number of schoolchildren available at the schools focused on in this sub-study, and set to allow for meaningful comparisons across all study sites.

New case detection rates (NCDR) by district for 2023 are presented in Supplementary table 1 [31].

**Demographic data collection.** Information regarding demographics and indicators of SES was obtained using a questionnaire. Body weight and height were measured and used to calculate the age-standardised z-scores of body mass index (z-BMI), according to WHO guidelines [32]. For the purpose of this study, the categorisation of BMI-for-age was simplified by classifying all children with a z-score lower than -2 as “underweight”, all children with a z-score between -2 and 1 as “normal weight” and all children with z-scores above 1 as “overweight”. BCG vaccination was assessed by examining the presence of a BCG scar on the upper arm, measured as the mean of two diameters using a ruler.

**Parasitological examination.** Same day stool specimens were obtained from each child and subjected to microscopic examination upon arrival at the laboratory. Stool specimens were examined using Kato-Katz technique in accordance with WHO recommendations for the detection of STH, such as *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm [33]. For stool specimens collected from the KM area (Java), additional microscopy techniques were employed: a direct smear and Haradamori (a culture-based microscopy technique) targeting the larvae of hookworms or *Strogylodes stercoralis* [29].

**Fingerstick blood collection.** FSB was collected using disposable 20 µl Minivette ® collection tubes (Heparin coated; Sarstedt) and directly mixed with 980 µl high salt finger stick (HSFS) buffer: 100 mM Tris pH 8.0, 270 mM NaCl, 1% (v/v) Triton X-100, and 1% (w/v) BSA providing a 50-fold dilution [22, 25].

**Plasma specimen collection.** The whole blood samples were collected from KM and HA areas, in 0.5 ml EDTA vacutainer tubes (BD, Franklin Lakes, NJ, USA). Tubes were

centrifuged at 2,500 rpm for 10 min and plasma was subsequently aliquoted and frozen (-80°C) until further use.

**PGL-I QURapid.** Individually packaged UCP-LFA cassettes for the detection of human anti-PGL-I IgM antibodies were produced by Maxim Biomedical Inc. (Rockville, MD, USA) as described previously [22]. The air-tight pouches with test cassettes contained silica dry packs for extended shelf life and protection against humidity. The Test (T) line on the LF strip (nitrocellulose membrane; Sartorius UniSart CN95) comprised 100 ng of synthetic PGL-I, phenolic trisaccharide functionalized with a hexanoic acid linker for conjugation to BSA (NPT1-H-BSA; Leiden, the Netherlands) [34]. The flow-control (FC) line comprised 100 ng rabbit anti-goat IgG (G4018; Sigma-Aldrich, Inc., St. Louis, MO, USA). Goat IgG against human IgM (I0759; Sigma-Aldrich, Inc., St. Louis, MO, USA) was conjugated to polyacrylic acid functionalized UCPs [200 nm, NaYF<sub>4</sub>:Yb<sup>3+</sup>, Er<sup>3+</sup>; Intelligent Material Solutions Inc. (IMS); Princeton, NJ, USA] according to previously described protocols at a concentration of 50 µg antibody per mg UCP [35]. To dry the UCPs onto the glass fiber conjugate-release pad, the material was diluted in a buffer containing 100 mM Tris pH 8.0, 270 mM NaCl, 10% (w/v) sucrose, 1% (w/v) BSA, 0.5% Tween-20, and striped at a density of 100 ng/mm.

**UCP-LFA.** 50 µl of a 50-fold diluted FSB- or plasma sample was added to the LF strip to initiate LF. PGL-I QURapid-tests were analyzed using a battery-operated UCP-adapted portable lightweight standalone reader (ESEQUant LFR adapted for UCP; DIALUNOX, Stockach, Germany) [36]. Results were calculated as the Ratio (R)-value between the test line (T) and the flow control line (FC) signal based on relative fluorescence units (RFUs) measured at the respective lines. A quality control (QC) protocol was designed to monitor test reproducibility using sera of clinically diagnosed leprosy patients selected based on their anti-PGL-I IgM levels in standard anti-PGL-I IgM ELISAs [24, 34]: a highly seropositive, a seropositive with an OD indicative for the cut-off for seropositivity with the corresponding anti-PGL-I IgM ELISAs (QC reference) and a seronegative serum sample (from a healthy Dutch blood bank donor without travel history to leprosy endemic areas). For direct comparison of FSB and plasma test data, results were normalized and presented as anti-PGL-I 'units'. A seropositivity cut-off of  $\geq 0.16$  was applied for serum/plasma, and  $\geq 0.12$  for FSB. The determination of these cut-offs was described previously [25]. In the reported study, the WHO TPP published in 2023 [37] was leading in terms of required minimum Sn/Sp for establishing a cut-off. Assuming a linear relation between R-value and concentration above the cut-off value, units were calculated by dividing individual R-values by the respective cut-off value, in analogy to methods used in anti-PGL-I ELISAs [38-41]. Units  $\geq 1$  were considered seropositive.

**Statistical analysis.** This study was conducted in four purposively selected districts and a limited number of schools within each district. Data clustering by school was not accounted for in the analyses. Consequently, all analyses should be considered exploratory, aimed at describing patterns rather than generating nationally representative estimates. The statistical software GraphPad Prism version 9.0.1 for Windows (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics for Windows version 29 (IBM Corp, Armonk, NY,

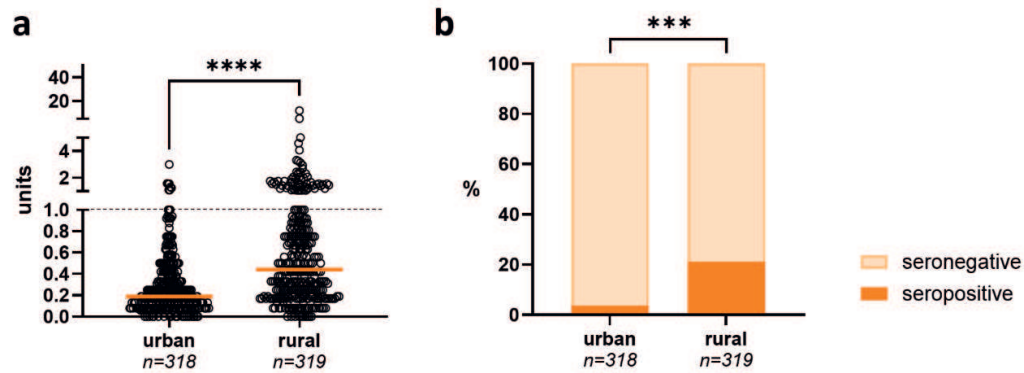
USA) were used to perform statistical analysis. Mann–Whitney U and Kruskal–Wallis tests were used to assess differences between two and three independent groups, respectively. Differences in seroprevalence between groups in cross-sectional analyses were evaluated using Chi-squared tests. When information regarding age, gender, helminth infection status, or z-BMI was missing, those individuals were excluded only from the analyses requiring that specific variable, but they remained included in all other analyses for which their data were complete.

## Results

**Study participants.** To assess *M. leprae* transmission in rural and urban areas with varying SES in Indonesia, we measured anti-PGL-I IgM seroprevalence using the PGL-I QURapid among 637 school-aged children from elementary schools located in North Jakarta, Makassar city, Pangkajene and Southwest Sumba. Of the participants, 51.2% were female, with a median age of 9 years (range: 6–15; Table 1). The majority (84.6%) attended low-SES schools, while 15.4% attended high-SES schools. Half of the children (50.1%) were from rural areas. Helminth infections were present in 28.3% of the participants, 20.1% were classified as underweight and 18.1% as overweight.

**Seroprevalence, gender differences and BCG.** Among the 637 children tested, 78 (12.2%) were seropositive for anti-PGL-I IgM (Table 2). Female children had significantly higher levels of anti-PGL-I IgM compared to male children (Supplementary figure 2A; Mann–Whitney:  $P < 0.0001$ ). Additionally, seropositivity rates were significantly higher in females ( $n = 50$ , 15.4%) than in males ( $n = 28$ , 9.0%; Chi-squared:  $P = 0.016$ ; Supplementary figure 2B). When analyzing the sites separately by gender, we found a similarly significant result for the urban sites, while only a trend was observed for the rural sites. No significant age-related differences in anti-PGL-I levels were observed (Supplementary figure 2C). Furthermore, BCG-scar size did not appear to influence seropositivity quantitatively as there was no correlation between size of the scar and levels of anti-PGL-I IgM (data not shown). The distribution of anti-PGL-I R-values is shown in Supplementary figure 3.

**Rural vs. urban areas.** Seroprevalence was lowest in urban areas, with Makassar city (3.1%) and North Jakarta (3.8%) showing the smallest proportions, while notably higher rates were observed in rural areas: Southwest Sumba (31.0%) and Pangkajene (11.2%; Table 2). Consistent with these findings, median anti-PGL-I IgM levels were significantly higher in children from rural areas (0.44; Southwest Sumba and Pangkajene) compared to those from urban settings (0.19; Makassar city and North Jakarta; Figure 1; Mann–Whitney;  $P < 0.0001$ ). Moreover, when focusing on the children with anti-PGL-I units  $\geq 2$  ( $n = 20$ ), the vast majority ( $n = 19$ ) 95.0% were derived from rural areas.

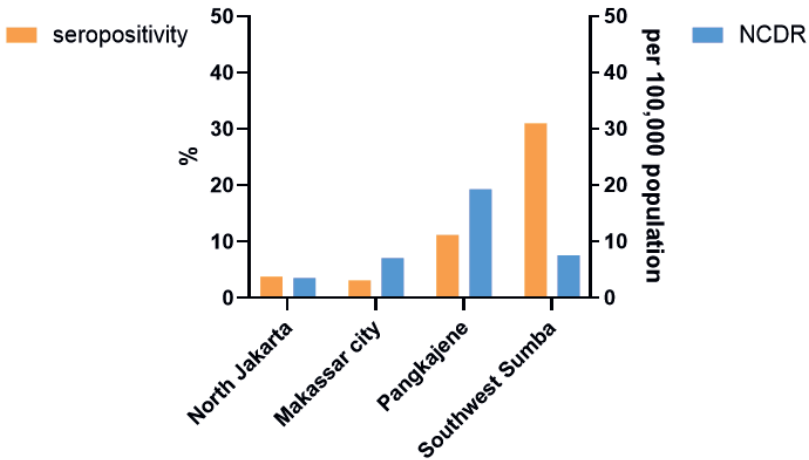


**Figure 1. Anti-PGL-I IgM in children in urban and rural areas across Indonesia.**

A: Anti-PGL-I IgM (in units; y-axis) of children from urban and rural areas (x-axis). Dots (open circles) represent anti-PGL-I units for individual samples. A Mann-Whitney U test was performed to determine differences between the two groups (\*\*\*\*  $P \leq 0.0001$ ). Anti-PGL-I units  $\geq 1$  (dotted line) were considered seropositive.

B: Percentages (%) of children testing seropositive (y-axis) for anti-PGL-I IgM stratified by urban and rural area (x-axis). Chi-squared tests were performed to test for differences in percentages between the two groups (\*\*  $P \leq 0.001$ ). IgM: immunoglobulin M; PGL-I: phenolic glycolipid-I.

When comparing seroprevalence in healthy young children with the leprosy patients' NCDR in the same area, we observed that the relatively lower NCDR in North Jakarta (3.5/100,000) and Makassar city (7.1/100,000) corresponded with lower seroprevalence (3.1-3.8%) of anti-PGL-I IgM in healthy young children, whereas the higher NCDR in Pangkajene (19.3/100,000) corresponded with higher seroprevalence (11.2%; Figure 2). In Southwest Sumba, on the other hand, this pattern did not hold true, as high seroprevalence (31.0%) was observed despite a relatively low NCDR (7.6/100,000). However, in this area in Sumba, the number of underreported cases is probably high, since dedicated leprosy control efforts are lacking. An active case finding study has identified 48 (80%) MB and 12 (20%) PB cases among newly diagnosed patients, of whom 20 (33%) already had visible grade 2 disabilities at diagnosis [25], indicating late diagnosis.

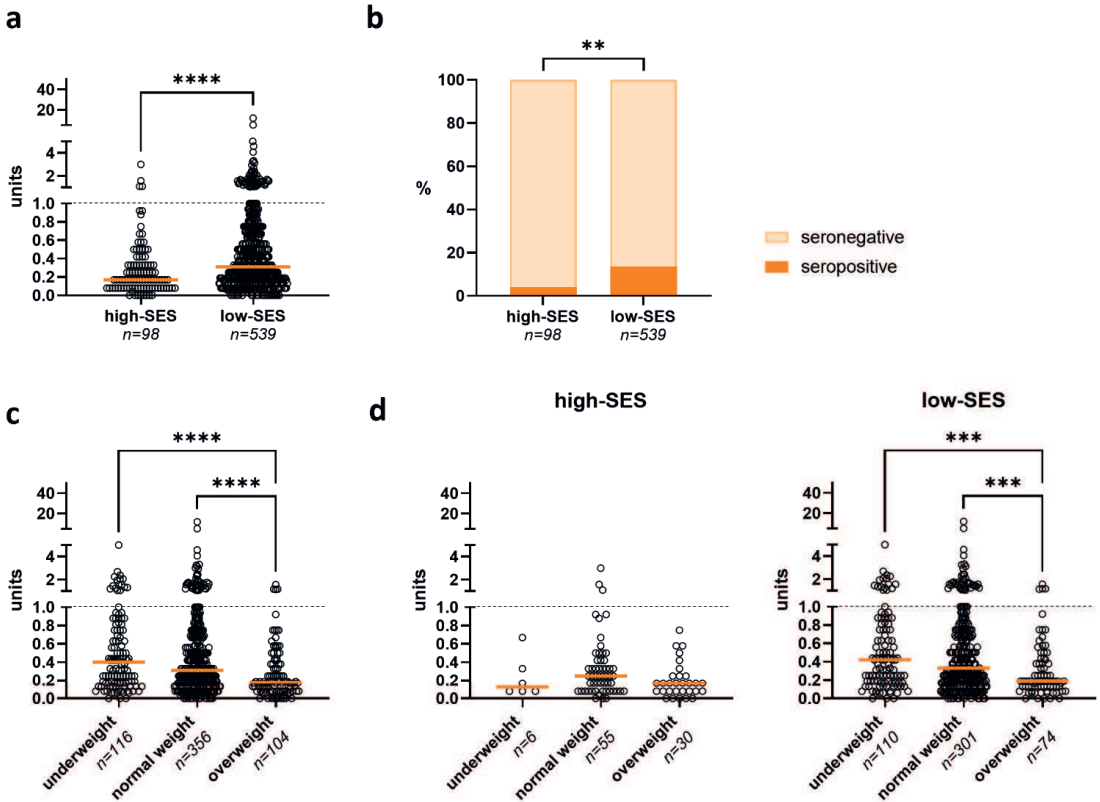


**Figure 2.** Anti-PGL-I IgM seropositivity in children, stratified by study district and compared with the corresponding leprosy new case detection rate (NCDR).

Seropositivity percentages are shown in orange (left y-axis) for North Jakarta, Makassar City, Pangkajene dan Kepulauan, and Southwest Sumba (x-axis). NCDR per district is shown in blue (right y-axis).

**High-SES vs. low-SES.** Given that leprosy is often associated with poverty [13, 42] as well as malnutrition [43], we examined *M. leprae* infection rates between children differing in z-BMI scores attending high-SES and low-SES schools. The data showed that children from low-SES schools had significantly higher anti-PGL-I levels than those from high-SES schools (Mann-Whitney:  $P < 0.0001$ ; Figure 3A). A significantly higher percentage (13.7%) of children from low-SES schools tested seropositive for anti-PGL-I IgM (high-SES: 4.1%; Figure 3B; Chi-squared:  $P = 0.007$ ). When investigating weight of the children as a parameter of well- or malnutrition, we observed that among high-SES students ( $n = 91$ ), 6.6% ( $n = 6$ ) were underweight, whereas among the 485 children from low-SES schools, 22.7% ( $n = 110$ ) were underweight. Anti-PGL-I IgM levels were significantly higher in children who were underweight or of normal weight compared to those who were overweight (Figure 3C; Kruskal-Wallis: both  $P < 0.0001$ ). After stratifying by SES, the association persisted only in the low-SES group (Figure 3D).





**Figure 3. Anti-PGL-I IgM in children attending high-SES and low-SES schools in Indonesia.**

A: Anti-PGL-I IgM (in units; y-axis) of children attending high-SES and low-SES schools (x-axis). Dots (open circles) represent anti-PGL-I units for individual samples. A Mann-Whitney U test was performed to determine differences between the two groups (\*\*\*\*  $P \leq 0.0001$ ).

B: Percentages (%) of children seropositive (y-axis) for anti-PGL-I IgM stratified for SES; high and low (x-axis). Anti-PGL-I units  $\geq 1$  (dotted line) were considered seropositive. Chi-squared tests were performed to test for differences in percentages between the two groups (\*\*  $P \leq 0.01$ ). IgM: immunoglobulin M; PGL-I: phenolic glycolipid-I.

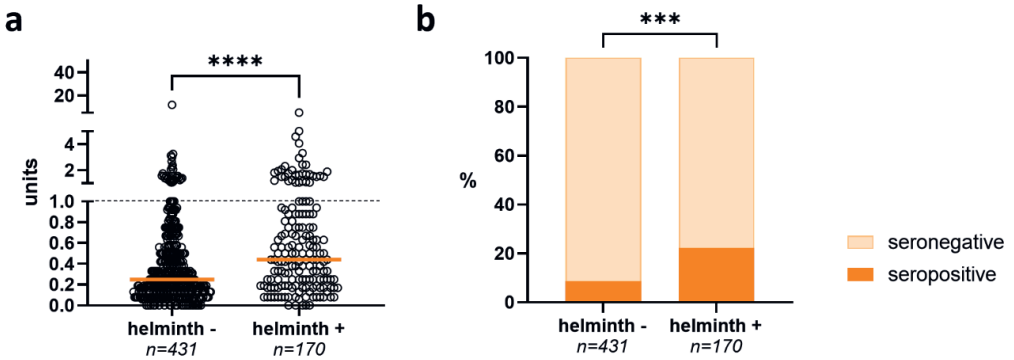
C: Anti-PGL-I IgM (in units; y-axis) of children classified as being underweight, having normal weight or being overweight (x-axis). PGL-I units  $\geq 1$  (dotted line) were considered seropositive. A Kruskal-Wallis test with Dunn's correction for multiple testing was performed to determine differences between the three groups (\*\*\*\*  $P \leq 0.0001$ ). z-BMI scores were used to classify weight groups. z-BMI < -2: underweight; z-BMI  $\geq -2$  and  $\leq 1$ : normal weight; z-BMI > 1: overweight. z-BMI scores were not recorded for 61 participants.

D: Anti-PGL-I IgM levels (y-axis) stratified by SES (left panel: high-SES; right panel: low-SES) for children classified as underweight, normal weight, or overweight (x-axis).

**Helminth infection.** Helminth infections such as *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm, are common in areas where leprosy is endemic [14, 44, 45]. Therefore, we

assessed anti-PGL-I levels in relation to helminth infection status. Helminth positivity was substantially higher in rural areas (Pangkajene: 42.6%; Southwest Sumba: 57.0%) in comparison to urban areas (Makassar city: 14.8%; North Jakarta: 0%; Table 3).

In helminth-infected children, median anti-PGL-I IgM levels were significantly higher (Figure 4A; Mann-Whitney:  $P < 0.0001$ ). Among these children ( $n = 170$ ), 22.4% ( $n = 38$ ) tested seropositive for anti-PGL-I IgM compared to only 8.8% ( $n = 38$  of 431) in those who were not helminth-infected (Figure 4B; Chi-squared:  $P < 0.001$ ).



**Figure 4. Anti-PGL-I IgM in children with and without helminth infection in Indonesia.**

A: Anti-PGL-I IgM (in units; y-axis) of children with and without helminth infection (x-axis). Dots (open circles) represent anti-PGL-I units for individual samples. A Mann-Whitney U test was performed to determine differences between the two groups (\*\*\*\*  $P \leq 0.0001$ ).

B: Percentages (%) of children testing seropositive (y-axis) for anti-PGL-I IgM stratified by helminth infection status (x-axis). Anti-PGL-I units  $\geq 1$  (dotted line) were considered seropositive. Chi-squared tests were performed to test for differences in percentages between the two groups (\*\* $P \leq 0.001$ ). IgM: immunoglobulin M; PGL-I: phenolic glycolipid-I.

## Discussion

This serosurvey contributes to the growing body of research assessing anti-PGL-I seroprevalence among children [8, 10, 46-49] as an indicator of *M. leprae* transmission, building on previous studies from other endemic regions while providing new data from Indonesia using a field-friendly, rapid test. The study also explores associations with SES and helminth infection. Our study reveals a significant burden of (past or present) *M. leprae* infection among school-aged children in Indonesia, particularly in rural and low-SES settings. The overall seroprevalence of 12.2% in children is in line with previously reported seroprevalence in leprosy endemic areas using our test [19, 22] and underscores that transmission is ongoing despite elimination efforts. Markedly higher seroprevalence was found in rural areas such as Southwest Sumba (31.0%) and Pangkajene (11.2%) compared to urban areas like Makassar city (3.1%) and North Jakarta (3.8%). The mechanisms underlying higher *M. leprae* infection rates in rural settings remain incompletely understood.

Transmission is thought to occur primarily from MB cases to susceptible individuals. However, several factors may contribute to higher seroprevalence in rural areas, including delayed diagnosis of index cases [28], limited access to healthcare and poor sanitation [50]. The markedly higher prevalence of helminth infections in these areas may further increase susceptibility through immunomodulation [45]. Recent studies have also suggested the potential role of soil [51] and water [52] as reservoirs of *M. leprae*, although more research is needed. While these hypotheses do not provide definitive explanations, they highlight the complex interplay of environmental, socioeconomic, and host factors that likely drive ongoing transmission in rural Indonesia.

Children attending low-SES schools exhibited seropositivity more frequently with significantly higher anti-PGL-I IgM levels than their high-SES counterparts, indicating higher transmission rates in the population where these groups originate from. Moreover, our findings further support the association between low-SES and increased exposure to *M. leprae*, highlighting that poverty-related factors indeed may contribute to heightened vulnerability to leprosy [13, 42]. This trend correlates with higher rates of undernutrition among low-SES children, supporting earlier findings that malnutrition impairs immune function and increases susceptibility to infection [43, 53, 54]. In relation to this, Feenstra et al. reported a rise in leprosy cases following a period of famine in Bangladesh, further underscoring the role of nutritional stress in amplifying disease vulnerability [43]. These data emphasize the need for holistic public health strategies that address both infectious diseases and nutritional deficiencies.

Our observation that female children exhibited significantly higher anti-PGL-I IgM levels than males is notable, given that males are generally reported to be more often affected by leprosy [55, 56]. However, leprosy in women is probably underreported due to sociocultural factors as well as stigma [56, 57] and elevated anti-PGL-I IgM levels among females have been documented previously [47, 48, 58, 59]. Nonetheless, the underlying reasons for this finding remain unclear and warrant further investigation.

Our findings indicate a clear association between helminth infection and elevated anti-PGL-I IgM levels. Seropositivity was 2.5 times higher among helminth-infected children (22.4%) compared to uninfected peers (8.8%). This reinforces the hypothesis that helminth-induced immunomodulation may increase susceptibility to *M. leprae* or delayed clearance of the pathogen [14, 15, 44]. Given this correlation, there is a strong rationale for incorporating helminth prophylaxis as part of integrated public health strategies in regions with concurrent burdens of leprosy and STH [16]. Improvements in sanitation may also contribute by lowering helminth prevalence, providing an additional mechanism for reductions in leprosy incidence. However, the introduction of helminth prophylaxis in leprosy-endemic settings must be approached with caution. Helminths are known to modulate host immune responses toward a Th2-biased profile [60], which may partially suppress Th1-type responses that are critical in leprosy control [14]. Sudden removal of helminth-induced immunoregulation may theoretically unmask subclinical leprosy or precipitate immunological reactions, such as reversal reactions (Type 1) [14] or erythema nodosum leprosum (Type 2) [61]. Therefore, any integration of deworming programs into leprosy control efforts warrants careful monitoring, along with clinical training to recognize and manage reactions effectively.

Southwest Sumba emerged in our study as the site with a striking seroprevalence of 31.0% among school-aged children, indicating high transmission. Although for the three other sites there seemed to be an association between the measured seroprevalence and NCDR, for Sumba seroprevalence in children did not align with the reported NCDR for the area [62]. However, this could be due to the lack of proper leprosy health care programs in that area as a recent study reported that between 2020 and 2024, 60 individuals were diagnosed with leprosy in Southwest Sumba and suggested significant underreporting of cases [28]. According to the same publication, the median interval between symptom onset and diagnosis was 36 months, and 33% of patients already had visible disabilities at diagnosis. These delays in diagnosis and treatment resulted from limited access to specialized dermatological care, with the nearest dermatologist located more than 24 hours away by boat. Given the intensity of transmission in Southwest Sumba, targeted public health interventions, such as active case finding, and contact tracing including the provision of single-dose rifampicin as post-exposure prophylaxis (PEP) are urgently needed [63–65]. Given the baseline data provided in this study, using serosurveillance, Sumba could serve as a sentinel site for evaluating the effects of integrated control strategies. Moreover, the findings of this study underscore the utility of serosurveys among children in detecting otherwise hidden transmission.

While this study was primarily descriptive, future studies incorporating multivariate regression analyses are needed to disentangle the independent effects of SES, helminth infection, and nutrition on *M. leprae* infection. Moreover, our study is exploratory and not intended to be nationally representative. Districts and schools were purposively selected and generalizations beyond the sampled districts are limited. Nonetheless, this study clearly demonstrated the operational feasibility and utility of the PGL-I QURapid in large-scale, field-based serosurveillance efforts. Preparations for a larger-scale investigation are currently underway in other regions of Indonesia. The performance of the PGL-I QURapid with FSB allows for efficient deployment in the field, providing reliable data that can inform risk mapping and help targeting of interventions. The test enabled the identification of seropositive children without clinical symptoms, suggesting a potential role in identifying hidden transmission hotspots.

## Conclusions

This study confirms ongoing *M. leprae* transmission in Indonesia, with particularly high seroprevalence among children in rural areas like Sumba. By detecting past or present infection, the PGL-I QURapid reveals transmission and offers a promising tool for both targeted prophylaxis guidance and post-intervention surveillance. Our findings support integrating serological surveillance into national leprosy programs, especially in low-SES and high endemic settings as well as areas thought to near or having reached elimination. Addressing factors like helminth infection and undernutrition alongside leprosy control may improve control effort outcomes. Monitoring child seroprevalence as a measure for recent transmission, as recommended by WHO, is key to tracking elimination progress and refining strategies.

## Data sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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## Declaration of interests

The authors declare that they have no conflict of interest.

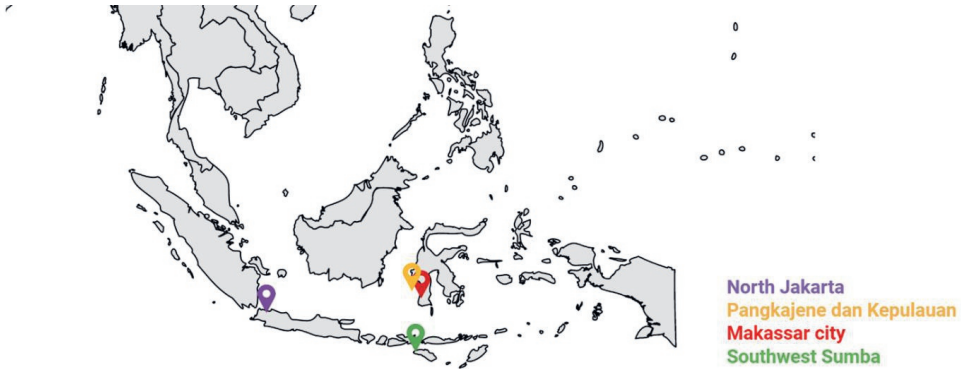
## References

1. WHO. Leprosy. Fact sheets 2025; Available from: <https://www.who.int/news-room/fact-sheets/detail/leprosy>.
2. Grijnsen, M.L., et al., Leprosy. *Nat Rev Dis Primers*, 2024. 10(1): p. 90.
3. WHO, Global leprosy (Hansen disease) update, 2023: Elimination of leprosy disease is possible – Time to act!, in *Weekly epidemiological record*. 2024, WHO..
4. Alter, A., et al., Leprosy as a genetic disease. *Mamm Genome*, 2011. 22(1-2): p. 19–31.
5. Araujo, S., et al., Molecular Evidence for the Aerial Route of Infection of *Mycobacterium leprae* and the Role of Asymptomatic Carriers in the Persistence of Leprosy. *Clin Infect Dis*, 2016. 63(11): p. 1412–1420.
6. Schuenemann, V.J., et al., Ancient genomes reveal a high diversity of *Mycobacterium leprae* in medieval Europe. *PLoS Pathog*, 2018. 14(5): p. e1006997.
7. Taggart, M., et al., Multibacillary leprosy with an incubation period exceeding 50 years. *BMJ Case Rep*, 2022. 15(7).
8. Barreto, J.G., et al., Spatial epidemiology and serologic cohorts increase the early detection of leprosy. *BMC Infect Dis*, 2015. 15: p. 527.
9. Goulart, I.M., et al., Risk and protective factors for leprosy development determined by epidemiological surveillance of household contacts. *Clin Vaccine Immunol*, 2008. 15(1): p. 101–5.
10. Bakker, M.I., et al., Risk factors for developing leprosy--a population-based cohort study in Indonesia. *Lepr Rev*, 2006. 77(1): p. 48–61.
11. Fine, P.E., et al., Household and dwelling contact as risk factors for leprosy in northern Malawi. *Am J Epidemiol*, 1997. 146(1): p. 91–102.
12. Velmurugan, H. and P. Thangaraju, Nutritional Status in Leprosy Patients: A Systematic Review and Meta-analysis. *Infect Disord Drug Targets*, 2024. 24(3): p. e211123223670.
13. Nery, J.S., et al., Socioeconomic determinants of leprosy new case detection in the 100 Million Brazilian Cohort: a population-based linkage study. *Lancet Glob Health*, 2019. 7(9): p. e1226–e1236.
14. Hagge, D.A., et al., Opening a Can of Worms: Leprosy Reactions and Complicit Soil-Transmitted Helminths. *EBioMedicine*, 2017. 23: p. 119–124.
15. Diniz, L.M., et al., Presence of intestinal helminths decreases T helper type 1 responses in tuberculoid leprosy patients and may increase the risk for multi-bacillary leprosy. *Clin Exp Immunol*, 2010. 161(1): p. 142–

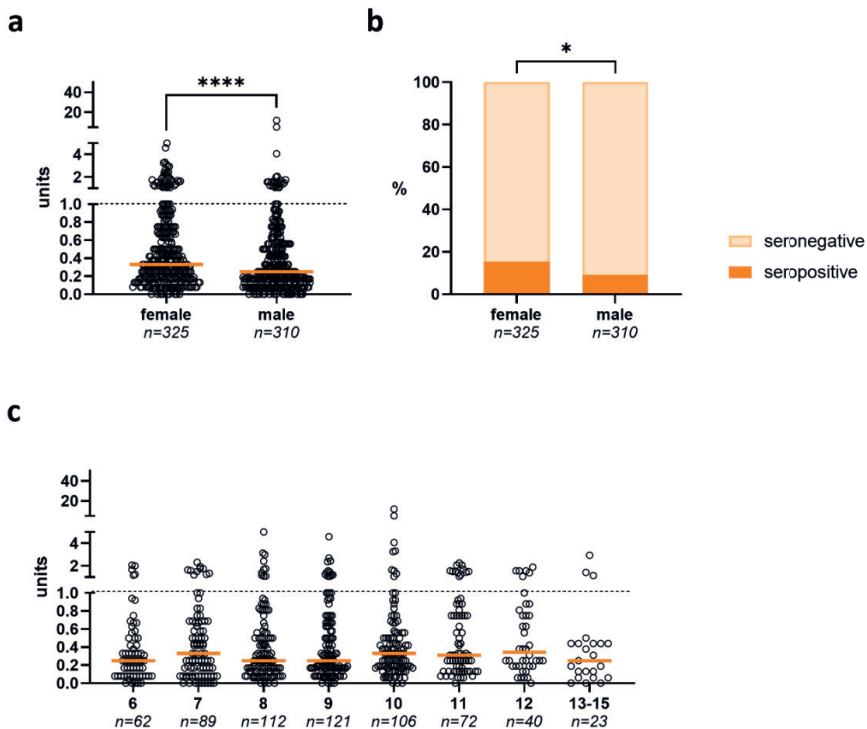
- 50.
16. Geluk, A., Correlates of immune exacerbations in leprosy. *Semin Immunol*, 2018. 39: p. 111–118.
17. Smith, W.C. and A. Aerts, Role of contact tracing and prevention strategies in the interruption of leprosy transmission. *Lepr Rev*, 2014. 85(1): p. 2–17.
18. WHO, R.O.f.S.-E.A., Interruption of Transmission and Elimination of Leprosy Disease - Technical Guidance. 2023, WHO Regional Office for South-East Asia.
19. Pierneef, L., et al., Detection of anti-M. leprae antibodies in children in leprosy-endemic areas: A systematic review. *PLoS Negl Trop Dis*, 2021. 15(8): p. e0009667.
20. van Brakel, W., et al., Operational approaches and recommendations to interrupt transmission of Mycobacterium leprae. *Leprosy Review*, 2025. 96(2).
21. Hasker, E., et al., Interrupting transmission of synthesis of new evidence and research recommendations. *Leprosy Review*, 2025. 96(2).
22. Pierneef, L., et al., Field-friendly anti-PGL-I serosurvey in children to monitor Mycobacterium leprae transmission in Bihar, India. *Front Med (Lausanne)*, 2023. 10: p. 1260375.
23. Pierneef, L., et al., Large-scale field application of a fingerstick blood test for Mycobacterium leprae infection: Monitoring population-wide effects of case finding and post-exposure prophylaxis on transmission in the Comoros and Madagascar. *PLOS Glob Public Health*, 2025. 5(12): p. e0005270.
24. van Hooij, A., et al., Field-friendly serological tests for determination of M. leprae-specific antibodies. *Sci Rep*, 2017. 7(1): p. 8868.
25. Pierneef, L., et al., Rapid test for Mycobacterium leprae infection: a practical tool for leprosy. *Infect Dis Poverty*, 2024. 13(1): p. 88.
26. WHO, Progress in leprosy control: Indonesia, 1991-2008, in *Weekly Epidemiological Record*. 2010, WHO. p. 249–262.
27. Pieter, Y. and M.L. Grijzen, Picturing health: the burden of leprosy in eastern Indonesia. *Lancet*, 2022. 399(10335): p. 1588–1599.
28. Siregar, G.O., et al., Leprosy identified in Sumba Island, eastern Indonesia: elimination targets under threat. *Lancet Reg Health Southeast Asia*, 2024. 26: p. 100409.
29. Narayani, D.A., et al., Common intestinal parasitic infections in an improved water access, sanitation, and hygiene profile setting in North Jakarta, Indonesia. *Narra J*, 2024. 4(3): p. e1264.
30. Amaruddin, A.I., et al., The Bacterial Gut Microbiota of Schoolchildren from High and Low Socioeconomic Status: A Study in an Urban Area of Makassar, Indonesia. *Microorganisms*, 2020. 8(6).
31. Ministry of Health Republic of Indonesia, Leprosy National Annual Report 2023. 2024.
32. de Onis, M., et al., Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ*, 2007. 85(9): p. 660–7.
33. WHO, Bench aids for the diagnosis of intestinal parasites: Second edition 2019. 2019, WHO.
34. van Dijk, J.H.M., et al., Synthetic Phenolic Glycolipids for Application in Diagnostic Tests for Leprosy. *ChemBiochem*, 2021. 22(8): p. 1487–1493.
35. van Hooij, A., et al., Evaluation of Immunodiagnostic Tests for Leprosy in Brazil, China and Ethiopia. *Sci Rep*, 2018. 8(1): p. 17920.
36. Corstjens, P.L., et al., Feasibility of a lateral flow test for neurocysticercosis using novel up-converting nanomaterials and a lightweight strip analyzer. *PLoS Negl Trop Dis*, 2014. 8(7): p. e2944.
37. WHO, Target product profile for a diagnostic test to detect Mycobacterium leprae infection among asymptomatic household and familial contacts of leprosy patients. 2023, WHO.
38. Lima, F.R., et al., Serological testing for Hansen's disease diagnosis: Clinical significance and performance of IgA, IgM, and IgG antibodies against Mce1A protein. *Front Med (Lausanne)*, 2023. 10: p. 1048759.
39. Frade, M.A., et al., Unexpectedly high leprosy seroprevalence detected using a random surveillance strategy in midwestern Brazil: A comparison of ELISA and a rapid diagnostic test. *PLoS Negl Trop Dis*, 2017. 11(2): p. e0005375.
40. Longoni, S.S., et al., ELISA Test Based on the Phenolic Glycolipid-I (PGL-I) of Mycobacterium leprae: A Reality of a Laboratory from a Non-Endemic Country. *Pathogens*, 2022. 11(8).
41. Lima, M.I.S., et al., Biotechnological and Immunological Platforms Based on PGL-I Carbohydrate-Like Peptide of Mycobacterium leprae for Antibodies Detection Among Leprosy Clinical Forms. *Front Microbiol*, 2020. 11: p. 429.
42. Oktaria, S., et al., Dietary diversity and poverty as risk factors for leprosy in Indonesia: A case-control study. *PLoS Negl Trop Dis*, 2018. 12(3): p. e0006317.
43. Feenstra, S.G., et al., Recent food shortage is associated with leprosy disease in Bangladesh: a case-control study. *PLoS Negl Trop Dis*, 2011. 5(5): p. e1029.
44. Weldegebreel, F., et al., Prevalence and associated factors of soil-transmitted helminth infections among children in previous leprosy and non-leprosy areas in Eastern Ethiopia: A community-based comparative study. *IJID Reg*, 2025. 15: p. 100633.

45. Oktaria, S., et al., Soil-transmitted helminth infections and leprosy: a cross-sectional study of the association between two major neglected tropical diseases in Indonesia. *BMC Infect Dis*, 2016. 16: p. 258.
46. Bakker, M.I., et al., Genetic, household and spatial clustering of leprosy on an island in Indonesia: a population-based study. *BMC Med Genet*, 2005. 6: p. 40.
47. Fine, P.E., et al., Seroepidemiological studies of leprosy in northern Malawi based on an enzyme-linked immunosorbent assay using synthetic glycoconjugate antigen. *Int J Lepr Other Mycobact Dis*, 1988. 56(2): p. 243–54.
48. Barreto, J.G., et al., High rates of undiagnosed leprosy and subclinical infection amongst school children in the Amazon Region. *Mem Inst Oswaldo Cruz*, 2012. 107 Suppl 1: p. 60–7.
49. Barreto, J.G., et al., Anti-PGL-I seroepidemiology in leprosy cases: household contacts and school children from a hyperendemic municipality of the Brazilian Amazon. *Lepr Rev*, 2011. 82(4): p. 358–70.
50. Emerson, L.E., et al., Poor WASH (Water, Sanitation, and Hygiene) Conditions Are Associated with Leprosy in North Gondar, Ethiopia. *Int J Environ Res Public Health*, 2020. 17(17).
51. Tio-Coma, M., et al., Detection of *Mycobacterium leprae* DNA in soil: multiple needles in the haystack. *Sci Rep*, 2019. 9(1): p. 3165.
52. Ploemacher, T., et al., Reservoirs and transmission routes of leprosy; A systematic review. *PLoS Negl Trop Dis*, 2020. 14(4): p. e0008276.
53. Wagenaar, I., et al., Diet-related risk factors for leprosy: a case-control study. *PLoS Negl Trop Dis*, 2015. 9(5): p. e0003766.
54. Morales, F., et al., Effects of Malnutrition on the Immune System and Infection and the Role of Nutritional Strategies Regarding Improvements in Children's Health Status: A Literature Review. *Nutrients*, 2023. 16(1).
55. Quilter, E.E.V., D.N.J. Lockwood, and C.R. Butlin, Rising percentage of new female leprosy cases since 2004. *Leprosy Review*, 2022. 93(4): p. 364–369.
56. Price, V.G., Factors preventing early case detection for women affected by leprosy: a review of the literature. *Glob Health Action*, 2017. 10(sup2): p. 1360550.
57. Dijkstra, J.I.R., W.H. Van Brakel, and M. Van Elteren, Gender and leprosy-related stigma in endemic areas: A systematic review. *Leprosy Review*, 2017. 88(3): p. 419–440.
58. Schuring, R.P., et al., Association between anti-pGL-I IgM and clinical and demographic parameters in leprosy. *Lepr Rev*, 2006. 77(4): p. 343–55.
59. Frota, C.C., et al., Seropositivity to anti-phenolic glycolipid-I in leprosy cases, contacts and no known contacts of leprosy in an endemic and a non-endemic area in northeast Brazil. *Trans R Soc Trop Med Hyg*, 2010. 104(7): p. 490–5.
60. Bass, A., et al., *Mycobacterium leprae* infection, Hansen's disease, and helminth infections: A cross-sectional study in southeastern Brazil. *medRxiv*, 2025.
61. Menaldi, S.L., et al., Unusual presentations of a severe type 2 leprosy reaction mimicking sepsis induced by helminth infection. *PLoS Negl Trop Dis*, 2021. 15(7): p. e0009453.
62. Ministry of Health Republic Indonesia, Evaluation of Leprosy Prevention and Control Program in 2023. 2024.
63. WHO, Guidelines for the diagnosis, treatment and prevention of leprosy. 2018. 2017, World Health Organization, Regional Office for South-East Asia: New Delhi.
64. Moraes, M.O. and N.C. Duppre, Leprosy post-exposure prophylaxis: innovation and precision public health. *Lancet Glob Health*, 2021. 9(1): p. e8–e9.
65. Moet, F.J., et al., Effectiveness of single dose rifampicin in preventing leprosy in close contacts of patients with newly diagnosed leprosy: cluster randomised controlled trial. *BMJ*, 2008. 336(7647): p. 761–4.

## Supplemental information



**Supplementary figure 1. Map of Indonesia showing the geographic distribution of study sites.** Map of Indonesia showing the four study locations where samples were collected from 637 children aged 6-15 years. Sites included schools in urban and rural areas in Sulawesi (Pangkajene: dark yellow; Makassar city: red), Java (North Jakarta: purple), and Sumba (Southwest Sumba: green). This figure was created using Biorender.



**Supplementary figure 2. Anti-PGL-I IgM in children stratified by gender and age.**

**A:** Anti-PGL-I IgM (in units; y-axis) of female and male children (x-axis). A Mann-Whitney U test was performed to determine differences between the two groups (\*\*\*\*  $P \leq 0.0001$ ). Anti-PGL-I units  $\geq 1$  (dotted line) were

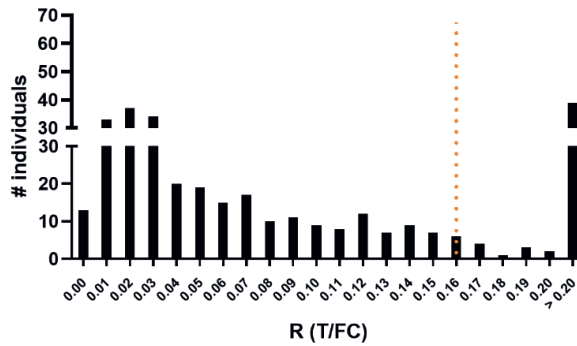


considered seropositive. Two individuals were excluded from the analysis due to missing information on gender.

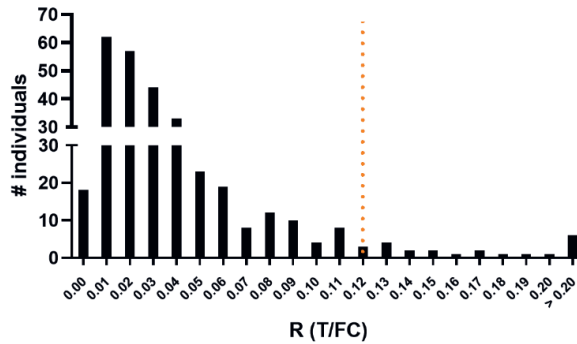
**B:** Percentages (%) of children testing seropositive (y-axis) for anti-PGL-I IgM stratified by gender (x-axis). Chi-squared tests were performed to test for differences in percentages between the two groups (\*  $P \leq 0.05$ ).

**C:** Anti-PGL-I IgM (in units; y-axis) per age group (x-axis). A Kruskal-Wallis test with correction for multiple testing was performed to determine differences between the groups. Twelve participants were excluded from the analysis due to missing age information. Ages 13 to 15 were grouped together because of the low number of participants in these age categories. Dots (open circles) represent anti-PGL-I units for individual samples. IgM: immunoglobulin M; PGL-I: phenolic glycolipid-I.

**a**



**b**



**Supplementary figure 3. Presence of anti-PGL-I IgM antibodies in plasma (A) and FSB (B) of schoolchildren in Indonesia.**

**A:** Histogram with a mode of 0.01 for R. The cut-off for positivity in plasma ( $R \geq 0.16$ ) for the UCP-LFA used in this study is indicated by the dotted line.

**B:** Histogram with a mode of 0.01 for R. The cut-off for positivity in FSB ( $R \geq 0.12$ ) for the UCP-LFA used in this study is indicated by the dotted line.

FC: flow control line; IgM: immunoglobulin M; PGL-I: phenolic glycolipid I; R: ratio value; T: test line.





# 6

## **Large-scale field application of a fingerstick blood test for *Mycobacterium leprae* infection: Monitoring population-wide effects of case finding and post-exposure prophylaxis on transmission in the Comoros and Madagascar**

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## Abstract

Since 2018, the WHO recommends provision of single-dose rifampicin post-exposure prophylaxis (SDR-PEP) to contacts of leprosy patients. In the Post-ExpOsure Prophylaxis for LEprosy (PEOPLE) trial that took place in the Comoros (Anjouan and Mohéli) and Madagascar (2019-2023), single double-dose rifampicin (SDDR)-PEP administration modalities were compared. Additionally, in 16/64 villages, anti-*M. leprae* phenolic glycolipid-I (anti-PGL-I) IgM levels were measured for three consecutive years, in newly diagnosed leprosy patients and more than 17,000 household- and neighbourhood contacts who had been screened for signs of leprosy. To study the impact of active case finding and SDDR-PEP on *M. leprae* infection rates in the population, fingerstick blood of contacts was sampled before, during and after the administration of SDDR-PEP. In this serosurvey, anti-PGL-I IgM levels were measured for the first time at such large scale by local staff using a field-friendly, quantitative lateral flow assay based on the UCP-platform (UCP-LFA). Out of 53 multibacillary patients with a bacterial index of 1 or higher, 92.5% (n = 49) tested seropositive for anti-*M. leprae* PGL-I IgM, confirming excellent performance of the test when executed by local staff. Before SDDR-PEP administration, seroprevalence among contacts in the different villages ranged from 21.9% to 27.4% across the three study sites. After two years of active case finding and SDDR-PEP, seroprevalence was significantly lower in the population in all three study sites (11.6% to 22.8%). Longitudinal serological monitoring in initially seropositive contacts showed significant reductions in anti-PGL-I IgM levels at an individual level. This reduction in seropositivity was observed irrespective of administration of SDDR-PEP. These serological data suggest that the implemented combination of active case finding with SDDR-PEP, led to a reduction in infection levels in the population in these study sites. This large-scale field serosurvey demonstrates the usefulness of the anti-PGL-I UCP-LFA to evaluate and monitor at a population level the effects of leprosy control interventions on *M. leprae* transmission.

## Introduction

Leprosy, caused by *Mycobacterium leprae* or *M. lepromatosis*, is a neglected tropical disease primarily affecting the skin and peripheral nerves [1, 2]. The disease remains a significant public health issue in many parts of the world, particularly in parts of Asia and South-America [3], but also Africa [4]. Although in the WHO African Region, almost all Member States have achieved leprosy elimination (prevalence rate less than 1 per 10,000 population), pockets of hotspots within this region persist with more than 20,000 new cases reported every year [4].

Household contacts of leprosy patients - especially those with a high bacterial load - are most at risk of contracting the disease [5-7]. Early diagnosis and treatment are crucial for preventing the onset of disabilities and controlling transmission [3]. However, leprosy diagnosis remains clinical and therefore prone to miss asymptomatic individuals [8], underscoring the need for improved diagnostic tools.

Since 2018, the WHO has recommended provision of post-exposure prophylaxis (PEP) using single-dose rifampicin (SDR) to contacts of leprosy patients [9]. SDR-PEP should halt infection with *M. leprae* from progressing into clinical leprosy disease [10]. However, in previous studies using rifampicin as prophylaxis, contradictory results have been found: in Bangladesh, the COLEP trial (2002-2007) found a significant reduction in the risk of leprosy after SDR was given to contacts [11]. In contrast, a study conducted on five islands in the Flores Sea of Indonesia (2000-2003) showed no protective effect from two doses of rifampicin, three months apart, to household- and social contacts of leprosy patients [12]. However, this same study in Indonesia showed a four-fold decrease in leprosy incidence when PEP was offered as a blanket intervention across the entire island population.

The combination of active case finding and provision of single double-dose rifampicin (SDDR)-PEP to contacts of leprosy patients is aimed at reducing disease as well as breaking the chain of transmission [10, 13]. The effectiveness of these interventions, however, requires monitoring through reliable tools that can detect changes in transmission and not only in the number of new cases. The PEOPLE trial, conducted on the Comoros and in Madagascar located in the Indian Ocean, was designed to explore SDDR-PEP administration modalities [14, 15]. In the trial, 64 villages were randomly assigned to four arms and inhabitants were screened once a year for leprosy [14, 15].

The current study describes the serological data collected using a quantitative, field-friendly test designed to detect present or past infection through measurement of *M. leprae*-specific anti-PGL-I IgM, which was included in one arm of the PEOPLE trial. The here assessed test utilizes luminescent, background-free, up-converting reporter particles (UCP) [16] and immunochromatography (i.e. the UCP-LF test platform) for accurate quantitation of anti-PGL-I IgM without operator bias [17]. Previously, it was demonstrated that the presence of anti-PGL-I IgM is highly specific for *M. leprae* infection, and that the level of anti-PGL-I IgM correlates strongly with the bacterial load present in an individual [18, 19]. In this study, we applied the anti-PGL-I UCP-LFA for the first time at such large scale to assess the feasibility of measuring seroprevalence among leprosy patients and their contacts. Moreover, we aimed to evaluate the possibility of monitoring the impact of the implementation of active

case finding and SDDR-PEP on *M. leprae*-specific antibody levels over time using our field-friendly test, providing insights into the effects of these interventions on *M. leprae* transmission.

## Materials and methods

**Study design and setting.** The set-up of the PEOPLE trial in the Comoros and Madagascar has been described in extensive detail (<https://clinicaltrials.gov/ct2/show/NCT03662022>) [14, 15]. The current study is focused on data gathered with the anti-PGL-I UCP-LFA and took place from January 11, 2019 to November 1, 2022. During this period, fingerstick blood (FSB) samples were collected from all consenting newly diagnosed leprosy cases on the Comoros (Anjouan and Mohéli; starting in 2019) and in Madagascar (Miandrivazo; starting in 2020) all newly diagnosed leprosy cases detected in the villages participating in the PEOPLE trial. Furthermore, FSB samples of leprosy contacts were collected in 16 villages that were assigned to arm 4 during the PEOPLE trial (Comoros: 9 on Anjouan; 3 on Mohéli; Madagascar: 4 on Miandrivazo). All samples were analysed using the anti-PGL-I UCP-LFA in local laboratories (Comoros: laboratory of the reference hospital Hombo in Mutsamudu on Anjouan; laboratory of the reference hospital in Fomboni on Mohéli; Madagascar: laboratory of the Centre d'Infectiologie Charles Mérieux in Antananarivo). From 2014-2019, the Comoros reported an annual incidence rate of 4.2 per 10,000 whereas on Madagascar 0.6 per 10,000 was reported [15, 20]. Transmission in the areas remains high, as reflected by 36% of new cases being children (2018; Comoros), while nearly 20% of newly diagnosed cases in Madagascar have grade-2 disabilities (G2D) [14, 20].

**Door-to-door screening.** Annual screening rounds were conducted over a four-year period in each village where participants were recruited. Permanent residents of the study villages were invited for leprosy screening by national tuberculosis and leprosy programme (NTLP) teams in the Comoros, and national leprosy programme teams in Madagascar accompanied by community health workers during household visits. During household visits, all consenting household members present were screened for leprosy, and their history of previous leprosy diagnosis or treatment, tuberculosis (TB), and presence of a Bacillus Calmette–Guérin vaccine (BCG) scar were assessed. Individual eligibility criteria were based on WHO recommendations for SDR-PEP - ie, healthy contacts aged 2 years and older, excluding possible patients with leprosy or tuberculosis, or both, in the absence of other contraindications.

**Leprosy patients.** Newly identified patients during door-to-door screening or routine activities were treated according to NTLP guidelines [15]. Diagnosis of leprosy was clinical, based on the presence at least one of three cardinal signs: patch with loss of sensation, enlarged peripheral nerves and/or slit-skin smear (SSS) positive for acid fast bacilli. Patients were classified as PB (one to five lesions) or MB (more than five lesions) per WHO criteria [3]. For most MB patients, the bacterial index (BI) was determined using slit skin smears. Patients with disease detected after being screened during at least one prior round were

classified as “incident cases” as they were already under contact-follow-up of the PEOPLE trial at time of their diagnosis.

**Contacts and SDDR-PEP.** In the study villages, all eligible contacts were offered double (20 mg/kg) the regular dose (10 mg/kg) of SDR-PEP, based on an increased early bactericidal effect observed for *Mycobacterium tuberculosis* with this dose [21, 22]. Paediatric dosages were adjusted according to body weight. Eligibility for SDDR-PEP was determined both on geospatial and individual criteria. All household contacts of newly diagnosed leprosy cases, as well as individuals living within 100m of a newly diagnosed case and testing seropositive for anti-PGL-I antibodies using the UCP-LFA were revisited. Those present and meeting individual eligibility criteria were offered SDDR-PEP. Individual eligibility criteria for SDDR-PEP included: healthy contacts aged two years and above, able and willing to provide informed consent. Exclusion criteria for SDDR-PEP included: signs of active leprosy, signs of active pulmonary TB (cough  $\geq$  two weeks), and rifampicin intake within the last 24 months (to avoid any theoretical risk of inducing rifampicin resistance). SDDR-PEP was aimed to be distributed within one month following the completion of the annual screening round in each village, based on the cases detected during these screening rounds and anti-PGL-I test results.

**Informed consent procedures.** Written informed consent was obtained for each individual participating in door-to-door screening. A separate written informed consent was collected for all leprosy patients prior to sampling. For those under 18 years old, written informed consent or parent/guardian approval was obtained, along with assent from minors aged 12 years or older.

**Fingerstick blood (FSB) collection.** Prior to field activities, tubes with 980  $\mu$ l high salt fingerstick (HSFS) buffer: 100mM Tris pH 8.0, 270mM NaCl, 1% (v/v) Triton X-100, and 1% (w/v) BSA were prepared. FSB was collected by local field staff using disposable 20  $\mu$ l Minivette collection tubes (Heparin coated; Sarstedt) and added to the prepared buffer tubes on which the participants unique pseudonymized identification number was written. After sampling, FSB was transported at ambient temperature (in black plastic bags) to local laboratories where FSB samples were tested using UCP-LF strips. On the Comoros, during door-to-door screening entire village populations were sampled, whereas in Madagascar, only household contacts and individuals residing within 100m of a new case were sampled.

**Lateral flow strips.** UCP-LF strips specific for anti-PGL-I IgM were produced in-house in four batches by Leiden University Medical Center as described previously [18, 23]; total production size was 47,000. Synthesized disaccharide epitope (3,6-di-O-methyl- $\beta$ -D-glucopyranosyl(1  $\rightarrow$  4)2,3-di-O-methylrhamnopyranoside), similar to *M. leprae* specific PGL-I glycolipid, coupled to human serum albumin (synthetic PGL-I; designated ND-O-HSA) was obtained through the Biodefense and Emerging Infections Research Resources Repository (<http://www.beiresources.org/TBVTRMResearchMaterials/tabid/1431/Default.aspx>).



The Test (T) line on the LF strip (nitrocellulose membrane; Sartorius UniSart CN95) comprised 100 ng of synthetic PGL-I. The flow-control (FC) line comprised 100 ng rabbit anti-goat IgG (G4018; Sigma-Aldrich, Inc., St. Louis, MO, USA). Goat IgG specific for anti-human IgM (I0759; Sigma-Aldrich, Inc., St. Louis, MO, USA) was conjugated to polyacrylic acid functionalized UCPs [200 nm, NaYF<sub>4</sub>:Yb<sup>3+</sup>, Er<sup>3+</sup>; Intelligent Material Solutions Inc. (IMS); Princeton, NJ, USA MS] according to previously described protocols at a concentration of 50 µg antibody per mg UCP [24]. UCP stock solutions were kept at 4°C until use. To dry the UCPs on the glass fiber conjugate-release pad, the material was diluted in a buffer containing 100 mmol/L Tris pH 8.0, 270 mmol/L NaCl, 10% (w/v) sucrose, 1% (w/v) BSA, 0.5% Tween-20, and striped at a density of 100 ng/mm. Components were mounted on plastic backing cards which were cut into LF strips of 4 mm width by 5 cm length.

**UCP-LFA.** In the local laboratories, each UCP-LF strip was labeled with the same written unique barcode (as on the tube) specific to a participant. 50 µl of the in the field stabilized, diluted, and lysed (with HSFS buffer) FSB sample was added to a 96-wells plate and LF was initiated by placing the UCP-LF strip into the well. Immunochromatography was allowed to continue until strips were dry (overnight). UCP-LF strips were scanned with a UCP dedicated benchtop reader (Madagascar; UPCON; Labrox Oy Turku, Finland) and a standard portable ESEQuant *LFR* adapted for UCP (Comoros; DIALUNOX, Stockach, Germany). Results were calculated as the ratio (R) value between Test (T) and Flow Control (FC) signal based on relative fluorescence units (RFUs) measured at the respective lines. A quality control (QC) protocol was designed to monitor test reproducibility over time using sera of clinically diagnosed leprosy patients selected based on their anti-PGL-I IgM levels in standard anti-PGL-I IgM ELISAs [18, 25]: a highly seropositive, a seropositive with an OD around the cut-off for seropositivity in anti-PGL-I IgM ELISAs (QC reference) and a seronegative serum sample (from a healthy Dutch blood bank donor without travel history to leprosy endemic areas). As described above, each site used their own UCP-reader which were from different suppliers. Sites were provided with UCP-LF strips, comprising different production lots, with regular shipments. To allow direct comparison of all test results from different batches and readers, results were normalized and presented as anti-PGL-I 'units' using the QC reference serum sample. First, cut-offs for different strip batches were determined as the median R-value of 20 measurements of the QC reference serum sample minus the standard deviation for that respective UCP-LF batch. Units were then determined by dividing individual R-values by the R-value of the cut-off for that respective UCP-LF strip batch, like normalization methods used in anti-PGL-I ELISAs [26-29]. Units  $\geq 1$  are considered seropositive.

**Ethics.** This study was performed according to the Helsinki Declaration (7<sup>th</sup> revision, 64<sup>th</sup> Meeting, 2013, Fortaleza). Ethical approval of the trial protocol was obtained through the 'Comité d'Éthique de la Recherche Biomédicale' (CERBM) in Madagascar and the 'Comité National d'Éthique pour les Sciences de la Vie et de la Santé' (CNESS) in the Comoros. Approval was also obtained from the Institutional Review Board (IRB) of ITM and the Ethics Committee (EC) of the University of Antwerp Hospital. Prior to the start, this trial was

included in the Clinicaltrials.gov public registry (NCT03662022, on September 7<sup>th</sup> 2018, <https://clinicaltrials.gov/ct2/show/NCT03662022>).

**Statistical analysis.** GraphPad Prism version 9.0.1 for Windows (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics for Windows version 29 (IBM Corp, Armonk, NY, USA) were used to perform statistical analysis. Analyses of quantitative anti-PGL-I units were performed using the Mann-Whitney U test for comparisons between two groups and the Kruskal-Wallis test with Dunn's correction for multiple testing for comparisons between three groups. Paired analyses of anti-PGL-I units were conducted using the Wilcoxon signed-rank test for two groups and the Friedman test with Dunn's correction for multiple testing for three groups. To evaluate statistical significance in seroprevalence between study years in cross-sectional analyses, the Chi-squared test was applied. For paired comparisons of seroprevalence between study years, the McNemar's test was used. The analysis on contact data included data from incident cases gathered prior to their leprosy diagnosis, but excluded data collected after their diagnosis.

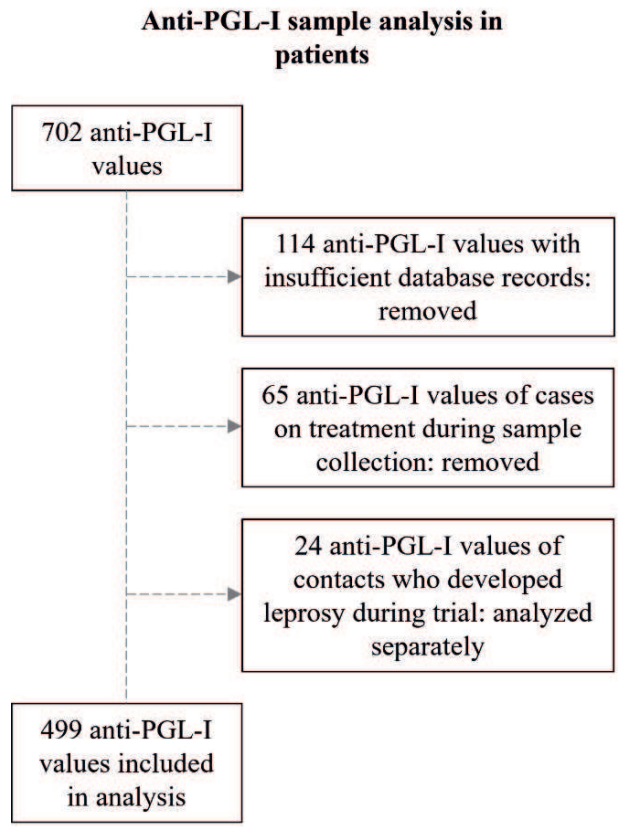
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## Results

To determine seroprevalence and quantitatively monitor anti-PGL-I IgM levels over time in the leprosy endemic areas where active case finding and SDDR-PEP administration were implemented during the PEOPLE trial, FSB samples from index cases ( $n = 702$ ) as well as their household and neighbourhood contacts ( $n > 17,000$ ) were obtained during field screenings.

### Patients

To first assess the field performance of the anti-PGL-I UCP-LFA in leprosy patients by local staff on the Comoros, leprosy patients detected during door-to-door screening or routine leprosy case detection were tested for anti-PGL-I IgM at field sites using the anti-PGL-I UCP-LFA. Out of the 1,369 patients diagnosed between 2019 and 2022 (Comoros: 1,241 on Anjouan, 128 on Mohéli), 702 (51%) anti-PGL-I values were available. Of these 702 anti-PGL-I values, 114 were excluded because they were missing key information such as date of diagnosis or sampling. Additionally, 65 anti-PGL-I values were removed from the analysis as the patients had already started multi-drug therapy (MDT) at the time of first sampling (Fig 1). Anti-PGL-I values of individuals who were initially included as contacts but developed leprosy during the study (incident cases;  $n = 24$ ) were also excluded from this evaluation as they were analysed separately. Of the 499 leprosy patients remaining, 162 (32.5%) were clinically diagnosed with MB and 337 (67.5%) with PB leprosy (Table 1).



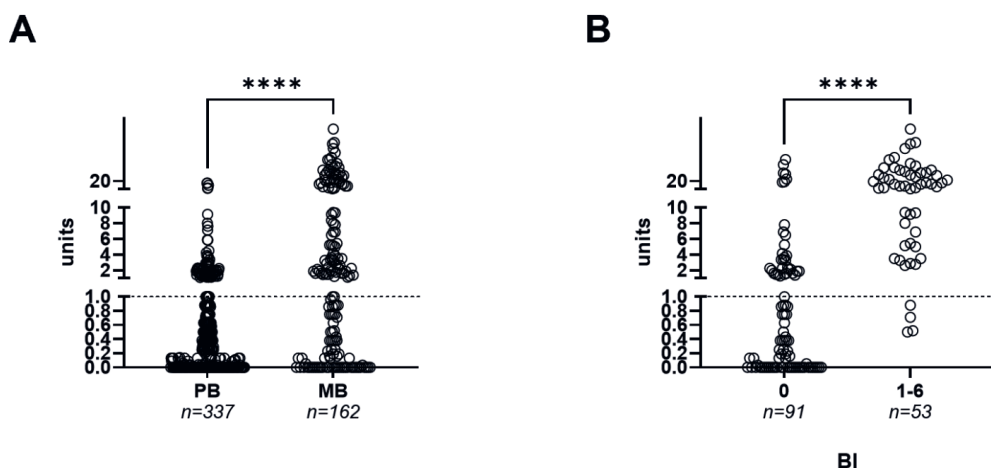
**Fig 1. Schematic representation of data preprocessing of FSB samples included in the analysis of anti-PGL-I values of leprosy patients.** Insufficient database records included missing date of diagnosis or sampling. PGL-I: phenolic glycolipid-I.

**Leprosy patients**

|              |    | <i>n</i>   | %           |
|--------------|----|------------|-------------|
| <b>MB</b>    | BI | <b>162</b> | <b>32.5</b> |
|              | 0  | 91         | 18.2        |
|              | 1  | 17         | 3.4         |
|              | 2  | 13         | 2.6         |
|              | 3  | 8          | 1.6         |
|              | 4  | 7          | 1.4         |
|              | 5  | 4          | 0.8         |
|              | 6  | 4          | 0.8         |
|              | nd | 18         | 3.6         |
|              |    |            |             |
| <b>PB</b>    | BI | <b>337</b> | <b>67.5</b> |
|              | nd | 337        | 67.5        |
|              |    |            |             |
| <b>Total</b> |    | <b>499</b> | <b>100</b>  |

**Table 1. Overview of leprosy patients from the Comoros tested for anti-PGL-I IgM at diagnosis between 2019 and 2022.** Leprosy type and bacterial index are indicated if available. BI: bacterial index; MB: multibacillary; PB: paucibacillary; nd: not determined.

Anti-PGL-I units among patients ranged from 0 to 86.29. 174 out of 499 patients (34.9%) tested seropositive for anti-PGL-I IgM; 80 out of 337 PB patients (23.7%) and 94 out of 162 MB patients (58.0%) (Fig 2). Patients with a BI of 1 and higher had significantly increased anti-PGL-I units compared to those with BI 0 (Mann-Whitney:  $P \leq 0.0001$ ). Importantly, among MB patients testing anti-PGL-I seronegative for which their BI was available ( $n = 61$ ), 93.4% ( $n = 57$ ) had a BI of 0. When only MB patients with a BI of 1 or higher were considered, 49 out of 53 (92.5%) tested seropositive for anti-PGL-I IgM, showing a strong association between anti-PGL-I antibodies and bacterial load. These results confirm the previously reported high sensitivity of the anti-PGL-I UCP-LFA in detecting MB leprosy cases [17].

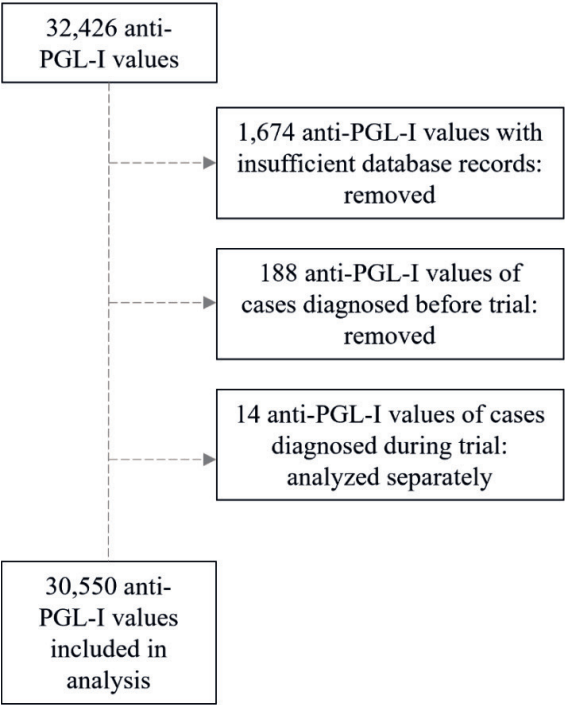


**Fig 2. Anti-PGL-I values (shown in units) in FSB of PB and MB leprosy patients.** A: Anti-PGL-I IgM (y-axis) units among PB ( $n = 337$ ) and MB ( $n = 162$ ) leprosy patients (x-axis). B: Anti-PGL-I IgM (y-axis) units among leprosy patients stratified per bacterial index (BI) group (0 and 1-6; x-axis). Open circles represent anti-PGL-I units for individual samples. Units above the dotted line (units  $\geq 1$ ) are considered seropositive. The Mann-Whitney U test was performed to test for statistical significance (\*\*\*\* $P \leq 0.0001$ ). BI: bacterial index; FSB: fingerstick blood; IgM: immunoglobulin M; MB: multibacillary; PB: paucibacillary; PGL-I: phenolic glycolipid-I.

## Contacts

To evaluate the feasibility of a large-scale field serosurvey among contacts of leprosy patients in the Comoros and Madagascar using the anti-PGL-I UCP-LFA, a total of 32,426 tests were conducted between 2019 and 2022 (Fig 3). A total of 1,674 anti-PGL-I values were excluded due to incomplete/errors in barcodes or missing date of sampling. Additionally, 188 anti-PGL-I values derived from individuals who were identified as (former) patients after their initial inclusion (as contacts), of which no data from prior to - or at timepoint of diagnosis were available, were removed from the dataset. Furthermore, 14 anti-PGL-I values (from after diagnosis) corresponding to contacts who were diagnosed with leprosy during the study period were moved to a separate analysis, as illustrated in S3 Fig. Consequently, 30,550 anti-PGL-I values remained for the analysis.

### Anti-PGL-I sample analysis in contacts

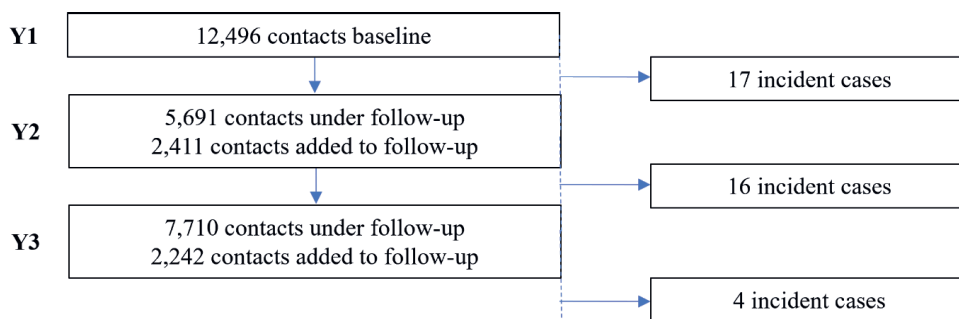


**Fig 3. Schematic representation of data preprocessing of FSB samples included for analyses of anti-PGL-I values of contacts.** Insufficient database records included incomplete/errors in barcodes or missing date of sampling. PGL-I: phenolic glycolipid-I.

**Table 2. Overview of contacts included and tested for anti-PGL-I IgM per study site and year on the Comoros and in Madagascar between 2019 and 2022.**

| Contacts   |                             | Year 1        | Year 2        | Year 3        |
|------------|-----------------------------|---------------|---------------|---------------|
| Anjouan    | Total                       | 9642          | 5547          | 7191          |
|            | Sex                         |               |               |               |
|            | Male                        | 4,201 (43.6%) | 2,192 (39.5%) | 2,893 (40.2%) |
|            | Female                      | 5,441 (56.4%) | 3,355 (60.5%) | 4,298 (59.8%) |
| Mohéli     | Median age (range in years) | 17 (2-100)    | 15 (2-100)    | 15 (2-100)    |
|            | Total                       | 2300          | 1984          | 2071          |
|            | Sex                         |               |               |               |
|            | Male                        | 926 (40.3%)   | 779 (39.3%)   | 787 (38.0%)   |
| Madagascar | Female                      | 1,374 (59.7%) | 1,205 (60.7%) | 1,284 (62.0%) |
|            | Median age (range in years) | 20 (2-93)     | 19 (2-92)     | 18 (2-91)     |
|            | Total                       | 554           | 571           | 690           |
|            | Sex                         |               |               |               |
| All sites  | Male                        | 252 (45.5%)   | 278 (48.7%)   | 330 (47.8%)   |
|            | Female                      | 302 (54.5%)   | 293 (51.3%)   | 360 (52.2%)   |
|            | Median age (range)          | 18 (2-92)     | 16 (2-90)     | 17 (2-90)     |
| Total      |                             | 12,496        | 8,102         | 9,952         |

A total of 12,496 individuals were included as contacts and tested for anti-PGL-I IgM in year 1: 9,642 from Anjouan, 2,300 from Mohéli and 554 from Madagascar (Table 2). In year 2 of the study, 8,102 contacts were tested for anti-PGL-I IgM, of whom 5,691 (70.2%) already had been tested in year 1 (Fig 4). In year 3 of the study, 9,952 contacts were tested and 7,710 (77.5%) of those had already been tested in year 1 and/or year 2.

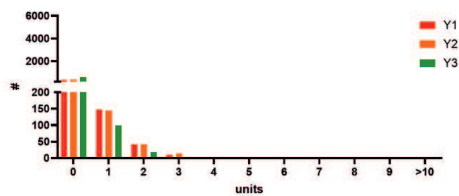
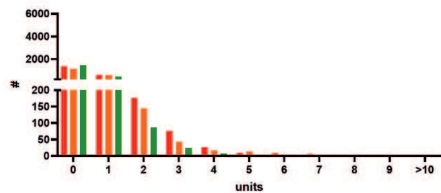
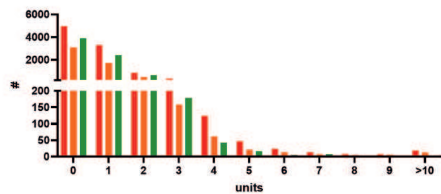


**Fig 4. Flow diagram showing follow-up of contacts per study year.** Patients with leprosy diagnosed after the first screening round were classified as “incident cases” if already under contact-follow-up of the PEOPLE trial at time of their diagnosis. A total of 37 incident cases were diagnosed among individuals included for anti-PGL-I testing (PB n = 25; MB n = 12; S1 Table).

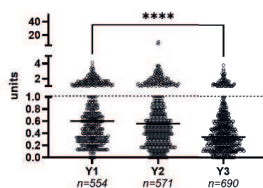
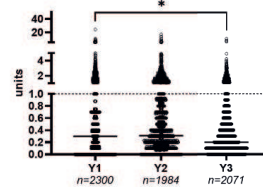
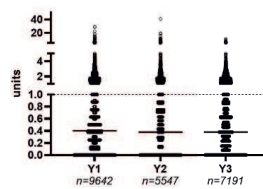
37 contacts of whom anti-PGL-I data were available were diagnosed with leprosy during the study period; 35 from Anjouan and 2 from Mohéli (Fig 4). These individuals were first included as contacts (showing no signs of leprosy) and at some point during the study period – after baseline inclusion – diagnosed with leprosy (incident cases; S1 Table). Seventeen contacts were diagnosed with leprosy between sample collections in year 1 and 2, whereas between sample collections in year 2 and 3, 16 contacts were diagnosed with leprosy. Four more contacts developed leprosy after the 3<sup>rd</sup> year of sample collection (up to November 2022). In total, 12 of these 37 (32.4%) incident cases were classified as MB leprosy and 25 (67.6%) as PB leprosy.

Anti-PGL-I units among contacts ranged from 0 to 40.86. Median anti-PGL-I units were 0.38 (IQR 0-0.90) and 0.30 (IQR 0.10-0.70) for Anjouan and Mohéli respectively, where the whole village was tested, and 0.47 (IQR 0.23-0.86) for Madagascar where only contacts within a 100m radius of new cases were tested. All three distributions were strongly skewed to the right. In addition, the majority of individuals tested seronegative (Fig 5A). Median anti-PGL-I units were highest among individuals aged 11-40 (S1 Fig), in line with previous studies [30, 31].

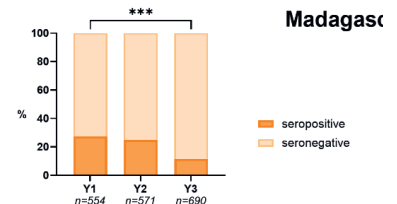
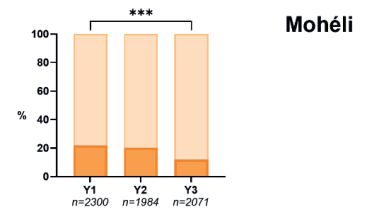
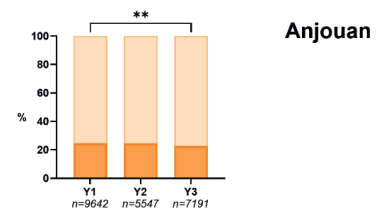
A



B



C



**Fig 5. Anti-PGL-I values (shown in units) in FSB among contacts on Anjouan, Mohéli and Madagascar.**

A: Distribution of anti-PGL-I IgM units among contacts on Anjouan (upper panel), Mohéli (middle panel) and Madagascar (lower panel). The number (#) of tests (y-axis) per unit range (0-0.99; 1-1.99; etc.) are indicated on the x-axis. B: Anti-PGL-I IgM (y-axis) units of contacts on Anjouan, Mohéli and Madagascar per study year (x-axis). Open circles represent anti-PGL-I units for individual samples. Units above dotted line (units  $\geq 1$ ) are considered seropositive. Kruskal-Wallis tests were performed to test for statistical significance between anti-PGL-I units at the beginning and end of the study (\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , \*\*\*\* $P \leq 0.0001$ ). C: Percentages (%) of contacts testing seropositive (y-axis) for anti-PGL-I IgM depicted per study year (x-axis). Chi-squared tests were performed to test for statistical significance between percentages at the beginning and end of the study (\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ ). FSB: fingerstick blood; IgM: immunoglobulin M; PGL-I: phenolic glycolipid-I; Y1: year 1; Y2: year 2; Y3: year 3.

To study the effect of the activities in the PEOPLE trial on *M. leprae* infection in the study population over time, analysis between anti-PGL-I IgM levels at the beginning (year 1) and the end (year 3) of the study was performed. On Anjouan, no significant difference in median antibody levels between year 1 and 3 was observed (Fig 5B; Kruskal-Wallis:  $P > 0.999$ ), whereas seroprevalence for anti-PGL-I IgM significantly decreased from 24.7% in year 1 to 22.8% in year 3 (Fig 5C; Chi-squared:  $P < 0.01$ ). On Mohéli, seroprevalence declined from

21.9% in year 1 to 12.0% in year 3 (Chi-squared:  $P < 0.001$ ) and median antibody levels also showed a significantly decreasing trend over the years (Kruskal-Wallis:  $P < 0.05$ ). In Madagascar, anti-PGL-I seroprevalence decreased from 27.4% (year 1) to 11.6% (year 3; Chi-squared:  $P < 0.001$ ), and median antibodies were significantly lower (Kruskal-Wallis:  $P < 0.0001$ ). Overall, the implemented interventions (including treatment of index cases) appeared to have a reducing impact on the population's infection levels.

## Children

To assess recent *M. leprae* transmission in the community, a sub-analysis on anti-PGL-I data from children (age 2-10 years) was conducted. Seroprevalence in young children reflects recent *M. leprae* infection and may thus be used to monitor transmission in an area [32-34]. Although median antibody levels significantly increased (Kruskal-Wallis:  $P < 0.01$ ; S2A Fig) over the years in children up to ten years old on Anjouan (the island reporting the highest leprosy incidence in the preceding years), seroprevalence in children remained around 20.0% (Chi-squared:  $P = 0.492$ ; S2B Fig). In contrast, median anti-PGL-I units in children on Mohéli and Madagascar were significantly decreased in year 3 compared to year 1 and/or 2 (Kruskal-Wallis:  $P < 0.0001$ ) and seroprevalence decreased from 25.5% to 13.7% and 22.7% to 9.4% (Chi-squared: both  $P < 0.001$ ), respectively.

## Incident leprosy cases

Anti-PGL-I levels before and at diagnosis of leprosy were compared in more detail: six out of 12 (50.0%) incident MB cases tested seropositive for anti-PGL-I IgM at some point during the study (S3 Fig); three incident cases who initially tested seronegative, were seropositive for anti-PGL-I IgM at the time of diagnosis. Another three individuals already tested seropositive before diagnosis. Out of the six MB patients testing seronegative, four (66.7%) had a BI of 0 and for the other two (33.3%) the BI was not available.

For incident PB cases, 20 out of 25 (80.0%) tested seronegative for anti-PGL-I IgM throughout the study, whereas five (20.0%) tested seropositive at least once. Although not significant (Mann-Whitney U:  $P = 0.08$ ), higher units of anti-PGL-I were found for incident MB patients ( $n = 7$ ) compared to incident PB patients ( $n = 14$ ; S4 Fig). These results again confirm good performance of the test among BI-positive individuals.

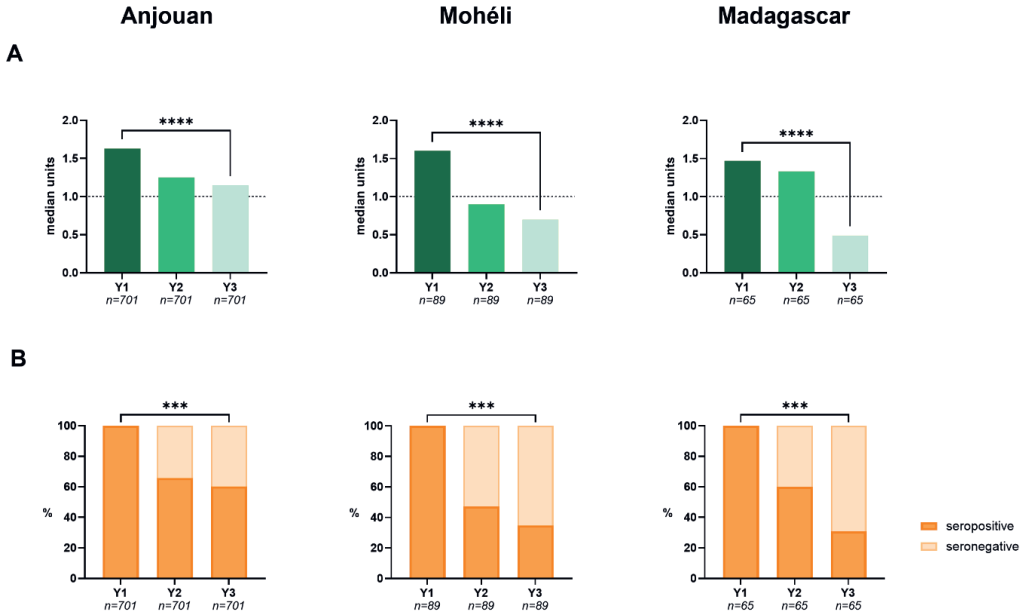
Five out of 37 (13.5%) incident cases had received SDDR-PEP prior to leprosy diagnosis, of whom four tested seropositive (S3 Fig). Among them, three (60.0%) were diagnosed with MB and two (40.0%) with PB leprosy.

## Longitudinal anti-PGL-I IgM and SDDR-PEP among contacts

When analysing anti-PGL-I data longitudinally to study the effects of the implemented interventions on an individual level (irrespective of SDDR-PEP intake), contacts testing seropositive for anti-PGL-I IgM in year 1, showed significantly decreased antibody levels in year 3 (Friedman:  $P < 0.0001$ ) and seroprevalence (McNemar:  $P < 0.001$ ) (Fig 6 and S5 Fig)



in all three study sites. While on Mohéli and Madagascar median anti-PGL-I units dropped below 1 (anti-PGL-I seronegative) in year 3, the median unit on Anjouan remained above 1.



**Fig 6. Longitudinal anti-PGL-I values (shown in units) in FSB of contacts on Anjouan, Mohéli and Madagascar testing seropositive in year 1.** A: Paired median anti-PGL-I IgM units of seropositive contacts (Y1; y-axis) on Anjouan (left panel), Mohéli (middle panel) and Madagascar (right panel) are depicted per study year (x-axis). Anti-PGL-I units above dotted line (units  $\geq 1$ ) are considered seropositive. Friedman tests were performed to assess statistical significance between anti-PGL-I units at the beginning and end of the study (\*\*\*\* $P \leq 0.0001$ ). B: Paired percentages (%) of contacts testing seropositive (y-axis) for anti-PGL-I IgM depicted per study year (x-axis). McNemar tests were performed to assess statistical significance between percentages at the beginning and end of the study (\*\*\* $P \leq 0.001$ ). FSB: fingerstick blood; IgM: immunoglobulin M; PGL-I: phenolic glycolipid-I; Y1: year 1; Y2: year 2; Y3: year 3.

As the prior analysis did not include individual SDDR-PEP intake, we next performed a paired analysis stratified by SDDR-PEP recipients and non-recipients. This analysis showed significant reductions in paired anti-PGL-I units in year 3 when compared to year 1 among seropositive contacts who received SDDR-PEP (S6A Fig; Wilcoxon: Anjouan and Madagascar  $P < 0.0001$ , Mohéli  $P \leq 0.01$ ). However, similar results were found among contacts who did not receive SDDR/PEP (Wilcoxon: Anjouan and Mohéli  $P < 0.0001$ , Madagascar  $P < 0.01$ ). Amongst contacts seropositive in year 1, seroprevalence declined (from 100%) to 25.4% on Madagascar, 61.1% on Mohéli, and 61.5% on Anjouan in year 3 among PEP-recipients (McNemar: Anjouan and Madagascar  $P < 0.001$ , Mohéli  $P < 0.05$ ). Among non-SDDR-PEP recipients, seroprevalence declined to 35.0%, 32.6%, and 62.6% on Madagascar, Mohéli, and Anjouan, respectively (McNemar:  $P < 0.001$ ; S6B Fig).

## Discussion

Despite the availability of MDT, around 200,000 new persons are affected by leprosy each year [35], highlighting the need for innovations in leprosy control efforts [36]. Given that household contacts of untreated leprosy patients are at the greatest risk of infection [37], and that previous studies have demonstrated the effectiveness and feasibility of SDR-PEP, the WHO recommends the screening of close contacts of new leprosy patients combined with administration of SDR-PEP to prevent disease progression in potentially infected individuals [9].

This study describes the largest field application of the anti-PGL-I UCP-LFA to date ( $n = 33,128$  tests), using humoral immunity in the population (anti-*M. leprae*-specific seroprevalence), to assess the impact of PEP implementation including repeated door-to-door screening on population infection levels, rather than merely evaluating the number of newly detected cases many years after PEP. While the primary objective of the PEOPLE trial was to explore the effects of various SDDR-PEP administration methods among contacts of leprosy patients across the Comoros and Madagascar, the focus of the present study was on the utility of the anti-PGL-I UCP-LFA in two use cases within this context. Our findings underscore the significant value of this quantitative field-friendly test in (1) diagnosing (MB) leprosy and (2) monitoring infection trends in populations undergoing leprosy control interventions.

The anti-PGL-I UCP-LFA exhibited excellent performance in detecting leprosy patients with a positive BI, with 92.5% (49/53) testing seropositive. As was also shown previously [17, 18], a higher bacterial load was associated with higher anti-PGL-I units. Additionally, for 37 contacts who developed leprosy during the PEOPLE trial (incident cases), all four incident MB cases with a positive BI, tested seropositive at the time of diagnosis. These findings are consistent with previous studies and support the use of anti-PGL-I UCP-LFA, alongside clinical evaluation, as an adjunctive diagnostic tool for MB, offering a less invasive alternative to slit-skin smears or biopsies through FSB sampling [17-19, 38]. Also in line with previous reports, the anti-PGL-I UCP-LFA demonstrated lower sensitivity in PB patients, of whom 23.7% (80/337) tested seropositive. This result reflects the challenge of detecting *M. leprae* infection in individuals with a negative or low BI using antibody-based tests, as these patients primarily mount a cellular immune response, and antibody levels - while sometimes detectable - are generally lower due to limited bacterial load and more efficient immune control [39]. Therefore, there is a need for complementary biomarkers for improved detection in this patient group [24, 40, 41].

Despite the fact that six out of 37 contacts (16.2%;  $n = 3$  MB;  $n = 3$  PB) who developed leprosy during the trial already tested seropositive (anti-PGL-I units 1 – 5) approximately one year (or more) before diagnosis, previous reports have shown that anti-PGL-I seropositivity is not predictive for leprosy disease [42-44]. In line with this, we found that 83.8% ( $n = 31$ ) of incident cases tested anti-PGL-I seronegative one or two years prior to their diagnosis. However, as a result of a serosurvey conducted in India, a nine-year-old child who tested seropositive for anti-PGL-I IgM during a door-to-door serosurvey in Bihar, was subsequently diagnosed with PB leprosy during follow-up one year after the serosurvey.

The opportunity for early detection of leprosy, including PB, through follow-up of seropositive individuals, underscores the additional benefit of serosurveys [45].

At the start of the study (year 1, before SDDR-PEP implementation), seroprevalence among contacts ranged from 21.9% to 27.4% across the three study sites. By year 3, seroprevalence on Anjouan was not very different from year 1 (8% reduction), while in Madagascar and Mohéli substantial reductions were observed (45% and 58%, respectively), suggesting a beneficial impact of active case finding and SDDR-PEP on infection rates. Despite decreased seroprevalence on Anjouan, no significant drop in anti-PGL-I levels was observed, possibly reflecting differences in transmission dynamics or intervention efficacy, potentially influenced by population density and leprosy incidence, both notably lower in Madagascar and Mohéli in comparison to Anjouan [19, 46].

Still, in all study sites, paired analysis of seropositive individuals showed reduced anti-PGL-I levels over time, regardless of SDDR-PEP intake. Similarly, a study in South Sumatra, Indonesia, reported reduced anti-PGL-I IgM among seropositive contacts of leprosy patients two years after SDR-PEP [47]. However, our data regarding individual anti-PGL-I levels before and after PEP, suggest that the combination of active case finding followed by treatment of new cases and PEP - rather than PEP alone - play a crucial role in reducing *M. leprae* transmission and that this could successfully be measured using our anti-PGL-I UCP-LFA. In the PEOPLE trial, household contacts and anyone living within 100m of an index case and who tested seropositive for anti-PGL-I received PEP. Including community controls not receiving PEP in future studies could be informative to assess only the effects of PEP on anti-PGL-I IgM levels among contacts.

Notably, five individuals who received SDDR-PEP before diagnosis still progressed to clinical disease. Incorporating additional immunological markers beyond anti-PGL-I may enhance evaluation of prophylactic interventions, as is currently being investigated in the INDIGO#2 trial in Bangladesh (NCT06222372) [48].

With numerous initiatives being implemented to combat leprosy, including the administration of SDR-PEP [49-51], it is important to investigate the (long-term) effects of such prophylactic measures on the transmission of *M. leprae* and globally compare the impact of PEP across areas with different levels of endemicity. As infection in young children per definition reflects recent transmission in a population [32-34], testing should be focused on young children to assess seroprevalence within a community prior to and following the implementation of interventions. An analysis of the data for children alone showed that seroprevalence remained stable on Anjouan, whereas it significantly decreased in the other two study sites after intervention implementation, again indicating varied effects on recent transmission as discussed earlier. While some countries are progressing towards or have already successfully achieved the elimination of leprosy as determined by the number of new child cases [52, 53], it remains essential to maintain robust surveillance systems [54]. In this context, the introduction of a serological rapid test that allows large-scale field-testing could represent a straightforward, yet effective approach to monitor transmission of *M. leprae*.

A key requirement for serosurveillance in leprosy endemic populations is the implementation of a standardized, quantitative assay that is user-friendly, allowing large-scale application in low resource field settings. When the PEOPLE trial began in 2019, a pre-final version of the

current anti-PGL-I UCP-LFA format was available, which consisted of LF strips and vertical flow. In 2024, the LUMC immunodiagnostic research group introduced the PGL-I QURapid, which, besides having the advances of the UCP-LFA format also includes a biodegradable cassette [17]. Unlike the methodology used in this study (2019-2023), this new version including the biodegradable cassette, will eliminate the need for a 96-well plate, allowing the diluted FSB sample to be directly applied to the test cassette, making it significantly easier for field staff to use in future studies. Moreover, pre-printed barcode stickers can be applied to the cassette, reducing the risks occurring during writing of participant IDs.

## Conclusions

Our findings confirm the high sensitivity of the anti-PGL-I UCP-LFA in detecting BI-positive individuals. Moreover, the data of the serological analysis of the effect of SDDR-PEP in the Comoros and Madagascar show that concurrent with active case finding and PEP implementation, anti-PGL-I seroprevalence in the population, representing the number of individuals with past or present infection, significantly decreased in all three study sites. The seroprevalence decreased in both contacts who did and did not receive SDDR-PEP, albeit not randomized, suggesting that active case finding, with treatment initiation rapidly decreasing infectiousness, is probably at least as important in reducing transmission, as is PEP. Simultaneously, *M. leprae*-specific antibody levels, representing the extent of infection, significantly decreased over the years in the populations of Mohéli and Madagascar. Thus, this study highlights the suitability of the low-invasive, field-friendly anti-PGL-I UCP-LFA, for large-scale implementation in leprosy control programs [17]. By enabling the detection of past or present infection with *M. leprae* in a quantitative fashion this test can play a vital role in monitoring the effectiveness on transmission of control interventions, thus providing an essential tool for the global effort to reduce the burden of leprosy.

## Data availability

The data supporting the findings of this study are retained at the Leiden University Medical Center (LUMC), Leiden, and are not openly accessible due to ethical and privacy considerations. However, data may be made available upon reasonable request. Interested researchers should submit a motivated and written request to Mycobacterial Research, Leiden University Center of Infectious Diseases, at: [Secretariaat.lucid-i@lumc.nl](mailto:Secretariaat.lucid-i@lumc.nl).

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## Declaration of interests

The authors have declared that no competing interests exist.

## References

1. Scollard, D.M., et al., *The continuing challenges of leprosy*. Clin Microbiol Rev, 2006. **19**(2): p. 338–81.
2. Deps, P. and S.M. Collin, *Mycobacterium lepromatosis as a Second Agent of Hansen's Disease*. Front Microbiol, 2021. **12**: p. 698588.
3. WHO. *Leprosy*. 2023; Available from: <https://www.who.int/news-room/fact-sheets/detail/leprosy>.
4. WHO Africa. *Leprosy*. 2022; Available from: <https://www.afro.who.int/health-topics/leprosy#:~:text=Leprosy%20is%20curable%20with%20Multiple,subject%20to%20discrimination%20and%20stigmatization>.
5. Bakker, M.I., et al., *Risk factors for developing leprosy--a population-based cohort study in Indonesia*. Lepr Rev, 2006. **77**(1): p. 48–61.
6. Goulart, I.M., et al., *Risk and protective factors for leprosy development determined by epidemiological surveillance of household contacts*. Clin Vaccine Immunol, 2008. **15**(1): p. 101–5.
7. Sales, A.M., et al., *Leprosy among Patient Contacts: A Multilevel Study of Risk Factors*. Plos Neglected Tropical Diseases, 2011. **5**(3).
8. Grijnsen, M.L., et al., *Leprosy*. Nat Rev Dis Primers, 2024. **10**(1): p. 90.
9. WHO, *Guidelines for the diagnosis, treatment and prevention of leprosy*, in 2017. 2018, World Health Organization, Regional Office for South-East Asia: New Delhi.
10. Richardus, J.H., et al., *Leprosy post-exposure prophylaxis with single-dose rifampicin (LPEP): an international feasibility programme*. Lancet Glob Health, 2021. **9**(1): p. e81–e90.
11. Moet, F.J., et al., *Effectiveness of single dose rifampicin in preventing leprosy in close contacts of patients with newly diagnosed leprosy: cluster randomised controlled trial*. BMJ, 2008. **336**(7647): p. 761–4.
12. Bakker, M.I., et al., *Prevention of leprosy using rifampicin as chemoprophylaxis*. Am J Trop Med Hyg, 2005. **72**(4): p. 443–8. *Large-scale field application of M. leprae infection test*
13. Moraes, M.O. and N.C. Duppre, *Leprosy post-exposure prophylaxis: innovation and precision public health*. Lancet Glob Health, 2021. **9**(1): p. e8–e9.
14. Ortuno-Gutierrez, N., et al., *Protocol, rationale and design of PEOPLE (Post ExpOsure Prophylaxis for LEprosy in the Comoros and Madagascar): a cluster randomized trial on effectiveness of different modalities of implementation of post-exposure prophylaxis of leprosy contacts*. BMC Infect Dis, 2019. **19**(1): p. 1033.
15. Hasker, E., et al., *Post-exposure prophylaxis in leprosy (PEOPLE): a cluster randomised trial*. Lancet Global Health, 2024. **12**(6): p. e1017–e1026.
16. Niedbala, R.S., et al., *Detection of analytes by immunoassay using up-converting phosphor technology*. Anal Biochem, 2001. **293**(1): p. 22–30.
17. Pierneef, L., et al., *Rapid test for Mycobacterium leprae infection: a practical tool for leprosy*. Infect Dis Poverty, 2024. **13**(1): p. 88.
18. van Hooij, A., et al., *Field-friendly serological tests for determination of M. leprae-specific antibodies*. Sci Rep, 2017. **7**(1): p. 8868.
19. Braet, S.M., et al., *Minimally invasive sampling to identify leprosy patients with a high bacterial burden in the Union of the Comoros*. PLoS Negl Trop Dis, 2021. **15**(11): p. e0009924.
20. WHO, *Weekly epidemiological record. Global leprosy update, 2018: moving towards a leprosy-free world*. 2019, WHO: Geneva.
21. Boeree, M.J., et al., *A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis*. Am J Respir Crit Care Med, 2015. **191**(9): p. 1058–65.
22. Diacon, A.H., et al., *Early bactericidal activity of high-dose rifampin in patients with pulmonary tuberculosis evidenced by positive sputum smears*. Antimicrob Agents Chemother, 2007. **51**(8): p. 2994–6.
23. van Hooij, A., et al., *Quantitative lateral flow strip assays as User-Friendly Tools To Detect Biomarker Profiles For Leprosy*. Sci Rep, 2016. **6**: p. 34260.

24. van Hooij, A., et al., *Evaluation of Immunodiagnostic Tests for Leprosy in Brazil, China and Ethiopia*. Scientific Reports, 2018. **8**.
25. van Dijk, J.H.M., et al., *Synthetic Phenolic Glycolipids for Application in Diagnostic Tests for Leprosy*. Chembiochem, 2021. **22**(8): p. 1487–1493.
26. Lima, F.R., et al., *Serological testing for Hansen's disease diagnosis: Clinical significance and performance of IgA, IgM, and IgG antibodies against Mce1A protein*. Front Med (Lausanne), 2023. **10**: p. 1048759.
27. Frade, M.A., et al., *Unexpectedly high leprosy seroprevalence detected using a random surveillance strategy in midwestern Brazil: A comparison of ELISA and a rapid diagnostic test*. PLoS Negl Trop Dis, 2017. **11**(2): p. e0005375.
28. Longoni, S.S., et al., *ELISA Test Based on the Phenolic Glycolipid-I (PGL-I) of Mycobacterium leprae: A Reality of a Laboratory from a Non-Endemic Country*. Pathogens, 2022. **11**(8).
29. Lima, M.I.S., et al., *Biotechnological and Immunological Platforms Based on PGL-I Carbohydrate-Like Peptide of Mycobacterium leprae for Antibodies Detection Among Leprosy Clinical Forms*. Front Microbiol, 2020. **11**: p. 429.
30. Fine, P.E., et al., *Seroepidemiological studies of leprosy in northern Malawi based on an enzyme-linked immunosorbent assay using synthetic glycoconjugate antigen*. Int J Lepr Other Mycobact Dis, 1988. **56**(2): p. 243–54.
31. Ulrich, M., et al., *IgM antibodies to native phenolic glycolipid-I in contacts of leprosy patients in Venezuela: epidemiological observations and a prospective study of the risk of leprosy*. Int J Lepr Other Mycobact Dis, 1991. **59**(3): p. 405–15.
32. Pierneef, L., et al., *Detection of anti-M. leprae antibodies in children in leprosy-endemic areas: A systematic review*. PLoS Negl Trop Dis, 2021. **15**(8): p. e0009667.
33. WHO, *Global leprosy (Hansen disease) update, 2021: moving towards interruption of transmission*. 2022.
34. Zhou, Z., Pierneef, L., van Hooij, A., Geluk, A., *Detection of anti-M. leprae antibodies in healthy children in China: A systematic review of Chinese literature*. Front. Trop. Dis., 2022. **3**.
35. WHO, *Global leprosy (Hansen disease) update, 2023: Elimination of leprosy disease is possible – Time to act!*, in *Weekly epidemiological record*. 2024, WHO.
36. Steinmann, P., et al., *The Leprosy Post-Exposure Prophylaxis (LPEP) programme: update and interim analysis*. Leprosy Review, 2018. **89**(2): p. 102–116.
37. Walker, S.L. and D.N. Lockwood, *The clinical and immunological features of leprosy*. Br Med Bull, 2006. **77-78**: p. 103–21.
38. Tio-Coma, M., et al., *Genomic Characterization of Mycobacterium leprae to Explore Transmission Patterns Identifies New Subtype in Bangladesh*. Front Microbiol, 2020. **11**: p. 1220. Large-scale field application of *M. leprae* infection test
39. Spencer, J.S., et al., *Identification of serological biomarkers of infection, disease progression and treatment efficacy for leprosy*. Mem Inst Oswaldo Cruz, 2012. **107 Suppl 1**: p. 79–89.
40. van Hooij, A., et al., *Application of new host biomarker profiles in quantitative point-of-care tests facilitates leprosy diagnosis in the field*. EBioMedicine, 2019. **47**: p. 301–308.
41. van Hooij, A., et al., *Prototype multi-biomarker test for point-of-care leprosy diagnostics*. iScience, 2021. **24**(1): p. 102006.
42. Richardus, R.A., et al., *Longitudinal assessment of anti-PGL-I serology in contacts of leprosy patients in Bangladesh*. PLoS Negl Trop Dis, 2017. **11**(12): p. e0006083.
43. Douglas, J.T., et al., *Prospective study of serological conversion as a risk factor for development of leprosy among household contacts*. Clin Diagn Lab Immunol, 2004. **11**(5): p. 897–900.
44. Duppre, N.C., et al., *Impact of PGL-I seropositivity on the protective effect of BCG vaccination among leprosy contacts: a cohort study*. PLoS Negl Trop Dis, 2012. **6**(6): p. e1711.
45. Pierneef, L., et al., *Field-friendly anti-PGL-I serosurvey in children to monitor Mycobacterium leprae transmission in Bihar, India*. Front Med (Lausanne), 2023. **10**: p. 1260375.
46. WHO, *ANNEX 4 TB burden estimates, notifications and treatment outcomes for individual countries and territories, WHO regions and the world.*, in *Global Tuberculosis Report 2018*. 2018, World Health Organization: Geneva.
47. Jusuf Fantoni, O.J., et al., *Comparison of antiphenolic glycolipid-1 antibody levels in seropositive contacts of leprosy after 2 years of single-dose rifampicin as postexposure prophylaxis*. Int J Mycobacteriol, 2023. **12**(4): p. 399–406.
48. Geluk, A. *Novel Interventions and Diagnostic Tests for Leprosy (INDIGO#2)*. 2024; Available from: <https://clinicaltrials.gov/study/NCT06222372>.
49. Global Partnership for Zero Leprosy | ITC, *Factsheet on SDR-PEP: The latest research on single-dose rifampicin post-exposure prophylaxis*. 2022.
50. Schoenmakers, A., et al., *PEP4LEP study protocol: integrated skin screening and SDR-PEP administration for leprosy prevention: comparing the effectiveness and feasibility of a community-based*

- intervention to a health centre-based intervention in Ethiopia, Mozambique and Tanzania*. Bmj Open, 2021. **11**(8).
51. Hinders, D.C., et al., *The PEP++ study protocol: a cluster-randomised controlled trial on the effectiveness of an enhanced regimen of post-exposure prophylaxis for close contacts of persons affected by leprosy to prevent disease transmission*. BMC Infect Dis, 2024. **24**(1): p. 226.
52. WHO, *Interruption of transmission and elimination of leprosy disease - Technical guidance*. 2023: India.
53. van Brakel, W.H., et al., *Strategies for leprosy services in low-endemic areas and countries*. Leprosy Review, 2024. **95**(3).
54. WHO *Jordan becomes first country to receive WHO verification for eliminating leprosy*. 2024.

## Supplemental information

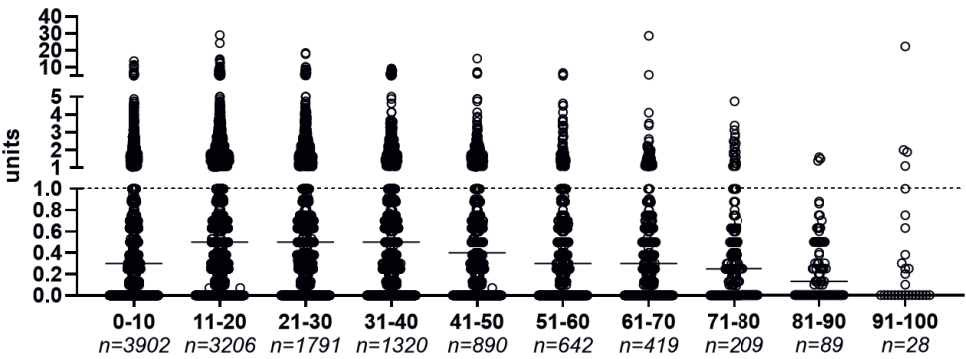
**S1 Table. Overview of contacts tested for anti-PGL-I prior to leprosy diagnosis.**

|         |                             |  |            |
|---------|-----------------------------|--|------------|
| Anjouan | total                       |  | 35         |
|         | sex                         |  |            |
|         | male                        |  | 20 (57.1%) |
|         | female                      |  | 15 (42.9%) |
|         | median age (range in years) |  | 16 (3-78)  |
|         | type of leprosy             |  |            |
|         | MB                          |  |            |
| Mohéli  | PB                          |  | 10 (28.6%) |
|         |                             |  | 25 (71.4%) |
|         | total                       |  | 2          |
|         | sex                         |  |            |
|         | male                        |  | 2 (100%)   |
|         | female                      |  | 0 (0%)     |
|         | median age (range in years) |  | 22 (11-33) |
| all     | type of leprosy             |  |            |
|         | MB                          |  |            |
|         | PB                          |  | 2 (100%)   |
|         |                             |  | 0 (0%)     |
| total   |                             |  | 37*        |

\*No incident cases in Madagascar were tested for anti-PGL-I IgM.

MB: multibacillary; PB: paucibacillary.

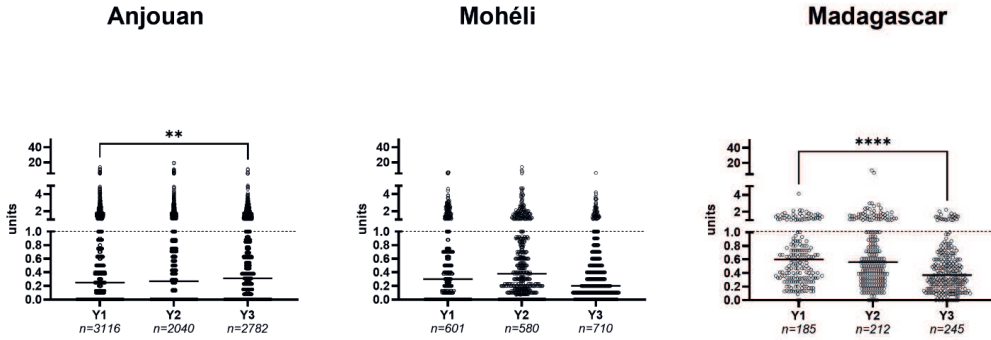




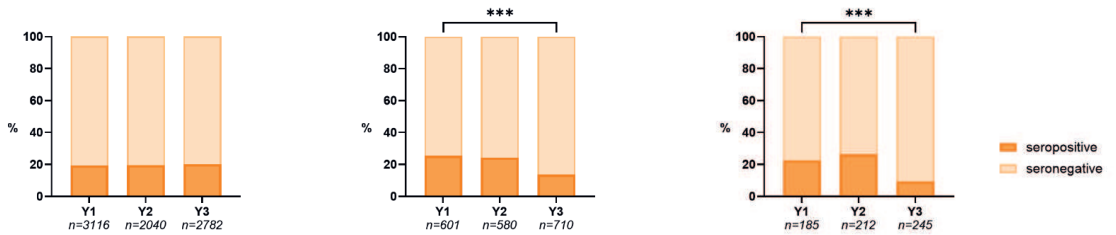
| Dunn's multiple comparisons test | Significance | P Value |
|----------------------------------|--------------|---------|
| 0-10 vs. 11-20                   | ****         | <0,0001 |
| 0-10 vs. 21-30                   | ****         | <0,0001 |
| 0-10 vs. 31-40                   | ****         | <0,0001 |
| 0-10 vs. 41-50                   | **           | 0,0057  |
| 0-10 vs. 81-90                   | **           | 0,0022  |
| 11-20 vs. 31-40                  | *            | 0,0304  |
| 11-20 vs. 41-50                  | ****         | <0,0001 |
| 11-20 vs. 51-60                  | ****         | <0,0001 |
| 11-20 vs. 61-70                  | ****         | <0,0001 |
| 11-20 vs. 71-80                  | ****         | <0,0001 |
| 11-20 vs. 81-90                  | ****         | <0,0001 |
| 11-20 vs. 91-100                 | *            | 0,0151  |
| 21-30 vs. 41-50                  | **           | 0,0033  |
| 21-30 vs. 51-60                  | ****         | <0,0001 |
| 21-30 vs. 61-70                  | ****         | <0,0001 |
| 21-30 vs. 71-80                  | ****         | <0,0001 |
| 21-30 vs. 81-90                  | ****         | <0,0001 |
| 21-30 vs. 91-100                 | *            | 0,0305  |
| 31-40 vs. 51-60                  | ****         | <0,0001 |
| 31-40 vs. 61-70                  | ****         | <0,0001 |
| 31-40 vs. 71-80                  | ****         | <0,0001 |
| 31-40 vs. 81-90                  | ****         | <0,0001 |
| 41-50 vs. 51-60                  | **           | 0,0066  |
| 41-50 vs. 61-70                  | *            | 0,0153  |
| 41-50 vs. 71-80                  | **           | 0,0053  |
| 41-50 vs. 81-90                  | ****         | <0,0001 |
| 51-60 vs. 81-90                  | *            | 0,0343  |

**S1 Fig. Anti-PGL-I values (shown in units) in FSB samples of contacts stratified by age.** Open circles represent anti-PGL-I IgM units of individual samples. Units above dotted line (units  $\geq 1$ ) are considered seropositive. Kruskal-Wallis tests with Dunn's correction for multiple testing were performed to assess statistical significance between anti-PGL-I units per age group.

A



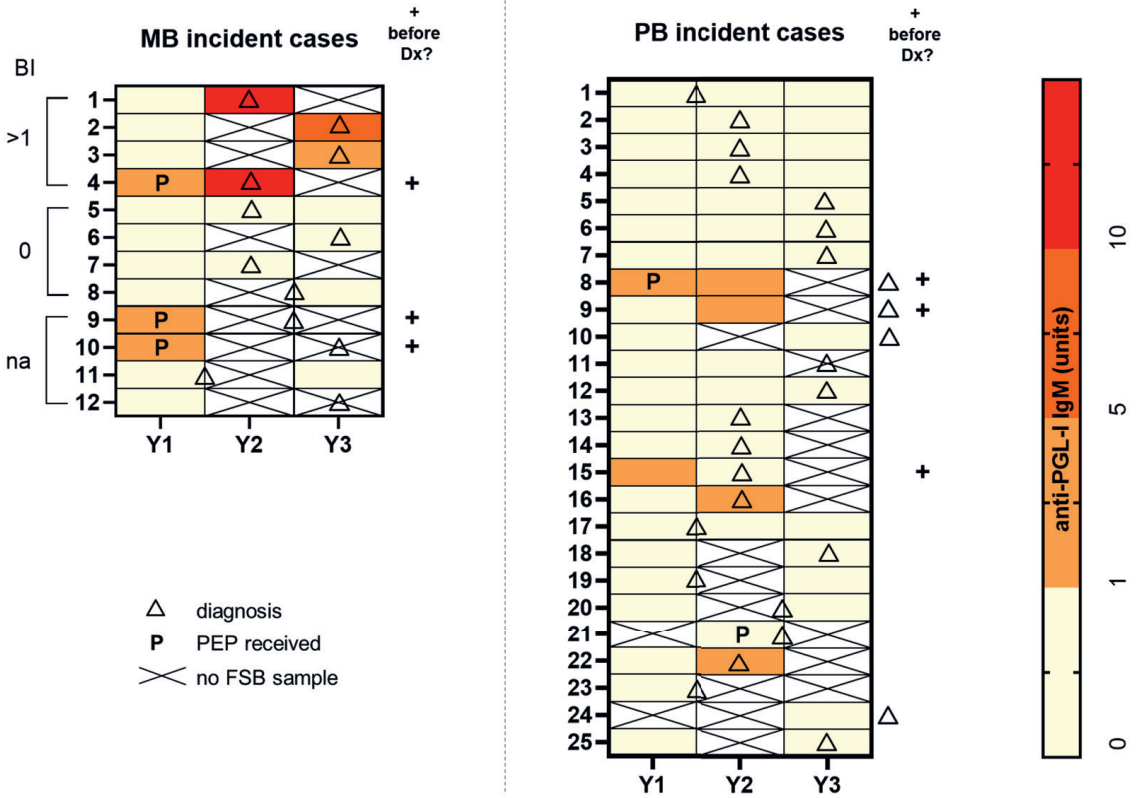
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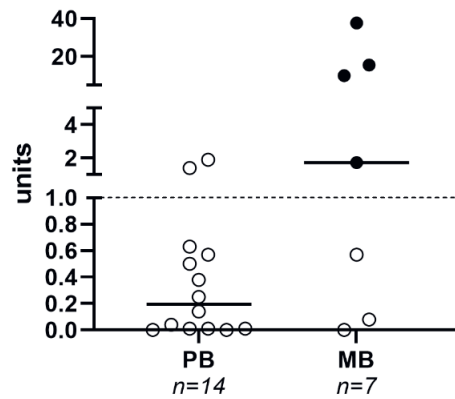
6

**S2 Fig. Anti-PGL-I values (shown in units) in FSB samples of child contacts on Anjouan, Mohéli and Madagascar.** A: Anti-PGL-I IgM (y-axis) units of children (2-10 years of age) who are contacts of leprosy patients on Anjouan (left panel), Mohéli (middle panel) and Madagascar (right panel) per study year (x-axis). Open circles represent anti-PGL-I units of individual samples. Units above dotted line (units  $\geq 1$ ) are considered seropositive. Kruskal-Wallis tests were performed to assess statistical significance between anti-PGL-I units at the beginning and end of the study (\*\* $P \leq 0.01$ , \*\*\*\* $P \leq 0.0001$ ). B: Percentages (%) of child contacts (2-10 years of age) testing seropositive for anti-PGL-I IgM (y-axis) depicted per study site and year (x-axis). Chi-squared tests were performed to assess statistical significance between percentages at the beginning and end of the study (\*\*\* $P \leq 0.001$ ).

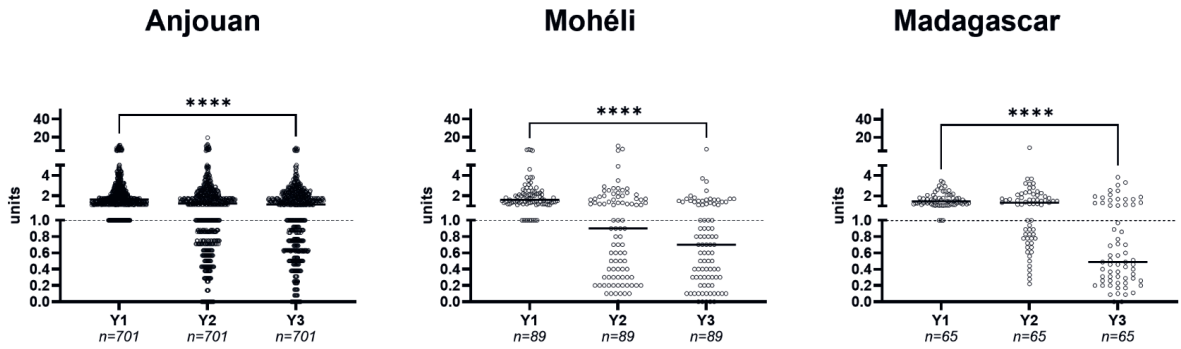
FSB: fingerstick blood; IgM: immunoglobulin M; PGL-I: phenolic glycolipid-I; Y1: year 1; Y2: year 2; Y3: year 3.



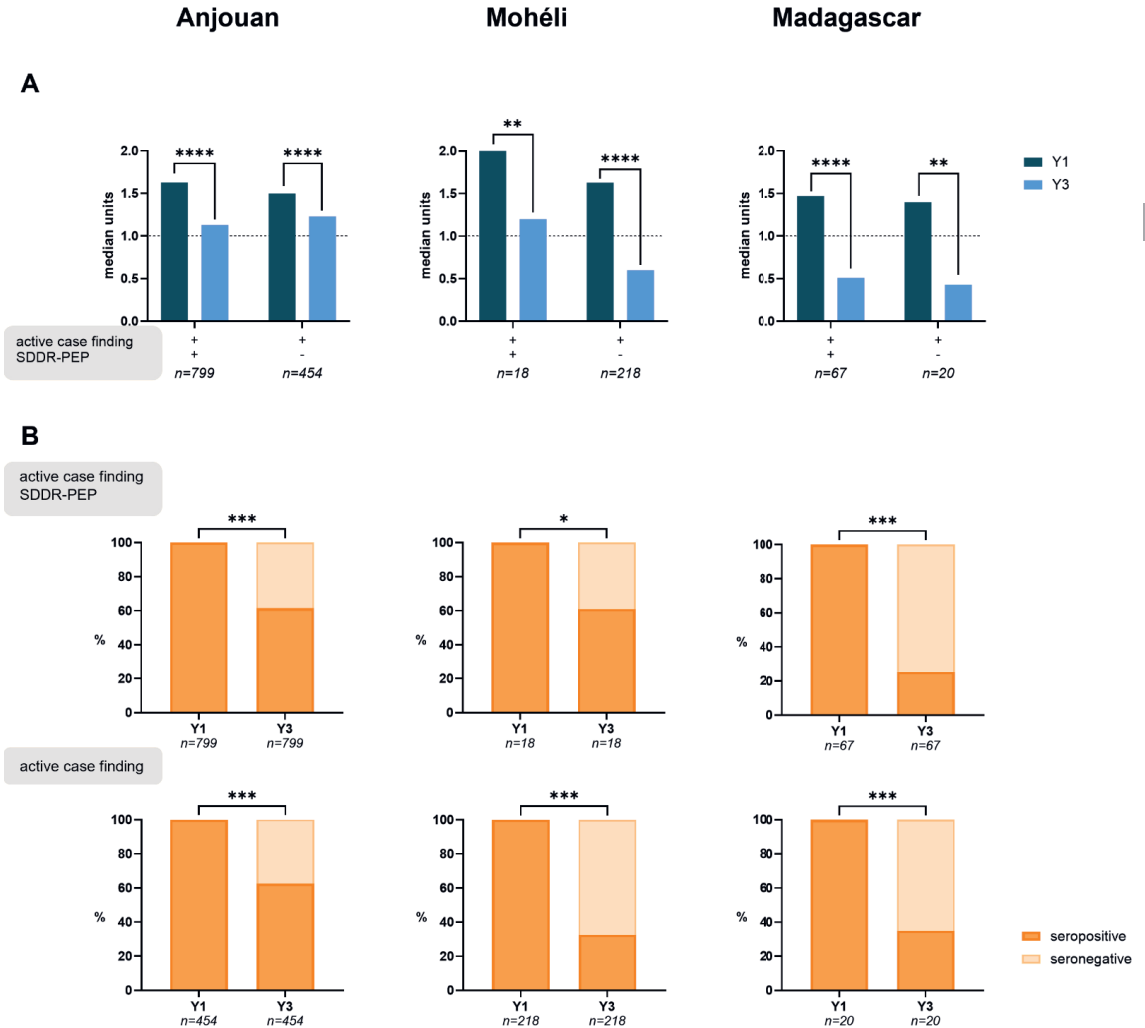
**S3 Fig. Longitudinal monitoring of anti-PGL-I values (shown in units) in FSB of incident leprosy cases.** Heat maps showing anti-PGL-I IgM units of MB (left panel; n = 12) and PB (right panel; n = 25) leprosy cases. Anti-PGL-I units  $\geq 1$  are considered seropositive. 'P' indicates timepoint of receiving SDDR-PEP. Triangles indicate timepoint of diagnosis. '×' indicates that no FSB sample was taken in that year. Four contacts were diagnosed with leprosy after the third year of the study. Anti-PGL-I units 0-0.99: light orange; units 1-4.99: orange; units 5-9.99: dark orange; units  $\geq 10$ : red. BI: bacterial index; FSB: fingerstick blood; MB: multibacillary; na: not available; PB: paucibacillary; PGL-I: phenolic glycolipid-I; SDDR-PEP: single double-dose rifampicin post exposure prophylaxis; Y1: year 1; Y2: year 2; Y3: year 3.



**S4 Fig. Anti-PGL-I values (shown in units) in FSB samples of incident PB (n = 14) and MB (n = 7) leprosy patients at diagnosis.** Open circles represent anti-PGL-I IgM units of individual samples. Units above dotted line (units  $\geq 1$ ) are considered seropositive. Solid black circles indicate BI-positive individuals. The Mann-Whitney U test was applied to assess statistical significance between the two groups. BI: bacterial index; MB: multibacillary; PB: paucibacillary.



**S5 Fig. Anti-PGL-I values (shown in units) of contacts on Anjouan, Mohéli and Madagascar testing seropositive in year 1 longitudinally.** Anti-PGL-I IgM (y-axis) units of contacts on Anjouan (left panel), Mohéli (middle panel) and Madagascar (right panel) testing seropositive in year 1 per study year (x-axis). Open circles represent anti-PGL-I units of individual samples. Units above dotted line (units  $\geq 1$ ) are considered seropositive. Friedman tests were performed to assess statistical significance between anti-PGL-I levels at the beginning and end of the study (\*\*\*\* $P \leq 0.0001$ ). PGL-I: phenolic glycolipid-I; Y1: year 1; Y2: year 2; Y3: year 3.



**S6 Fig. Longitudinal anti-PGL-I values (shown in units) among contacts on Anjouan, Mohéli and Madagascar testing seropositive in year 1 separated by SDDR-PEP intake.** In both groups (SDDR-PEP and no PEP), active case finding took place. A: Paired median anti-PGL-I IgM (y-axis) units of seropositive contacts (Y1) that received SDDR-PEP or not (x-axis) on Anjouan (left panel), Mohéli (middle panel) and Madagascar (right panel) longitudinally. Units above dotted line (units  $\geq 1$ ) are considered seropositive. Wilcoxon tests were performed to test for statistical significance between two groups ( $**P \leq 0.01$ ,  $****P \leq 0.0001$ ). B: Paired percentages (%) of individuals testing seropositive for anti-PGL-I IgM (y-axis) per study year (x-axis) divided per study site and SDDR-PEP intake (upper panel: active case finding + SDDR-PEP; lower panel: active case finding). McNemar tests were performed to test for statistical significance between percentages ( $*P \leq 0.05$ ,  $***P \leq 0.001$ ). IgM: immunoglobulin M; PGL-I: phenolic glycolipid-I; SDDR-PEP: single double-dose rifampicin post-exposure prophylaxis; Y1: year 1; Y3: year 3.



# 7

## **Host biomarker-based quantitative rapid tests for detection and treatment monitoring of tuberculosis and COVID-19**

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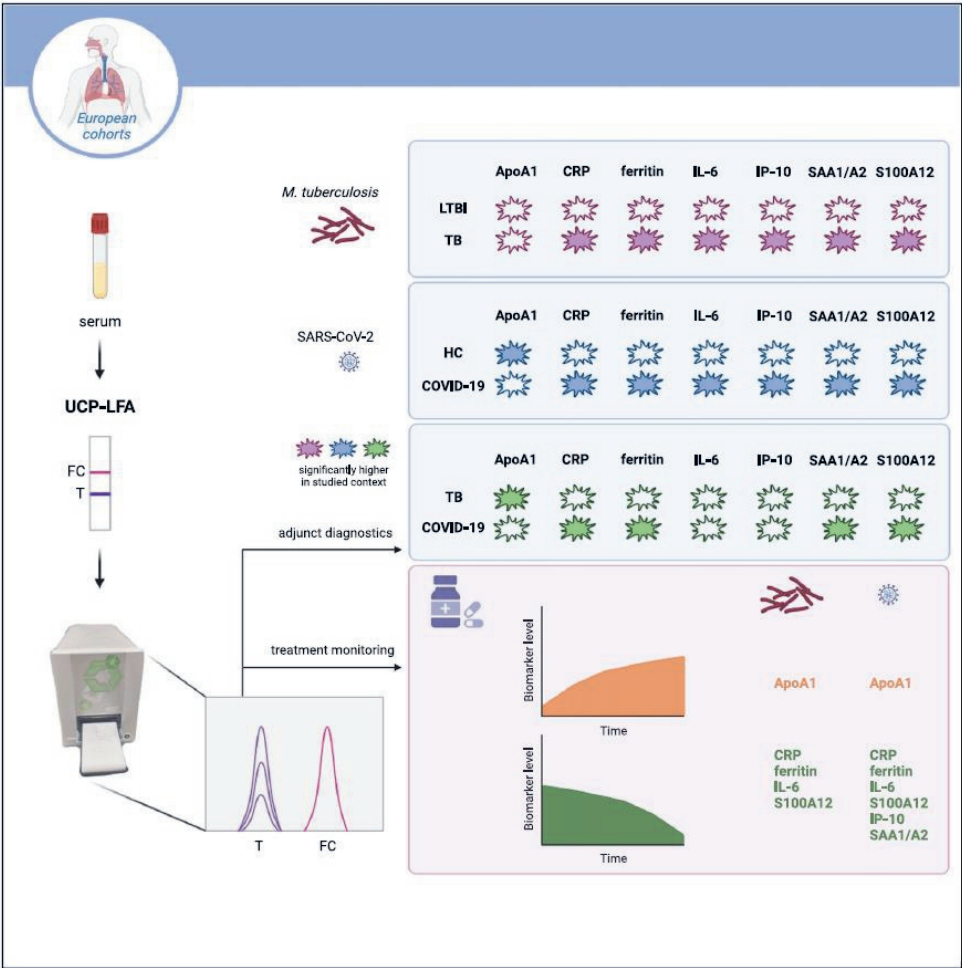
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# Abstract

Diagnostic services for tuberculosis (TB) are not sufficiently accessible in low-resource settings, where most cases occur, which was aggravated by the COVID-19 pandemic. Early diagnosis of pulmonary TB can reduce transmission. Current TB-diagnostics rely on detection of *Mycobacterium tuberculosis* (*Mtb*) in sputum requiring costly, time-consuming methods, and trained staff. In this study, quantitative lateral flow (LF) assays were used to measure levels of seven host proteins in sera from pre-COVID-19 TB patients diagnosed in Europe and latently *Mtb*-infected individuals (LTBI), and from COVID-19 patients and healthy controls. Analysis of host proteins showed significantly lower levels in LTBI versus TB (AUC: 0.94) and discriminated healthy individuals from COVID-19 patients (0.99) and severe COVID-19 from TB. Importantly, these host proteins allowed treatment monitoring of both respiratory diseases. This study demonstrates the potential of non-sputum LF assays as adjunct diagnostics and treatment monitoring for COVID-19 and TB based on quantitative detection of multiple host biomarkers.



## Introduction

Since the end of 2019, COVID-19, the devastating respiratory disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has plagued humans, swiftly resulting in a global pandemic. This has led to over 500 million cases and 6.3 million deaths (July 2022) [1]. Currently, reverse transcription polymerase chain reaction (RT-PCR) specific for SARS-CoV-2 based on nasopharyngeal swabs are used for diagnosis [2]. SARS-CoV-2 is transmitted via the respiratory route with an average incubation time of days [3]. Whereas most individuals infected with this virus are asymptomatic or experience mild to moderate disease, [3,4] still a substantial number of patients is hospitalized because of severe respiratory problems [5-7]. Moreover, in individuals with severe COVID-19, a proinflammatory cytokine storm can be observed inducing respiratory distress [4, 8].

Despite the COVID-19 pandemic, tuberculosis (TB) remains one of the most lethal infectious diseases, mainly in low-income countries, but also presenting an unignorable threat in Europe [1,9]. In 2020, around 10 million individuals developed TB and 1.5 million deaths were attributed to this disease [9]. It is estimated that one-quarter of the global population is latently infected with *Mycobacterium tuberculosis* (*Mtb*) and approximately 3–10% of those individuals are at risk of developing active TB during their lifetime [10]. As a poverty-associated disease, TB places a huge burden on health care services of low- and middle-income countries. Of the estimated 10 million patients, 3.6 million active TB cases are not diagnosed or reported [11]. Early diagnosis, followed by prompt and successful treatment will reduce *Mtb* transmission [11] and prevent disease-associated mortality [10]. Active TB is diagnosed by detection of the pathogen in sputum using microbiological, microscopic or genetic methods, which are often expensive, time-consuming, resource intense, require specially trained staff, and are less sensitive in HIV co-infected individuals [12]. Besides, sputum has relatively large sampling error resulting in false negative outcomes as it is difficult to obtain, especially from children [13-16]. Also, sputum cultures carry the risk of infection resulting in unusable data. Another hurdle of the use of sputum-based diagnostics is its lack of point-of-care (POC) application, therefore requiring additional clinic visits before treatment initiation. This is associated with a considerable risk of loss to follow-up as this may be a lengthy process, thereby sustaining transmission. TB diagnostic services are not sufficiently accessible, as exemplified by the fact that the WHO endorsed Xpert MTB/RIF (GeneXpert; Cepheid Inc., Sunnyvale, CA, USA) test for TB [17,18] is not sufficiently available to people living in remote, TB endemic areas.

Furthermore, it is important to note that COVID-19 contributed to disrupt TB services and therefore local disease control by reducing diagnosis and treatment of active TB and latently *Mtb* infected individuals (LTBI) [19]. Therefore, in the near future, an increase of active TB cases including multidrug-resistant cases, is likely to be observed [20-22]. Considering that the risk of death because of TB has been estimated as 1.4 times higher in COVID-19 patients [23], it is vital to develop new tools to identify patients with TB and/or SARS-CoV-2 infection. As signs and symptoms of COVID-19 might resemble those that are associated with active TB in areas where both are endemic or in “western” settings encountering refugees from TB endemic areas, biomarkers that can rapidly discriminate between these viral and bacterial respiratory diseases can be useful for triage.

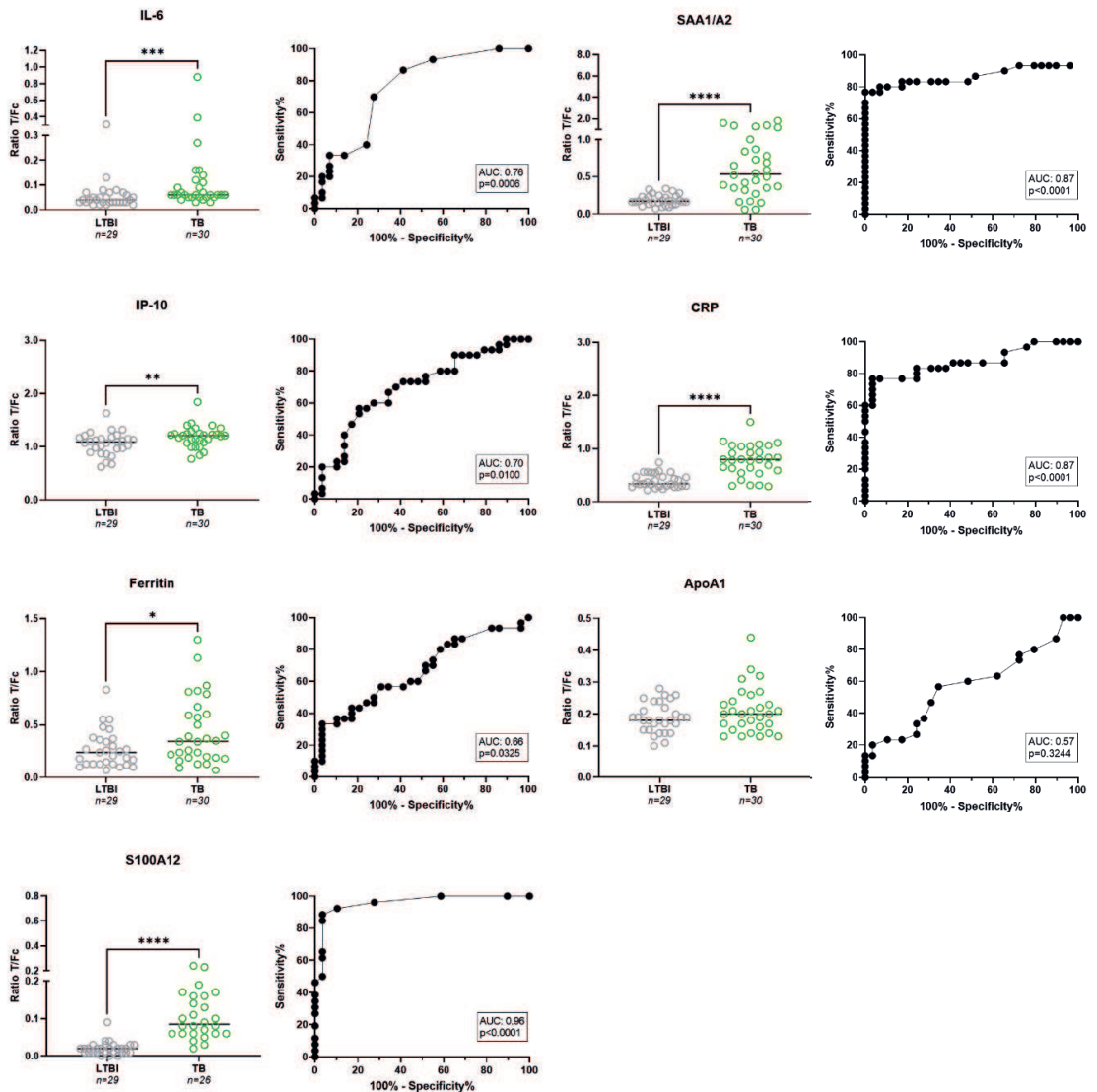
Using the luminescent upconverting reporter particle (UCP) technology combined with low-cost, field-friendly immune-chromatography, we have previously developed and field-evaluated quantitative lateral flow assays (LFAs) [24–31]. These UCP-LFAs are suitable for accurate quantification of cytokines, acute phase proteins and antibodies in serum, stimulated whole blood, pleural fluid, saliva, and fingerstick blood [29, 32–35]. The user-friendly, low complexity UCP-LFAs do not require sophisticated analytical laboratory equipment. Portable battery-operated readers provide full instrument-assisted analysis which also avoid potential operator bias. The UCP-LFAs represent POC alternatives for the more elaborate and time consuming, laboratory-based enzyme linked immunosorbent assay (ELISA). Another advantage of the UCP-LFA is that it permits multiplexing to measure several markers simultaneously allowing a biomarker signature to be assessed in field settings [27,31].

Exploratory proteomics previously identified a promising host protein signature that distinguished active TB patients from other respiratory diseases (ORD) with signs and symptoms suggestive of TB in an African setting [36]. Based on this signature, we have applied C-reactive protein (CRP), apolipoprotein-A1 (ApoA1), inducible protein (IP)-10/C-X-Cmotif chemokine 10 (CXCL-10), and serum amyloid A (SAA) to the UCP-LFA format [29]. In addition, we have developed UCP-LFAs for interleukin-6 (IL-6), S100 calcium-binding protein A12 (S100A12), and ferritin in view of their role in tuberculous meningitis [37,38], inflammatory disorders [39], leprosy [29,31] and iron homeostasis in *Mtb* [36,40] respectively. In this study, we have used UCP-LFAs to rapidly assess serum levels of these host proteins in European TB and COVID-19 patients to investigate to what extent these can identify and discriminate between the old and new respiratory disease.

## Results

### Assessment of host biomarkers for active TB in a European cohort

Previously, host biomarkers were identified by Luminex as discriminatory between TB and ORD in African settings [37]. The aim of the present study was to assess whether these host biomarkers could also allow the identification of TB in a European hospital setting, UCP-LF strips were developed for quantitative measurement of seven cytokines [24–26,35,41,42] and used for analysis of banked sera from LTBI and pulmonary TB patients collected in Europe (TB cohort 1, Figure 1). Serum levels for CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 were significantly higher in the TB group ( $p < 0.0001$ ,  $p = 0.0325$ ,  $p = 0.0006$ ,  $p = 0.01$ ,  $p < 0.0001$ , and  $p < 0.0001$ , respectively), but no significant difference was found for ApoA1 ( $p = 0.3244$ ). CRP, SAA1/A2, and S100A12 were the most discriminatory when comparing TB to LTBI (AUCs: 0.87–0.96). Chest X-ray severity did not affect the levels for any of the cytokines (Figure S1).



**Figure 1. Evaluation of host biomarkers for TB and LTBI in a European cohort.** Levels of IL-6, IP-10, ferritin, SAA1/A2, CRP, ApoA1, and S100A12 were measured by UCP-LFA in serum samples of TB patients (n = 30; green dots) and LTBI (n = 29; gray dots) from Europe. Median values for each group are indicated by horizontal bars. Mann-Whitney U tests were performed to determine the statistical significance between groups (p-values: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001, \*\*\*\*p≤0.0001). Green dots: TB cohort 1; gray dots: LTBI cohort 1. AUC: area under the curve; Fc: flow control line; LTBI: latent tuberculosis infection; T: test line; TB: tuberculosis.

The total number of six biomarkers (ApoA1, CRP, ferritin, IL-6, IP-10, and SAA1/A2) scoring above the cut-off value (NUMscore), based on the Youden's index for each marker, was calculated [31]. Using a cut-off of ≥3 positive markers, accuracy for active TB (with LTBI as control group) with a sensitivity of 83% (CI: 66.4 to 92.7) and specificity of 97% (CI: 82.8 to 99.8) (AUC: 0.94; Figure S2) was found. An overview of medians for each marker per test

group/comparison and the cut-off values for each biomarker are displayed in Tables 1 and 2. Other NUM score combinations and corresponding AUC and Sn/Sp were evaluated and shown in Table S3. Biomarkers were deleted from the NUM score based on their contribution (AUC); the biomarker with the lowest AUC was removed first and this was repeated until the most discriminatory marker for that comparison remained. Noteworthy is that a 2-marker NUM score of CRP and SAA1/A2 (using a cut-off of  $\geq 1$ ) resulted in an AUC of 0.91 and similar Sn/Sp (83%/97%) as the 6-marker NUM score, in the comparison of LTBI versus TB.

Moreover, comparison of available QuantiFERON data and NUMscore results in TB cohort 1 showed that a NUM score based on the levels of six (ApoA1, CRP, ferritin, IL-6, IP-10, and SAA1/A2) as well as two (CRP and SAA1/A2) host proteins, allowed identification of individuals with active TB whose QuantiFERON test was negative (Figure S3). In addition, whereas QuantiFERON was not able to accurately distinguish between active TB and LTBI, both 2- and 6-marker NUM scores successfully discriminated active from latent TB in QuantiFERON-positive individuals.

### **Analysis of host proteins for COVID-19 in a European cohort**

In 2020, COVID-19 and SARS-CoV-2 infection posed TB diagnostics with an additional respiratory disease in the differential diagnosis. Therefore, UCP-LFA for the same seven biomarkers were also used to analyze hospitalized Dutch COVID-19 patients ( $n = 102$ ) and healthy controls ( $n = 39$ ); the latter sampled either in ( $n = 12$ ) or before 2020 ( $n = 27$ ; Figure 2). UCP-LFAs showed that for all seven proteins, serum levels were significantly different between the COVID-19 patients and healthy controls, with p-values ranging from  $p = 0.0008$  to  $p < 0.0001$  (Figure 2). Of note was that for six markers (CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12) higher values were detected in COVID-19 sera whereas for ApoA1, significantly lower levels were detected in the COVID-19 group ( $p < 0.0001$ ). The markers with the highest discriminatory potential between these two groups included CRP, ferritin, and SAA1/A2 (AUCs ranging from 0.94 to 0.98). For ApoA1, ferritin, IP-10, and SAA1/A2, no significantly different levels were observed at hospital admission – between COVID-19 patients with moderate disease, severe disease or fatal outcome (Figures 2 and S1). Cytokines CRP, IL-6, and S100A12 were significantly increased in patients with fatal outcome compared to those with moderate disease. In addition, higher CRP levels were observed in severe compared to moderate disease. Moreover, CRP, ferritin, IL-6, IP-10, and SAA1/A2 did not show any significant differences in serum samples at hospital admission of COVID-19 patients who had already received anti-inflammatory treatment before the first sample collection compared to those who had not received any treatment yet (Figure S4). S100A12, on the other hand, significantly decreased on anti-inflammatory treatment, whereas ApoA1 increased.

**Table 1. Overview of medians including interquartile range (IQR) for each marker per test group/comparison**

| Marker   | Test group        | Median | IQR    |
|----------|-------------------|--------|--------|
| ApoA1    | LTBI cohort 1     | 0 · 18 | 0 · 07 |
|          | TB cohort 1       | 0 · 20 | 0 · 08 |
|          | TB cohort 1 + 2   | 0 · 24 | 0 · 13 |
|          | Healthy controls  | 0 · 28 | 0 · 07 |
|          | COVID-19          | 0 · 19 | 0 · 08 |
| CRP      | LTBI cohort 1     | 0 · 34 | 0 · 18 |
|          | TB cohort 1       | 0 · 80 | 0 · 41 |
|          | TB cohort 1 + 2   | 0 · 80 | 0 · 32 |
|          | Healthy controls  | 0 · 55 | 0 · 50 |
|          | COVID-19          | 1 · 43 | 0 · 56 |
| Ferritin | LTBI cohort 1     | 0 · 24 | 0 · 24 |
|          | TB cohort 1       | 0 · 35 | 0 · 40 |
|          | TB cohort 1 + 2   | 0 · 35 | 0 · 36 |
|          | Healthy controls  | 0 · 06 | 0 · 09 |
|          | COVID-19          | 0 · 53 | 0 · 32 |
| IL-6     | LTBI cohort 1     | 0 · 04 | 0 · 03 |
|          | TB cohort 1       | 0 · 06 | 0 · 05 |
|          | TB cohort 1 + 2   | 0 · 07 | 0 · 10 |
|          | Healthy controls  | 0 · 03 | 0 · 04 |
|          | COVID-19          | 0 · 07 | 0 · 14 |
| IP-10    | LTBI cohort 1     | 1 · 09 | 0 · 21 |
|          | TB cohort 1       | 1 · 21 | 0 · 15 |
|          | TB cohort 1 + 2   | 1 · 11 | 0 · 33 |
|          | Healthy controls  | 0 · 67 | 0 · 38 |
|          | COVID-19          | 1 · 02 | 0 · 60 |
| SAA1/A2  | LTBI cohort 1     | 0 · 17 | 0 · 09 |
|          | TB cohort 1       | 0 · 54 | 0 · 51 |
|          | TB cohort 1 + 2   | 0 · 54 | 0 · 56 |
|          | Healthy controls  | 0 · 25 | 0 · 21 |
|          | COVID-19          | 1 · 78 | 0 · 77 |
| S100A12  | LTBI cohort 1 + 2 | 0 · 02 | 0 · 02 |
|          | TB cohort 1       | 0 · 09 | 0 · 10 |
|          | TB cohort 1 + 2   | 0 · 07 | 0 · 08 |
|          | Healthy controls  | 0 · 03 | 0 · 06 |
|          | COVID-19          | 0 · 17 | 0 · 16 |

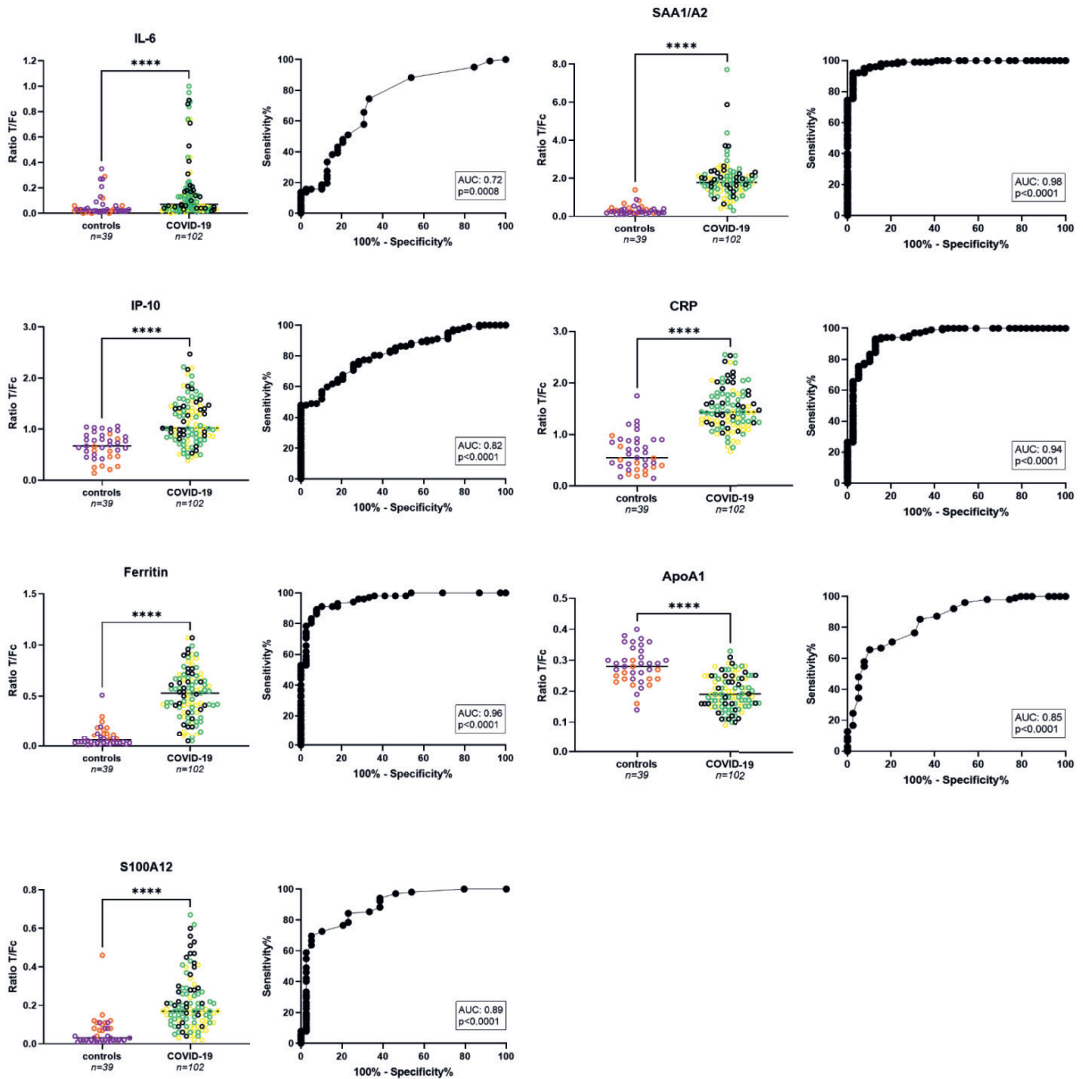
Overview of the medians including IQR per test group for each individual marker. COVID-19: coronavirus disease 2019; IQR: interquartile range; LTBI: latent tuberculosis infection; TB: tuberculosis.

An in-sample validation of the seven biomarkers (ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12) combined through calculation of a NUMscore yielded a sensitivity of 93% (CI: 86.5 to 96.6) with specificity of 100% (CI: 91.0 to 100), applying a cut-off of at least four positive markers in the comparison of COVID-19 patients to healthy controls (AUC: 0.99; Figure S5; Table 2). Only combining the three most discriminatory markers (CRP, ferritin, and SAA1/A2) resulted in Sn/Sp of 95%/97% (cut-off  $\geq 2$ ) and an AUC of 0.99 (Table S3).

### **Comparison UCP-LFAs for COVID-19 and TB**

To assess whether the seven markers could also distinguish TB from COVID-19, UCP-LFA data of sera from 26 untreated, pulmonary TB patients collected in Italy (TB cohort 1, Figure 3) were combined with 20 additional sera from former TB patients collected in the Netherlands (TB cohort 2, Figure 3) to increase group size, and compared to 102 serum samples from COVID-19 patients at hospitalization. For CRP, ferritin, SAA1/A2, and S100A12, serum cytokine levels were significantly higher in the COVID-19 group ( $p < 0.0001$ ,  $p = 0.0038$ ,  $p < 0.0001$  and  $p < 0.0001$ , respectively). In contrast, ApoA1 levels were significantly lower in COVID-19 patients ( $p < 0.0001$ ). However, no significant differences were found for IL-6 and IP-10 between the two groups. SAA1/A2 and CRP showed the highest discriminatory potential (AUCs of 0.92 and 0.93, respectively).

When comparing COVID-19 to TB patients, the calculation of a NUM score based on seven biomarkers (ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12), resulted in a sensitivity of 91% (CI: 84.1 to 95.3) with specificity of 87% (CI: 74.3 to 93.9) applying a cut-off of at least four positive markers (AUC: 0.95; Figure S6). A NUM score combining CRP, SAA1/A2, and S100A12 yielded Sn/Sp of 90%/87% applying a cut-off of at least 2 positive markers (AUC: 0.94).



**Figure 2. Evaluation of host biomarkers for Dutch COVID-19 patients and healthy controls.** Levels of IL-6, IP-10, ferritin, SAA1/A2, CRP, ApoA1, and S100A12 were measured by UCP-LFA in serum samples of COVID-19 patients ( $n = 102$ ) and healthy controls ( $n = 39$ ;  $n = 27$  from before (May) 2019 ( $n = 12$  from after 2019 (June/July 2020)) from the Netherlands. Median values for each group are indicated by horizontal bars. Mann-Whitney U tests were performed to determine the statistical significance between groups ( $p$ -values:  $*p \leq 0.05$ ,  $**p \leq 0.01$ ,  $***p \leq 0.001$ ,  $****p \leq 0.0001$ ). Orange dots: healthy controls from before 2019; purple dots: healthy controls from after 2019; black dots: COVID-19 patients with a fatal outcome; yellow dots: COVID-19 patients with severe disease; turquoise dots: COVID-19 patients with moderate disease. AUC: area under the curve; COVID-19: coronavirus disease 2019; Fc: flow control line; T: test line.



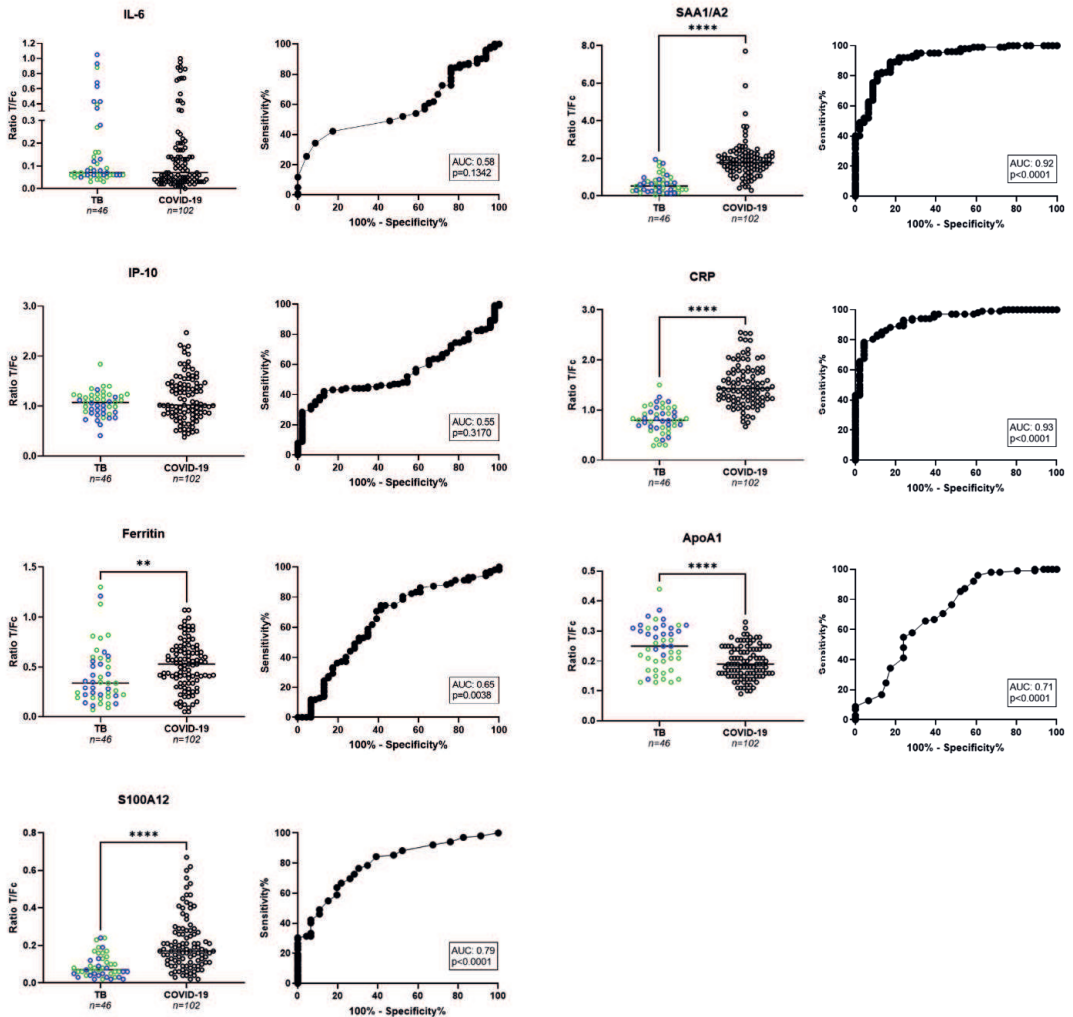
**Table 2.** Overview of cut-off ratios used for each marker per cohort comparison with corresponding sensitivity and specificity.

| Marker   | Cohort comparison                | AUC  | Cut-off ratio | Sn/Sp    |
|----------|----------------------------------|------|---------------|----------|
| ApoA1    | TB versus LTBI                   | 0.57 | >0.195        | 57%/66%  |
|          | COVID-19 versus healthy controls | 0.85 | <0.215        | 66%/90%  |
|          | COVID-19 versus TB               | 0.71 | <0.285        | 96%/36%  |
| CRP      | TB versus LTBI                   | 0.87 | >0.585        | 77%/97%  |
|          | COVID-19 versus healthy controls | 0.94 | >0.990        | 93%/87%  |
|          | COVID-19 versus TB               | 0.93 | >1.175        | 78%/96%  |
| Ferritin | TB versus LTBI                   | 0.66 | >0.560        | 33%/97%  |
|          | COVID-19 versus healthy controls | 0.96 | >0.205        | 89%/92%  |
|          | COVID-19 versus TB               | 0.65 | >0.405        | 71%/62%  |
| IL-6     | TB versus LTBI                   | 0.76 | >0.045        | 87%/59%  |
|          | COVID-19 versus healthy controls | 0.72 | >0.035        | 75%/67%  |
|          | COVID-19 versus TB               | 0.58 | <0.045        | 34%/92%  |
| IP-10    | TB versus LTBI                   | 0.70 | >1.190        | 57%/79%  |
|          | COVID-19 versus healthy controls | 0.82 | >0.855        | 75%/74%  |
|          | COVID-19 versus TB               | 0.55 | >1.260        | 41%/84%  |
| SAA1/A2  | TB versus LTBI                   | 0.87 | >0.345        | 100%/77% |
|          | COVID-19 versus healthy controls | 0.98 | >0.900        | 92%/97%  |
|          | COVID-19 versus TB               | 0.92 | >1.005        | 89%/82%  |
| S100A12  | TB versus LTBI                   | 0.96 | >0.045        | 88%/97%  |
|          | COVID-19 versus healthy controls | 0.89 | >0.125        | 70%/95%  |
|          | COVID-19 versus TB               | 0.79 | >0.105        | 76%/70%  |

Overview of the cut-off ratios used per each individual marker in three different comparisons: TB vs. LTBI, COVID-19 versus healthy controls, and COVID-19 versus TB. The corresponding AUC, sensitivity and specificity are shown for each biomarker. A cut-off ratio for positivity for each biomarker was determined by calculating the maximal Youden's index. Using the cut-offs for positivity, a NUM score was calculated; the number of biomarkers that scored above the threshold of positivity. AUCs in bold indicate the marker with the most discriminatory potential for a specific comparison; AUCs in italic font indicate a non-significant result. AUC: area under the curve; COVID-19: coronavirus disease 2019; LTBI: latent tuberculosis infection; Sn/Sp: sensitivity/specificity; TB: tuberculosis.

## Treatment monitoring

To assess the applicability of UCP-LFAs for these markers to monitor TB treatment efficacy, serum samples from 22 confirmed TB patients (from TB cohort 1) taken at month 2–4 (t1), and month 5–9 (t2) after onset of treatment were analyzed and compared to baseline data before initiation of treatment (t0) (Figures 4 and S7). At t2, serum levels of IL-6, ferritin, CRP, and S100A12 were significantly reduced compared to participants' serum levels at t0 ( $p = 0.0208$ ,  $p = 0.0002$ ,  $p = 0.0133$  and  $p = 0.0004$ , respectively). In contrast, ApoA1 levels significantly increased at t2 compared to t0 ( $p = 0.0133$ ), IP-10 and SAA1/A2 showed no significant differences between t0 and t2 ( $p = 0.1409$  and  $p > 0.9999$ , respectively).

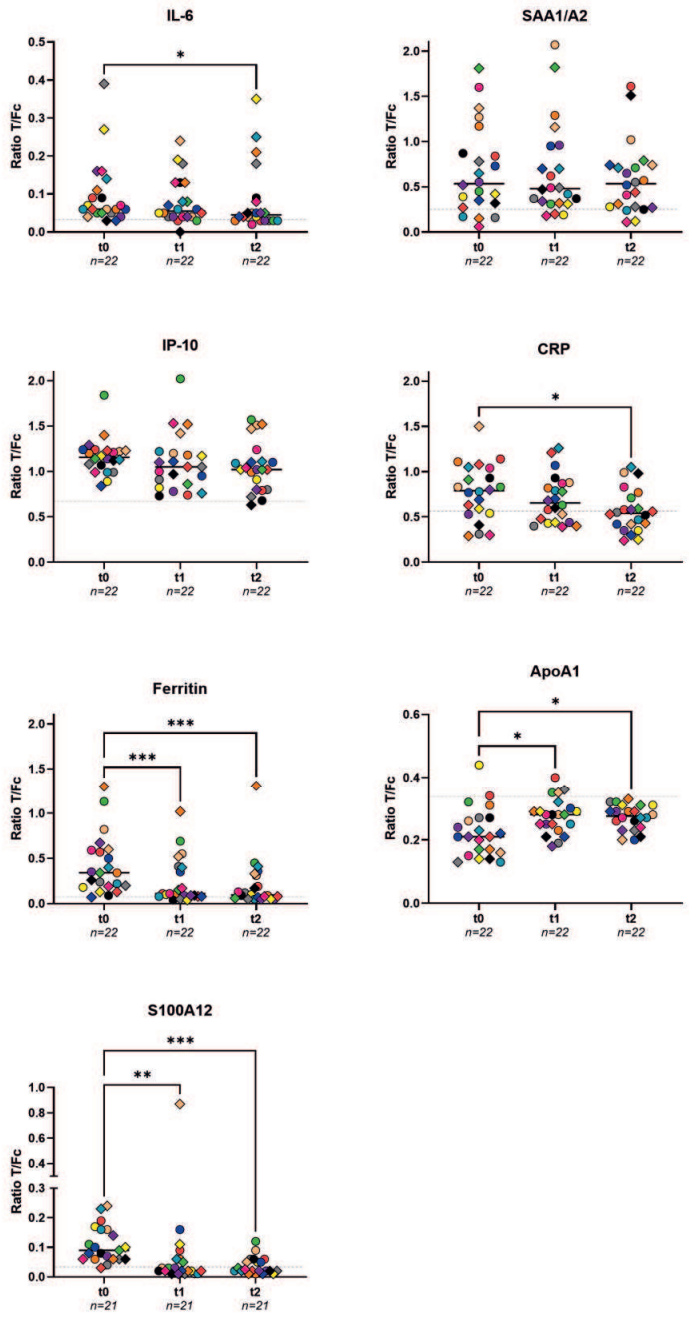


**Figure 3. Evaluation of host biomarkers for TB and COVID-19 patients.** Levels of IL-6, IP-10, ferritin, SAA1/A2, CRP, ApoA1, and S100A12 were measured by UCP-LFA in serum samples of TB patients (n = 46) and COVID-19 patients (n = 102) collected in European hospitals. Median values for each group are indicated by horizontal bars. Mann-Whitney U tests were performed to determine the statistical significance between groups (p-values: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001). Green dots: TB cohort 1; blue dots: TB cohort 2; black dots: COVID-19 patients. AUC: area under the curve; COVID-19: coronavirus disease 2019; Fc: flow control line; TB: tuberculosis.

A similar analysis was performed for 25 successfully treated COVID-19 patients with samples taken at hospital admission (t0) and at follow up around 6 weeks after hospital discharge (t2 – Figure 5). At t2, serum levels of CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 were significantly lower (p<0.0001, p = 0.0001, p<0.0001, p = 0.0005, p<0.0001

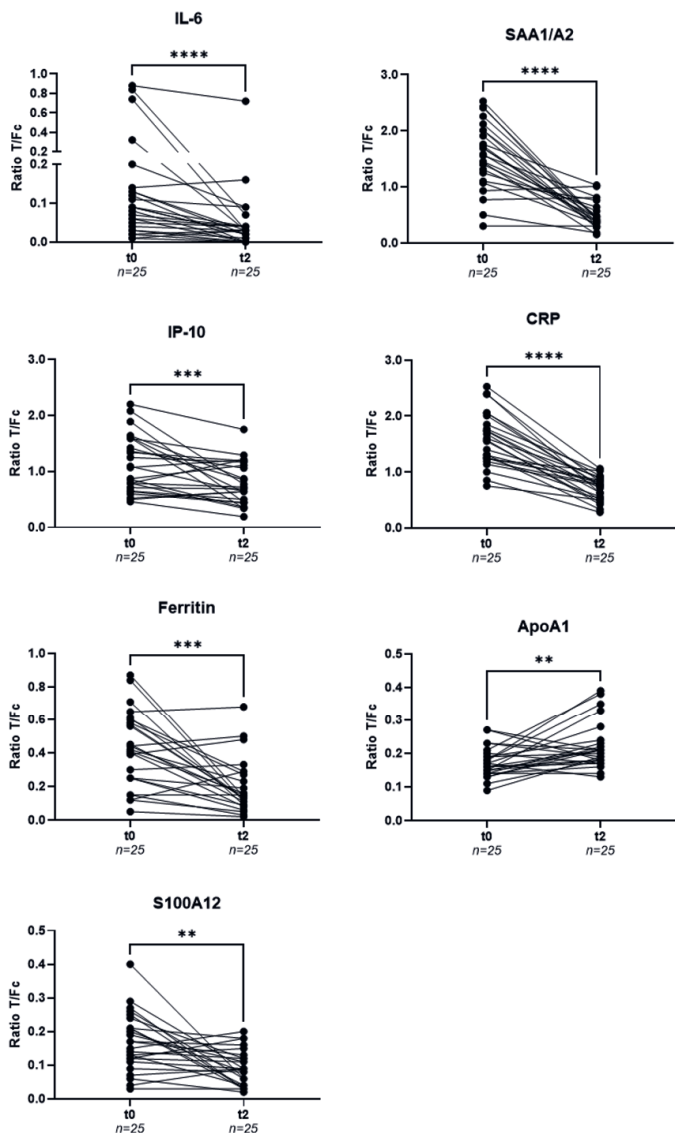
and  $p = 0.0022$ , respectively). However, ApoA1 levels were significantly higher in comparison to t0 ( $p = 0.0063$ ) in line with the finding of reduced ApoA1 serum levels for COVID-19 patients versus healthy controls.

**TB patients**



**Figure 4. Treatment monitoring for TB.** Levels of IL-6, IP-10, ferritin, SAA1/A2, CRP, ApoA1, and S100A12 were measured by UCP-LFA in serum samples of pulmonary TB patients ( $n = 22$ ) before treatment ( $t_0$ ), at months 2–4 ( $t_1$ ), and months 5–9 ( $t_2$ ) of treatment. Median values for each group are indicated by horizontal bars. The gray dotted lines represent the median value of the corresponding marker measured for 39 healthy controls. S100A12 data were missing for one patient. Friedman test with Dunn's correction for multiple testing was performed to determine the statistical significance between timepoints (p-values: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ ). Fc: flow control line; T: test line; TB: tuberculosis;  $t_0$ : first timepoints;  $t_1$ : 2–4 months after the beginning of treatment;  $t_2$ : 5–9 months after the beginning of treatment.

### COVID-19 patients



**Figure 5. Treatment monitoring for COVID-19.** Levels of IL-6, IP-10, ferritin, SAA1/A2, CRP, ApoA1, and S100A12 were measured by UCP-LFA in serum samples from COVID-19 patients ( $n = 25$ ) at hospital admission ( $t_0$ ) and follow-up ( $t_2$ ). Median values for each group are indicated by horizontal bars. The gray dotted lines represent the median value of the corresponding marker measured for 39 healthy controls. Wilcoxon matched pairs signed rank tests were performed to determine the statistical significances between timepoints ( $p$ -values:  $*p \leq 0.05$ ,  $**p \leq 0.01$ ,  $***p \leq 0.001$ ,  $****p \leq 0.0001$ ). COVID-19: coronavirus disease 2019; Fc: flow control line; T: test line;  $t_0$ : timepoint of hospital admission;  $t_2$ : follow-up around 6 weeks after hospital discharge.

## Discussion

Early detection and treatment of communicable diseases, particularly those spread via aerosols to the respiratory tract, is vital to stop transmission. As shown in WHO records, migrants traveling to Europe may also carry a higher risk of *Mtb* infection [43–46]. Thus, in view of the COVID-19 pandemic and the continuous migration to (Western) Europe from areas with multi- and even extensively drug-resistant TB [47,48], specific tools for screening and (rapid) diagnosis of TB, become even more crucial.

This study describes the performance of the rapid and quantitative measurement of seven markers in the UCP-LFA format. This POC platform is highly adaptable toward implementation of the number and variety of biomarkers and was developed for (simultaneous) assessment of cytokines, acute phase proteins [49,50], growth factors [51], antibodies, and complement markers [52]. In this study, LF strips for detection of one biomarker were applied for seven host serum proteins, representing a tentative signature relevant for TB triage, comprising ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12.

In comparison to sera from individuals with latent TB, we found significantly increased levels of CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 in those from active TB patients. CRP, SAA, and ferritin, all acute phase proteins (APPs) synthesized in the liver [53], are associated with TB [54–60] and inflammation [36,61,62]. Once secreted by monocytes, endothelial cells or fibroblasts, IP-10 can act as a chemotactic mediator for both innate and adaptive immune cells [63] and is recognized as a marker in HIV-positive TB patients [64]. IL-6 is a proinflammatory cytokine produced by macrophages [53,65]. In line with previous literature, these five markers were also elevated in COVID-19 patients compared to healthy controls [5,65–70]. Furthermore, two additional markers ApoA1 and S100A12, also showed diagnostic potential for COVID-19 confirming the earlier reported downregulation of ApoA1 and the upregulation of S100A12 in COVID-19 patients in France and China [71–73]. ApoA1 is believed to play an important role in modulating inflammation as it can inhibit monocyte activation by binding to T cells [74] resulting in decreased ApoA1 serum levels during inflammation [75]. On the other hand, the phagocytic protein S100A12 can exhibit proinflammatory effects and is found in high concentrations at sites of inflammation [39].

When levels of host proteins were compared between COVID-19 and TB patients, five (ApoA1, CRP, ferritin, SAA1/A2, and S100A12) showed promising discriminatory potential as all but ApoA1 were significantly higher in COVID-19 patients' sera. IL-6 and IP-10 seemed promising biomarkers successfully discriminating TB from LTBI and COVID-19 from healthy

controls. However, these two markers did not show potential in distinguishing TB from COVID-19 disease. Although CRP is generally described as a biomarker for bacterial infection [76,77], higher levels of this acute phase protein were observed in COVID-19 patients compared to TB. The excessive levels of these biomarkers could possibly be explained by the acute and exorbitant nature of local and systemic inflammation and immune activation observed in COVID-19 patients [78–80].

Despite the fact that certain host proteins are detectable in healthy as well as diseased individuals, the quantitative nature of the UCP-LFA allows the discrimination of TB and COVID-19 at POC because levels varied significantly between the test groups. In this respect, it should be noted that for each comparison (TB versus LTBI; COVID-19 versus HC; TB versus COVID-19) a distinct cut-off was required per biomarker. Besides allowing quantification of host biomarkers at POC, the addition of pathogen-specific biomarkers to a signature based on proteins that are not disease-specific (such as the host proteins evaluated here), increases the diagnostic potential. This was recently demonstrated for leprosy diagnostics in which the simultaneous detection of the cytokines described in this study and anti-*Mycobacterium leprae* PGL-I IgM [29] into a multi-biomarker test (MBT) allowed the discrimination of patients with both paucibacillary and multibacillary leprosy from controls in high- but also in non-endemic areas [31]. In the case of *Mtb* infection, however, a specific and sensitive antibody has not yet been identified [81,82].

This study aimed to evaluate whether serum levels of the selected host proteins can be used to detect TB and COVID-19 using in-sample validation. These markers need to be validated in an independent cohort, in which both patient groups are recruited prospectively at the same site and time. However, in-sample validation using NUM scores was assessed. This approach, based on serum levels of ApoA1, CRP, ferritin, IL-6, IP-10, and SAA1/A2, yielded an 83% sensitivity and 97% specificity for detection of TB versus LTBI. Noteworthy is that not all six markers might be necessary, as a 4-marker NUM score combining CRP, IL-6, IP-10, and SAA1/A2 in this study yields a sensitivity of 87%, which nears the WHO-recommended sensitivity (90%) for triage TB tests [83]. Similarly, using a NUM score based on 7 host proteins (additionally including S100A12), COVID-19 patients were separated from healthy controls with sensitivity of 93% and specificity of 100%, respectively. A 3-marker NUM score (CRP, ferritin, and SAA1/A2) resembles the above-mentioned test performance with sensitivity of 95% and specificity of 97%. The seven markers could also distinguish TB from COVID-19 with 91% sensitivity and 87% specificity. A combination of CRP and SAA1/A2 might already be sufficiently discriminatory, with Sn/Sp of 94%/80%.

Longitudinal analysis of both TB and COVID-19 cohorts, indicated that biomarker analysis allows immunomonitoring of treatment for both groups. CRP, ferritin, IL-6, and S100A12 all declined during TB treatment, whereas ApoA1 levels increased over time. Furthermore, in line with the use of IL-6 inhibitors for treatment of COVID-19 patients attempting to mediate inflammation [65,80], IL-6 levels declined significantly in these patients on treatment. Of interest, baseline IL-6 levels were significantly higher in patients with fatal outcome compared to those with moderate disease. Nevertheless, contradictory effects on mortality in several clinical trials using IL-6-blocking agents were reported [65]. Markers described to be valuable in predicting clinical outcome for COVID-19 in other studies included CRP, SAA,

ferritin, and S100A12 [62,69,73,84,85]. For two of those markers, CRP and S100A12, increased levels were indeed detected in our study at hospital admission in patients with fatal outcome compared to those with moderate disease.

Although some patients had been treated with anti-inflammatory medication before hospital admission, this did not affect biomarker levels for CRP, ferritin, IL-6, IP-10, and SAA1/A2. However, only S100A12 and to a lesser extent also ApoA1 already showed a significant effect, arguing for the potential of these proteins as biomarkers for monitoring of early treatment effect.

Our study shows that the UCP-LFA format cannot only provide (adjunct) rapid diagnostics for (triage of) chronic diseases such as TB and leprosy [31,34,36], but also for more acute diseases including COVID-19. Of note, we demonstrated that in contrast to QuantiFERON-TB Gold which detects *Mtb* infection but cannot discriminate between active TB and LTBI [86], the host proteins assessed here showed significant differences between active TB and LTBI (AUC: 0.88–1.00). Application of these biomarkers in UCP-LFA as adjunct diagnostic tools for triage of TB, can be useful to assess whether further diagnostic testing is warranted thereby reducing the costs for referrals for SARS-CoV-2 PCR and/or GeneXpert.

### Limitations of the study

It should be noted that the COVID-19 cohort studied concerned patients hospitalized in 2020 who were all severely ill but admitted at various COVID-19 stages, which was reflected by the detected range in host biomarker levels. Therefore, in areas endemic for both TB and COVID-19, it will be feasible to triage TB (accepting lower sensitivity) but challenging to diagnose TB based on the studied biomarkers, because these reflect an individual's disease and inflammation state which may be comparable for these diseases. Consequently, the outcome of non-disease specific, host biomarker-based diagnostics should always be considered in the context of the individual's clinical presentation and the burden of diseases in an area.

Future studies should thus be focused at simultaneous recruitment of TB as well as all ORD, including other, non-European settings. To this end, the UCP-LFA platform can facilitate replacement of biomarkers to generate more optimal signatures for various use-cases including discrimination of TB and COVID-19.

### Consortium

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## Declaration of interests

The authors declare that they have no conflict of interest.



# STAR Methods

## Key resources table

| REAGENT or RESOURCE                                                                                   | SOURCE                                                                              | IDENTIFIER               |
|-------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------|
| <b>Antibodies</b>                                                                                     |                                                                                     |                          |
| Goat-anti-human ApoA1                                                                                 | R&D systems                                                                         | AF3664; RRID: AB_2242717 |
| Mouse-anti-human CRP                                                                                  | Labned.com                                                                          | C5                       |
| Mouse-anti-human ferritin                                                                             | Novus Biologicals                                                                   | F31                      |
| Rat-anti-human IL-6                                                                                   | Biologend                                                                           | MQ2-39C3                 |
| Mouse-anti-human IP-10                                                                                | Diaclone Research                                                                   | B-C55                    |
| Mouse-anti-human SAA1/A2                                                                              | R&D systems                                                                         | 865504                   |
| Goat-anti-human S100A12                                                                               | R&D systems                                                                         | AF1052; RRID: AB_2183610 |
| Goat-anti-mouse                                                                                       | Sigma-Aldrich                                                                       | M8642; RRID: AB_260698   |
| Goat-anti-rabbit                                                                                      | Sigma-Aldrich                                                                       | R4880; RRID: AB_261349   |
| Goat-anti-rat                                                                                         | Sigma-Aldrich                                                                       | R5130; RRID: AB_261356   |
| Rabbit-anti-goat                                                                                      | Sigma-Aldrich                                                                       | G4018; RRID: AB_259895   |
| Rabbit-anti-ApoA1                                                                                     | R&D systems                                                                         | 2083A                    |
| Mouse-anti-CRP                                                                                        | Labned.com                                                                          | CRP135                   |
| Mouse-anti-ferritin                                                                                   | Novus Biologicals                                                                   | F23                      |
| Mouse-anti-IP-10                                                                                      | Diaclone Research                                                                   | B-C50                    |
| Mouse-anti-SAA1/A2                                                                                    | R&D systems                                                                         | 924903                   |
| Goat-anti-S100A12                                                                                     | R&D systems                                                                         | AF1052; RRID: AB_2183610 |
| Rat-anti-IL-6                                                                                         | Biologend                                                                           | MQ2-13A5                 |
| <b>Biological samples</b>                                                                             |                                                                                     |                          |
| Serum samples from individuals with TB and LTBI                                                       | the National Institute for Infectious Diseases "L. Spallanzani", IRCCS, Rome, Italy |                          |
| Serum samples from individuals with TB and LTBI                                                       | Leiden University Medical Center (LUMC), the Netherlands                            |                          |
| Serum samples from healthy individuals and COVID-19 patients                                          | Leiden University Medical Center (LUMC), the Netherlands                            |                          |
| <b>Software and algorithms</b>                                                                        |                                                                                     |                          |
| UCP dedicated benchtop reader                                                                         | Labrox Oy                                                                           | Upcon                    |
| GraphPad Prism version 9.0.1                                                                          | GraphPad Software                                                                   |                          |
| <b>Other</b>                                                                                          |                                                                                     |                          |
| Polyacrylic acid functionalized UCPs (200 nm, NaYF <sub>4</sub> :Yb <sup>3+</sup> ,Er <sup>3+</sup> ) | Intelligent Material Solutions Inc.                                                 |                          |

## Resource availability

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Annemieke Geluk (A.Geluk@lumc.nl).

### Materials availability

This study did not generate new unique reagents.

## Data and code availability

- All biomarker data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

7

### Study participants

Study participants were consenting adults from three different cohorts (Tables S1 and S2):

#### TB cohort 1

Pre-COVID-19 biobanked serum samples from 30 pulmonary TB patients and 29 LTBI patients were recruited at the National Institute for Infectious Diseases “L. Spallanzani”, IRCCS, Rome, Italy, as described earlier [52,87,88]. Pulmonary TB patients were diagnosed based on sputum-culture (BACTEC, MGIT, Becton, Dickinson and Company (BD), Franklin Lakes, NJ, USA) or positive Xpert Mtb/RIF assay (Cepheid Inc., Sunnyvale, CA, USA) and included within 7 days after treatment was initiated. LTBI was identified by QuantiFERON-TB Gold-in tube positivity (Qiagen, The Netherlands) in healthy subjects without radiological signs of active disease. All individuals were HIV-negative.

#### TB cohort 2

20 pre-COVID-19 biobanked serum samples were obtained from 20 TB patients during or after treatment recruited at the Leiden University Medical Center (LUMC) in the Netherlands, as described earlier [89,90]. TB patients were either HIV-1 seronegative or had no risk factors for exposure to HIV. Ten TB patients were of European origin, three of Asian, and seven of African origin. Since the UCP-LFA for S100A12 was developed at a later point in time than the other six UCP-LFAs, it was evaluated with available LTBI samples from TB cohort 1 (n=11) combined with LTBI samples (n=18) from another European cohort (from TB cohort 2) and compared to 26 TB samples (TB cohort 1).

### COVID-19 patients from the LUMC BEAT-COVID cohort study

From April 2020 until January 2021, 102 adult participants were admitted to the LUMC with PCR-confirmed SARS-CoV-2 infection and recruited before or during treatment into the LUMC BEAT-COVID cohort study [91]. Of those 102 individuals, 30 were non-intensive care unit (ICU) patients (moderate disease), 44 were ICU patients (severe disease), and 28 had a fatal outcome due to COVID-19 during hospital stay, as described earlier.<sup>92</sup> 50 COVID-19 patients had already received anti-inflammatory treatment (i.e. betamethasone,

dexamethasone, hydrocortisone, methylprednisolone, and prednisolone) before the first timepoint of blood sample collection, whereas 52 had not. Informed consent was obtained and longitudinal serum sampling was performed for the duration of hospital admission. When possible, convalescent samples were obtained at outpatient follow-up appointments (n=25), around 6 weeks after hospital discharge. All COVID-19 patients were Dutch citizens.

## **Healthy controls**

Sera from 39 healthy controls were sampled before (n=27) and during (n=12) the COVID-19 pandemic (June/July 2020) at the LUMC, the Netherlands. The latter were sex (male:female ratio of 2:1) and age-matched to the COVID-19 patients, had no recent history of symptoms of airway infection (fever, cough, hypoxia, rhinorrhea, myalgia, anosmia and/or ageusia or fatigue) and were included in the BEAT-COVID study after confirmed negative SARS-CoV-2-PCR and IgG testing. All healthy controls were Dutch citizens.

## **Ethics**

This study was performed according to the Helsinki Declaration (7th revision, 64th Meeting, 2013, Fortaleza). Ethical approval of the study protocol was obtained through the Medical Ethical Committee Leiden-Den Haag-Delft (NL73740.058.20), registered in the Dutch Trial Registry as NL8589 (COVID-19 patients/healthy controls); the Ethical Committee of the L. Spallanzani National Institute of Infectious diseases (INMI; 02/2007 and 72/2015; TB cohort 1); the local Medical Ethics Committee of the Leiden University Medical Center (METC project nr: P07.048 & P207/99; TB cohort 2). Participants were informed about the study objectives, sampling protocol and their right to refuse to take part or withdraw from the study without consequences for their treatment at any point in time. Written informed consent was obtained before enrollment.

## **Method details**

### **Serum collection**

Venous blood samples were collected, in 4 ml plain BD vacutainer serum tubes (BD, Franklin Lakes, NJ, USA). Tubes were centrifuged at 2500 rpm for 10 min and sera were subsequently aliquoted and frozen (−80°C) until use.

### **Lateral flow strips**

4 mm width UCP-LF strips specific for a single host protein – ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 - were produced as described earlier.<sup>24,28,29</sup> For ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 LF strips, each Test (T) line comprised 200 ng of the following antibodies: goat-anti-human ApoA1 pAb (AF3664; R&D systems, Minneapolis, MN, USA), mouse-anti-human CRP mAb (C5; Labned.com, Amstelveen, the Netherlands), mouse-anti-human ferritin mAb (F31; Novus Biologicals, Littleton, CO, USA),

rat-anti-human IL-6 mAb (MQ2-39C3; Biolegend, San Diego, CA, USA), mouse-anti-human IP-10 mAb (B-C55; Diaclone Research, Besancon, France), mouse-anti-human SAA1/A2 mAb (865504; R&D systems, Minneapolis, MN, USA), and goat-anti-human S100A12 pAb (AF1052; R&D systems, Minneapolis, MN, USA), respectively. To detect non-target bound UCP-conjugated antibodies, Flow-Control (FC) lines comprised 100 ng goat-anti-mouse (M8642; Sigma-Aldrich, St. Louis, MO, USA; for CRP, ferritin, IP-10, and SAA1/A2), goat-anti-rabbit (R4880; Sigma-Aldrich, St. Louis, MO, USA; for ApoA1), goat-anti-rat (R5130; Sigma-Aldrich, St. Louis, MO, USA; for IL-6), or rabbit-anti-goat (G4018; Sigma-Aldrich, St. Louis, MO, USA; for S100A12).

### UCP conjugates

Antibodies were conjugated to luminescent up-converting reporter particles (UCP) allowing quantitative measurements [93–95]. Polyacrylic acid functionalized UCPs (200 nm, NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup>; Intelligent Material Solutions Inc., Princeton, NJ, USA) were conjugated according to previously described protocols [96]. Rabbit-anti-ApoA1 (2083A; R&D systems, Minneapolis, MN, USA), mouse-anti-CRP (CRP135; Labned.com, Amstelveen, Netherlands), mouse-anti-ferritin (F23; Novus Biologicals, Littleton, CO, USA), mouse-anti-IP-10 (B-C50; Diaclone Research, Besancon, France), mouse-anti-SAA1/A2 (924903; R&D systems, Minneapolis, MN, USA), and goat-anti-S100A12 (AF1052; R&D systems, Minneapolis, MN, USA) were bound at a concentration of 50 mg antibody per mg UCP. Rat-anti-IL-6 (MQ2-13A5; Biolegend, San Diego, CA, USA) was bound at a concentration of 25 mg per mg UCP. Stock solutions were kept at 4°C until use. UCPs were incorporated in the sample/conjugate pad at a density of 200 ng per 4mm (ApoA1, CRP, ferritin, IL-6, SAA1/A2, and S100A12) or 400 ng per 4 mm (IP-10).

### UCP-LFA

10-fold (ferritin, IL-6, and IP-10), 100-fold (S100A12), 1,000-fold (CRP and SAA1/A2), and 10,000-fold (ApoA1) serum dilutions were prepared in high salt buffer (100mM Tris pH 8, 270 mM NaCl, 1% (v/v) Triton X-100, 1% (w/v) BSA). 100 ml of diluted serum samples was added to a 96-wells plate and LF was initiated by placing the UCP-LF strip into the well. Immunochromatography was allowed to continue until strips were dry. UCP-LF strips were scanned with a UCP dedicated benchtop reader (UPCON; Labrox Oy Turku, Finland). Results are calculated as the ratio value (R) between Test and Flow Control signal based on relative fluorescence units (RFUs) measured at the respective lines.

### Quantification and statistical analysis

GraphPad Prism version 9.0.1 for Windows (GraphPad Software, San Diego, CA, USA) was used to perform statistical analysis. Mann-Whitney U tests and Kruskal-Wallis tests were performed to determine the statistical significance between two and three independent groups, respectively. Wilcoxon matched pairs signed rank tests and Friedman tests with

Dunn's correction were performed to determine the statistical significance between two and three paired timepoints, respectively. Plot receiver operating characteristic (ROC) curves were created and sensitivity (Sn), specificity (Sp) and the area under the curve (AUC) were calculated to evaluate test performance. A cut-off for positivity for each biomarker was determined by calculating the maximal Youden's index [97].

For each of the individuals tested, an extra parameter (NUM score) [31], was calculated, representing the number of biomarkers that scored above the threshold of positivity based on the Youden's index. Three comparisons were made: TB vs. LTBI, COVID-19 vs. healthy controls, and COVID-19 vs. TB. For each comparison, a NUM score was calculated with the number of biomarkers used ranging from 1 to 7.

## References

1. WHO Coronavirus (COVID-19) Dashboard (2021) (WHO). <https://covid19.who.int/>.
2. Sethuraman, N., Jeremiah, S.S., and Ryo, A. (2020). Interpreting diagnostic tests for SARS-CoV-2. *JAMA* 323, 2249–2251.
3. Coronavirus disease (COVID-19) (2021) (WHO). [https://www.who.int/health-topics/coronavirus#tab=tab\\_1](https://www.who.int/health-topics/coronavirus#tab=tab_1).
4. Huang, C.L., Wang, Y.M., Li, X.W., Ren, L.L., Zhao, J.P., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., and Cheng, Z. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506.
5. Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513.
6. Ferrando, C., Suarez-Sipmann, F., Mellado-Artigas, R., Hernandez, M., Gea, A., Arruti, E., Aldecoa, C., Martínez-Pallí, G., Martínez-González, M.A., Slutsky, A.S., and Villar, J. (2021). Clinical features, ventilatory management, and outcome of ARDS caused by COVID-19 are similar to other causes of ARDS. *Intensive Care Med.* 47, 144–146.
7. Grasselli, G., Zangrillo, A., Zanella, A., Antonelli, M., Cabrini, L., Castelli, A., Cereda, D., Coluccello, A., Foti, G., Fumagalli, R., et al. (2020). Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the lombardy region. *JAMA* 323, 1574–1581.
8. Petrone, L., Petruccioli, E., Vanini, V., Cuzzi, G., Fard, S.N., Alonzi, T., Castilletti, C., Palmieri, F., Gualano, G., Vittozzi, P., and Nicastrì, E. (2021). A whole blood test to measure SARS-CoV-2-specific response in COVID-19 patients. *Clin. Microbiol. Infect.* 27.
9. Tuberculosis (2021) (WHO). [https://www.who.int/health-topics/tuberculosis#tab=tab\\_1](https://www.who.int/health-topics/tuberculosis#tab=tab_1).
10. Houben, R.M.G.J., and Dodd, P.J. (2016). The global burden of latent tuberculosis infection: a Re-estimation using mathematical modelling. *PLoS Med.* 13, e1002152.
11. Global Tuberculosis Report 2019 (2021) (WHO). [http://www.who.int/tb/publications/global\\_report/en/](http://www.who.int/tb/publications/global_report/en/).
12. Johnson, J.L., Vjecha, M.J., Okwera, A., Hatanga, E., Byekwaso, F., Wolski, K., Aisu, T., Whalen, C.C., Huebner, R., Mugerwa, R.D., and Ellner, J.J. (1998). Impact of human immunodeficiency virus type-1 infection on the initial bacteriologic and radiographic manifestations of pulmonary tuberculosis in Uganda. *Int. J. Tubercul. Lung Dis.* 2, 397–404.
13. Goletti, D., Lee, M.R., Wang, J.Y., Walter, N., and Ottenhoff, T.H.M. (2018). Update on tuberculosis biomarkers: from correlates of risk, to correlates of active disease and of cure from disease. *Respirology* 23, 455–466.
14. Togun, T., Hoggart, C.J., Agbla, S.C., Gomez, M.P., Egere, U., Sillah, A.K., Saidy, B., Mendy, F., Pai, M., and Kampmann, B. (2020). A three-marker protein biosignature distinguishes tuberculosis from other respiratory diseases in Gambian children. *EBioMedicine* 58, 102909.
15. Brent, A.J., Mugo, D., Musyimi, R., Mutiso, A., Morpeth, S.C., Levin, M., and Scott, J.A.G. (2018). Bacteriological diagnosis of childhood TB: a prospective observational study. *Sci. Rep.* 8, 7223.
16. Goletti, D., Petruccioli, E., Joosten, S.A., and Ottenhoff, T.H. (2016). Tuberculosis biomarkers: from diagnosis to protection. *Infect. Dis. Rep.* 8, 6568–6632.
17. Cazabon, D., Pande, T., Kik, S., Van Gemert, W., Sohn, H., Denking, C., Qin, Z.Z., Waning, B., and Pai, M. (2018). Market penetration of Xpert MTB/RIF in high tuberculosis burden countries: a trend analysis from 2014-2016. *Gates Open Res.* 2, 35.

18. Helb, D., Jones, M., Story, E., Boehme, C., Wallace, E., Ho, K., Kop, J., Owens, M.R., Rodgers, R., Banada, P., et al. (2010). Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J. Clin. Microbiol.* 48, 229–237.
19. Global Tuberculosis Report 2021 (2021) (WHO). <https://www.who.int/publications/item/9789240037021>.
20. Casco, N., Jorge, A.L., Palmero, D.J., Alffenaar, J.W., Denholm, J., Fox, G.J., Wafaa, E., Jin-Gun, C., Alena, S., Varvara, S., and Pierre, B. (2022). Tuberculosis and COVID-19 co-infection: description of the global cohort. *Eur. Respir. J.* 59.
21. Migliori, G.B., Thong, P.M., Alffenaar, J.W., Denholm, J., Tadolini, M., Alyaquobi, F., Blanc, F.X., Buonsenso, D., Cho, J.G., Codecasa, L.R., et al. (2021). Gauging the impact of the COVID-19 pandemic on tuberculosis services: a global study. *Eur. Respir. J.* 58, 2101786.
22. Visca, D., Ong, C.W.M., Tiberi, S., Centis, R., D'Ambrosio, L., Chen, B., Mueller, J., Mueller, P., Duarte, R., D'alcolmo, M., et al. (2021). Tuberculosis and COVID-19 interaction: a review of biological, clinical and public health effects. *Pulmonology* 27, 151–165.
23. Gao, Y., Liu, M., Chen, Y., Shi, S., Geng, J., and Tian, J. (2021). Association between tuberculosis and COVID-19 severity and mortality: a rapid systematic review and meta-analysis. *J. Med. Virol.* 93, 194–196.
24. van Hooij, A., Tjon Kon Fat, E.M., Richardus, R., van den Eeden, S.J.F., Wilson, L., de Dood, C.J., Faber, R., Alam, K., Richardus, J.H., Corstjens, P.L.A.M., and Geluk, A. (2016). Quantitative lateral flow strip assays as user-friendly tools to detect biomarker profiles for leprosy. *Sci. Rep.* 6, 34260.
25. van Hooij, A., Tjon Kon Fat, E.M., van den Eeden, S.J.F., Wilson, L., Batista da Silva, M., Salgado, C.G., Spencer, J.S., Corstjens, P.L.A.M., and Geluk, A. (2017). Field-friendly serological tests for determination of *M. leprae*-specific antibodies. *Sci. Rep.* 7, 8868.
26. Corstjens, P.L.A.M., van Hooij, A., Tjon Kon Fat, E.M., van den Eeden, S.J.F., Wilson, L., and Geluk, A. (2016). Field-friendly test for monitoring multiple immune response markers during onset and treatment of exacerbated immunity in leprosy. *Clin. Vaccine Immunol.* 23, 515–519.
27. Bobosha, K., Tjon Kon Fat, E.M., van den Eeden, S.J.F., Bekele, Y., van der Ploeg-van Schip, J.J., de Dood, C.J., Dijkman, K., Franken, K.L.M.C., Wilson, L., Aseffa, A., et al. (2014). Field-Evaluation of a new lateral flow assay for detection of cellular and humoral immunity against *Mycobacterium leprae*. *PLoS Neglected Trop. Dis.* 8, e2845.
28. van Hooij, A., Tjon Kon Fat, E.M., Batista da Silva, M., Carvalho Bouth, R., Cunha Messias, A.C., Gobbo, A.R., Lema, T., Bobosha, K., Li, J., Weng, X., et al. (2018). Evaluation of immunodiagnostic tests for leprosy in Brazil, China and Ethiopia. *Sci. Rep.* 8, 17920.
29. van Hooij, A., van den Eeden, S., Richardus, R., Tjon Kon Fat, E., Wilson, L., Franken, K.L.M.C., Faber, R., Khatun, M., Alam, K., Sufian Chowdhury, A., et al. (2019). Application of new host biomarker profiles in quantitative point-of-care tests facilitates leprosy diagnosis in the field. *EBioMedicine* 47, 301–308.
30. Schilling, A.K., van Hooij, A., Corstjens, P., Lurz, P.W.W., DelPozo, J., Stevenson, K., Meredith, A., and Geluk, A. (2019). Detection of humoral immunity to mycobacteria causing leprosy in Eurasian red squirrels (*Sciurus vulgaris*) using a quantitative rapid test. *Eur. J. Wildl. Res.* 65, 49.
31. van Hooij, A., Tjon Kon Fat, E.M., de Jong, D., Khatun, M., Soren, S., Chowdhury, A.S., Chandra Roy, J., Alam, K., Kim, J.P., Richardus, J.H., et al. (2021). Prototype multibiomarker test for point-of-care leprosy diagnostics. *iScience* 24, 102006.
32. Sutherland, J.S., Mendy, J., Gindeh, A., Walzl, G., Togun, T., Owolabi, O., Donkor, S., Ota, M.O., Kon Fat, E.T., Ottenhoff, T.H.M., et al. (2016). Use of lateral flow assays to determine IP-10 and CCL4 levels in pleural effusions and whole blood for TB diagnosis. *Tuberculosis* 96, 31–36.
33. Chen, Z., Abrams, W.R., Geva, E., de Dood, C.J., González, J.M., Tanke, H.J., Niedbala, R.S., Zhou, P., Malamud, D., and Corstjens, P.L.A.M. (2013). Development of a generic microfluidic device for simultaneous detection of antibodies and nucleic acids in oral fluids. *BioMed Res. Int.* 2013, 543294.
34. Corstjens, P.L.A.M., van Hooij, A., Tjon Kon Fat, E.M., Alam, K., Vrolijk, L.B., Dlamini, S., da Silva, M.B., Spencer, J.S., Salgado, C.G., Richardus, J.H., et al. (2019). Fingerstick test quantifying humoral and cellular biomarkers indicative for *M. leprae* infection. *Clin. Biochem.* 66, 76–82.
35. Corstjens, P.L.A.M., Tjon Kon Fat, E.M., de Dood, C.J., van der Ploeg-van Schip, J.J., Franken, K.L.M.C., Chegou, N.N., Sutherland, J.S., Howe, R., Mihret, A., Kassa, D., et al. (2016). Multi-center evaluation of a user-friendly lateral flow assay to determine IP-10 and CCL4 levels in blood of TB and non-TB cases in Africa. *Clin. Biochem.* 49, 22–31.
36. Chegou, N.N., Sutherland, J.S., Malherbe, S., Crampin, A.C., Corstjens, P.L.A.M., Geluk, A., Mayanja-Kizza, H., Loxton, A.G., van der Spuy, G., Stanley, K., et al. (2016). Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB. *Thorax* 71, 785–794.
37. Misra, U.K., Kalita, J., Srivastava, R., Nair, P.P., Mishra, M.K., and Basu, A. (2010). A study of cytokines in tuberculous meningitis: clinical and MRI correlation. *Neurosci. Lett.* 483, 6–10.



38. Manyelo, C.M., Solomons, R.S., Snyders, C.I., Mutavhatsindi, H., Manngo, P.M., Stanley, K., Walzl, G., and Chegou, N.N. (2019). Potential of host serum protein biomarkers in the diagnosis of tuberculous meningitis in children. *Front. Pediatr.* 7, 376.
39. Foell, D., Wittkowski, H., Vogl, T., and Roth, J. (2007). S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. *J. Leukoc. Biol.* 81, 28–37.
40. Pandey, R., and Rodriguez, G.M. (2012). A ferritin mutant of *Mycobacterium tuberculosis* is highly susceptible to killing by antibiotics and is unable to establish a chronic infection in mice. *Infect. Immun.* 80, 3650–3659.
41. Corstjens, P.L.A.M., de Dood, C.J., van der Ploeg-van Schip, J.J., Wiesmeijer, K.C., Riuttama<sup>ki</sup>, T., van Meijgaarden, K.E., Spencer, J.S., Tanke, H.J., Ottenhoff, T.H.M., and Geluk, A. (2011). Lateral flow assay for simultaneous detection of cellular- and humoral immune responses. *Clin. Biochem.* 44, 1241–1246.
42. Corstjens, P.L.A.M., Zuiderwijk, M., Tanke, H.J., van der Ploeg-van Schip, J.J., Ottenhoff, T.H.M., and Geluk, A. (2008). A user-friendly, highly sensitive assay to detect the IFN-gamma secretion by T cells. *Clin. Biochem.* 41, 440–444.
43. TB and migration (2022 (WHO). <https://www.euro.who.int/en/health-topics/communicable-diseases/tuberculosis/areas-of-work/vulnerable-populations-risk-factors-and-social-determinants/tb-and-migration>.
44. Tuberculose (2022 (Rijksinstituut voor Volksgezondheid en Milieu). <https://lci.rivm.nl/richtlijnen/tuberculose>.
45. Ukraine TB Patients First To Receive Groundbreaking New Drug Regimen 2020 (2022) (KNCV Tuberculosis Foundation). <https://www.kncvtbc.org/en/2020/11/23/ukraine-tb-patients-first-to-receivegroundbreaking-new-drug-regimen/>.
46. Rustage, K., Lobe, J., Hayward, S.E., Kristensen, K.L., Margineanu, I., Stienstra, Y., Goletti, D., Zenner, D., Noori, T., Pareek, M., et al. (2021). Initiation and completion of treatment for latent tuberculosis infection in migrants globally: a systematic review and meta-analysis. *Lancet Infect. Dis.* 21, 1701–1712.
47. Holt, E. (2022). A new treatment for drug-resistant tuberculosis in Ukraine. *Lancet Infect. Dis.* 22, 23.
48. World Tuberculosis Day (2022). Supporting Ukraine in Scaling Up TB Diagnosis And Treatment 2021 (WHO). [https://www.euro.who.int/en/countries/ukraine/news/news/2021/3/world-tuberculosis-day-supportingukraine-in-scaling-up-tb-diagnosis-andtreatment#:~:text=Drug%2Dresistant%20TB%20\(DR%2D,46%25%20of%20previously%20treated%20patients](https://www.euro.who.int/en/countries/ukraine/news/news/2021/3/world-tuberculosis-day-supportingukraine-in-scaling-up-tb-diagnosis-andtreatment#:~:text=Drug%2Dresistant%20TB%20(DR%2D,46%25%20of%20previously%20treated%20patients).
49. Essone, P.N., Chegou, N.N., Loxton, A.G., Stanley, K., Kriel, M., van der Spuy, G., Franken, K.L., Ottenhoff, T.H., and Walzl, G. (2014). Host cytokine responses induced after overnight stimulation with novel *M. tuberculosis* infection phase-dependent antigens show promise as diagnostic candidates for TB disease. *PLoS One* 9, e102584.
50. Santos, V.S., Goletti, D., Kontogianni, K., Adams, E.R., Molina-Moya, B., Dominguez, J., Crudu, V., Martins-Filho, P.R.S., Ruhwald, M., Lawson, L., et al. (2019). Acute phase proteins and IP-10 as triage tests for the diagnosis of tuberculosis: systematic review and meta-analysis. *Clin. Microbiol. Infect.* 25, 169–177.
51. Smith, S.G., Kleinnijenhuis, J., Netea, M.G., and Dockrell, H.M. (2017). Whole blood profiling of *Bacillus calmette-guerin*-induced trained innate immunity in infants identifies epidermal growth factor, IL-6, platelet derived growth factor-AB/BB, and natural killer cell activation. *Front. Immunol.* 8, 644.
52. Lubbers, R., Sutherland, J.S., Goletti, D., de Paus, R.A., van Moorsel, C.H.M., Veltkamp, M., Vestjens, S.M.T., Bos, W.J.W., Petrone, L., Del Nonno, F., et al. (2018). Complement component C1q as serum biomarker to detect active tuberculosis. *Front. Immunol.* 9, 2427.
53. Ehrling, C., Wolf, S.D., and Bode, J.G. (2021). Acute-phase protein synthesis: a key feature of innate immune functions of the liver. *Biol. Chem.* 402, 1129–1145.
54. Debeer, F.C., Nel, A.E., Gie, R.P., Donald, P.R., and Strachan, A.F. (1984). Serum amyloid-a protein and C-R. protein-levels in pulmonary tuberculosis - relationship to amyloidosis. *Thorax* 39, 196–200.
55. Visser, A., and van de Vyver, A. (2011). Severe hyperferritinemia in mycobacteria tuberculosis infection. *Clin. Infect. Dis.* 52, 273–274.
56. Kotru, U., Rusia, U., Sikka, M., Chaturvedi, S., and Jain, A.K. (2004). Evaluation of serum ferritin in screening for iron deficiency in tuberculosis. *Ann. Hematol.* 83, 95–100.
57. Blauenfeldt, T., Petrone, L., del Nonno, F., Baiocchi, A., Falasca, L., Chiacchio, T., Bondet, V., Vanini, V., Palmieri, F., Galluccio, G., et al. (2018). Interplay of DDP4 and IP-10 as a potential mechanism for cell recruitment to tuberculosis lesions. *Front. Immunol.* 9, 1456.
58. Petrone, L., Bondet, V., Vanini, V., Cuzzi, G., Palmieri, F., Palucci, I., Delogu, G., Ciccocanti, F., Fimia, G.M., Blauenfeldt, T., et al. (2019). First description of agonist and antagonist IP-10 in urine of patients with active TB. *Int. J. Infect. Dis.* 78, 15–21.
59. Petrone, L., Cannas, A., Aloï, F., Nsubuga, M., Sserumkuma, J., Nazziwa, R.A., Jugheli, L., Lukindo, T., Girardi, E., Reither, K., and Goletti, D. (2015). Blood or urine IP-10 cannot discriminate between active tuberculosis and respiratory diseases different from tuberculosis in children. *BioMed Res. Int.*

60. Petrone, L., Cannas, A., Vanini, V., Cuzzi, G., Aloï, F., Nsubuga, M., Sserunkuma, J., Nazziwa, R.A., Jugheli, L., Lukindo, T., et al. (2016). Blood and urine inducible protein 10 as potential markers of disease activity. *Int. J. Tubercul. Lung Dis.* 20, 1554–1561.
61. Geluk, A., and Corstjens, P. (2017). tell-tale biomarker or common denominator? *Lancet Infect. Dis.* 17, 1225–1227.
62. Zinellu, A., Paliogiannis, P., Carru, C., and Mangoni, A.A. (2021). Serum amyloid A concentrations, COVID-19 severity and mortality: an updated systematic review and meta-analysis. *Int. J. Infect. Dis.* 105, 668–674.
63. Chen, Y., Wang, J., Liu, C., Su, L., Zhang, D., Fan, J., Yang, Y., Xiao, M., Xie, J., Xu, Y., et al. (2020). IP-10 and MCP-1 as biomarkers associated with disease severity of COVID-19. *Mol. Med.* 26, 97.
64. Vanini, V., Petruccioli, E., Gioia, C., Cuzzi, G., Orchi, N., Rianda, A., Alba, L., Giancola, M.L., Conte, A., Schinina, V., et al. (2012). IP-10 is an additional marker for tuberculosis (TB) detection in HIV-infected persons in a low-TB endemic country. *J. Infect.* 65, 49–59.
65. Rubin, E.J., Longo, D.L., and Baden, L.R. (2021). Interleukin-6 receptor inhibition in covid-19-cooling the inflammatory soup. *N. Engl. J. Med.* 384, 1564–1565.
66. Gao, Y., Li, T., Han, M., Li, X., Wu, D., Xu, Y., Zhu, Y., Liu, Y., Wang, X., and Wang, L. (2020). Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J. Med. Virol.* 92, 791–796.
67. Mo, P., Xing, Y., Xiao, Y., Deng, L., Zhao, Q., Wang, H., Xiong, Y., Cheng, Z., Gao, S., Liang, K., et al. (2021). Clinical characteristics of refractory coronavirus disease 2019 in wuhan, China. *Clin. Infect. Dis.* 73, E4208–E4213.
68. Pretorius, E., Vlok, M., Venter, C., Bezuidenhout, J.A., Laubscher, G.J., Steenkamp, J., and Kell, D.B. (2021). Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. *Cardiovasc. Diabetol.* 20, 172.
69. Kaushal, K., Kaur, H., Sarma, P., Bhattacharyya, A., Sharma, D.J., Prajapat, M., Pathak, M., Kothari, A., Kumar, S., Rana, S., et al. (2022). Serum ferritin as a predictive biomarker in COVID-19. A systematic review, meta-analysis and meta-regression analysis. *J. Crit. Care* 67, 172–181.
70. Yang, Y., Shen, C., Li, J., Yuan, J., Wei, J., Huang, F., Wang, F., Li, G., Li, Y., Xing, L., et al. (2020). Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. *J. Allergy Clin. Immunol.* 146, 119–127.e4.
71. Poynard, T., Deckmyn, O., Rudler, M., Peta, V., Ngo, Y., Vautier, M., Akhavan, S., Calvez, V., Franc, C., Castille, J.M., et al. (2020). Performance of serum apolipoprotein-A1 as a sentinel of Covid-19. *PLoS One* 15, e0242306.
72. Nie, S., Zhao, X., Zhao, K., Zhang, Z., Zhang, Z., and Zhang, Z. (2020). Metabolic disturbances and inflammatory dysfunction predict severity of coronavirus disease 2019 (COVID-19): a retrospective study. Preprint at medRxiv. <https://doi.org/10.1101/2020.03.24.20042283>.
73. Lei, H. (2021). A single transcript for the prognosis of disease severity in COVID-19 patients. *Sci. Rep.* 11, 12174.
74. Hyka, N., Dayer, J.M., Modoux, C., Kohno, T., Edwards, C.K., Roux-Lombard, P., and Burger, D. (2001). Apolipoprotein A-I inhibits the production of interleukin-1 beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood* 97, 2381–2389.
75. Montecucco, F., Favari, E., Norata, G.D., Ronda, N., Nofer, J.R., and Vuilleumier, N. (2015). Impact of systemic inflammation and autoimmune diseases on apoA-I and HDL plasma levels and functions. *Handb. Exp. Pharmacol.* 224, 455–482.
76. Simon, L., Gauvin, F., Amre, D.K., Saint-Louis, P., and Lacroix, J. (2004). Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clin. Infect. Dis.* 39, 206–217.
77. Sproston, N.R., and Ashworth, J.J. (2018). Role of C-reactive protein at sites of inflammation and infection. *Front. Immunol.* 9, 754.
78. Fajgenbaum, D.C., and June, C.H. (2020). Cytokine storm. *N. Engl. J. Med.* 383, 2255–2273.
79. Hojyo, S., Uchida, M., Tanaka, K., Hasebe, R., Tanaka, Y., Murakami, M., and Hirano, T. (2020). How COVID-19 induces cytokine storm with high mortality. *Inflamm. Regen.* 40, 37.
80. Ferraccioli, G., Gremese, E., Goletti, D., Petrone, L., Cantini, F., Ugel, S., Canè, S., and Bronte, V. (2022). Immune-guided therapy of COVID-19. *Cancer Immunol. Res.* 10, 384–402.
81. Rijnink, W.F., Ottenhoff, T.H.M., and Joosten, S.A. (2021). B-cells and antibodies as contributors to effector immune responses in tuberculosis. *Front. Immunol.* 12, 640168.
82. Melkie, S.T., Arias, L., Farroni, C., Jankovic Makek, M., Goletti, D., and Vilaplana, C. (2022). The role of antibodies in tuberculosis diagnosis, prophylaxis and therapy: a review from the ESGMYC study group. *Eur. Respir. Rev.* 31, 210218.
83. WHO Consolidated Guidelines On Tuberculosis (2021). Module 2: Screening – Systematic Screening For Tuberculosis Disease (WHO). <https://www.who.int/publications/i/item/9789240022676>.



84. Ali, N. (2020). Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19. *J. Med. Virol.* 92, 2409–2411.
85. Cheng, L., Yang, J.Z., Bai, W.H., Li, Z.Y., Sun, L.F., Yan, J.J., Zhou, C.L., and Tang, B.P. (2020). Prognostic value of serum amyloid A in patients with COVID-19. *Infection* 48, 715–722.
86. QuantiFERON-TB Gold. QIAGEN. <https://www.quantiferon.com/products/quantiferon-tb-gold/>.
87. Lubbers, R., Sutherland, J.S., Goletti, D., de Paus, R.A., Dijkstra, D.J., van Moorsel, C.H.M., Veltkamp, M., Vestjens, S.M.T., Bos, W.J.W., Petrone, L., et al. (2020). Expression and production of the SERPING1-encoded endogenous complement regulator C1-inhibitor in multiple cohorts of tuberculosis patients. *Mol. Immunol.* 120, 187–195.
88. Joosten, S.A., van Meijgaarden, K.E., del Nonno, F., Baiocchi, A., Petrone, L., Vanini, V., Smits, H.H., Palmieri, F., Goletti, D., and Ottenhoff, T.H.M. (2016). Patients with tuberculosis have a dysfunctional circulating B-cell compartment, which normalizes following successful treatment. *PLoS Pathog.* 12, e1005687.
89. Lin, M.Y., Geluk, A., Smith, S.G., Stewart, A.L., Friggen, A.H., Franken, K.L.M.C., Verduyn, M.J.C., van Meijgaarden, K.E., Voskuil, M.I., Dockrell, H.M., et al. (2007). Lack of immune responses to *Mycobacterium tuberculosis* DosR regulon proteins following *Mycobacterium bovis* BCG vaccination. *Infect. Immun.* 75, 3523–3530.
90. Leyten, E.M.S., Lin, M.Y., Franken, K.L.M.C., Friggen, A.H., Prins, C., van Meijgaarden, K.E., Voskuil, M.I., Weldingh, K., Andersen, P., Schoolnik, G.K., et al. (2006). Human T-cell responses to 25 novel antigens encoded by genes of the dormancy regulon of *Mycobacterium tuberculosis*. *Microb. Infect.* 8, 2052–2060.
91. Roukens, A.H.E., Pothast, C.R., König, M., Huisman, W., Dalebout, T., Tak, T., Azimi, S., Kruize, Y., Hagedoorn, R.S., Zlei, M., and Staal, F.J. (2021). Prolonged activation of nasal immune cell populations and development of tissue-resident SARS-CoV-2 specific CD8 T cell responses following COVID-19. Preprint at medRxiv. <https://doi.org/10.1101/2021.04.19.21255727>.
92. Pongracz, T., Nouta, J., Wang, W., van Meijgaarden, K.E., Linty, F., Vidarsson, G., Joosten, S.A., Ottenhoff, T.H.M., Hokke, C.H., de Vries, J.J.C., et al. (2022). Immunoglobulin G1 Fc glycosylation as an early hallmark of severe COVID-19. *EBioMedicine* 78, 103957.
93. Corstjens, P.L.A.M., Zuiderwijk, M., Nilsson, M., Feindt, H., Sam Niedbala, R., and Tanke, H.J. (2003). Lateral-flow and up-converting phosphor reporters to detect single-stranded nucleic acids in a sandwich-hybridization assay. *Anal. Biochem.* 312, 191–200.
94. Corstjens, P.L.A.M., Li, S., Zuiderwijk, M., Kardos, K., Abrams, W.R., Niedbala, R.S., and Tanke, H.J. (2005). Infrared up-converting phosphors for bioassays. *IEE Proc. - Nanobiotechnol.* 152, 64–72.
95. Zuiderwijk, M., Tanke, H.J., Sam Niedbala, R., and Corstjens, P.L.A.M. (2003). Sam Niedbala R, Corstjens PL. An amplification-free hybridization-based DNA assay to detect *Streptococcus pneumoniae* utilizing the upconverting phosphor technology. *Clin. Biochem.* 36, 401–403.
96. Corstjens, P.L.A.M., de Dood, C.J., Priest, J.W., Tanke, H.J., and Handali, S.; Cysticercosis Working Group in Peru (2014). Feasibility of a lateral flow test for neurocysticercosis using novel up-converting nanomaterials and a lightweight strip analyzer. *PLoS Neglected Trop. Dis.* 8, e2944.
97. Fluss, R., Faraggi, D., and Reiser, B. (2005). Estimation of the Youden Index and its associated cutoff point. *Biom. J.* 47, 458–472.

## Supplemental information

| SUPPLEMENTARY TABLE S1    |                     | Description of the cohorts, Related to Figures 1-4 and Tables 1-2 |     |                  |                |
|---------------------------|---------------------|-------------------------------------------------------------------|-----|------------------|----------------|
| Cohort                    | Country of sampling | Group                                                             | N   | Age mean (range) | Sex (% female) |
| TB cohort 1               | Italy               | TB                                                                | 30  | 34 (18 – 52)     | 43             |
|                           |                     | LTBI                                                              | 29  | 45 (19 – 75)     | 69             |
| TB cohort 2               | The Netherlands     | TB                                                                | 20  | 44 (15 – 84)     | 20             |
|                           |                     | LTBI                                                              | 18  | 35 (18 – 60)     | 61             |
| COVID-19/healthy controls | The Netherlands     | COVID-19                                                          | 102 | 63 (18 – 91)     | 23             |
|                           |                     | healthy controls                                                  | 39  | 42 (22 – 72)     | 59             |
|                           |                     | pre-COVID-19                                                      | 27  | 33 (22 – 60)     | 70             |
|                           |                     | during COVID-19                                                   | 12  | 64 (60 – 72)     | 33             |

Overview of the different cohorts including country of sampling, group, number of samples per group, age, and sex. COVID-19: coronavirus disease 2019; LTBI: latent tuberculosis infection; TB: tuberculosis.

| SUPPLEMENTARY TABLE S2    |       | Country of origin of the participants,<br>Related to Figures 1-4 and Tables 1-2 |     |
|---------------------------|-------|---------------------------------------------------------------------------------|-----|
| Cohort                    | Group | Country of origin                                                               | N   |
| TB cohort 1               | TB    | Albania                                                                         | 2   |
|                           |       | Colombia                                                                        | 1   |
|                           |       | Georgia                                                                         | 1   |
|                           |       | Italy                                                                           | 10  |
|                           |       | Kosovo                                                                          | 1   |
|                           |       | Moldova                                                                         | 1   |
|                           |       | Romania                                                                         | 14  |
|                           | LTBI  | Afghanistan                                                                     | 1   |
|                           |       | Colombia                                                                        | 1   |
|                           |       | Italy                                                                           | 20  |
|                           |       | Lithuania                                                                       | 1   |
|                           |       | Peru                                                                            | 1   |
|                           |       | Romania                                                                         | 5   |
| TB cohort 2               | TB    | Angola                                                                          | 2   |
|                           |       | Italy                                                                           | 1   |
|                           |       | Jakarta                                                                         | 2   |
|                           |       | Morocco                                                                         | 2   |
|                           |       | the Netherlands                                                                 | 9   |
|                           |       | Somalia                                                                         | 2   |
|                           |       | South Africa                                                                    | 1   |
|                           | LTBI  | Sri Lanka                                                                       | 1   |
|                           |       | France                                                                          | 1   |
|                           |       | the Netherlands                                                                 | 14  |
|                           |       | Netherlands Antilles                                                            | 1   |
|                           |       | Sri Lanka                                                                       | 2   |
|                           |       |                                                                                 |     |
| COVID-19/healthy controls |       | Dutch citizens*                                                                 | 141 |

Overview of the country of origin for the participants in the different cohorts. \*Individuals from the COVID-19/healthy controls cohort were all Dutch citizens. COVID-19: coronavirus disease 2019; LTBI: latent tuberculosis infection; TB: tuberculosis.

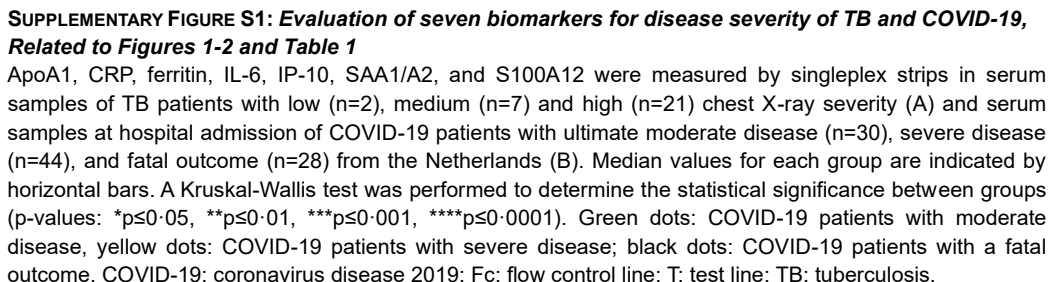
**SUPPLEMENTARY TABLE S3** *Overview of different NUM scores per cohort comparison with corresponding AUC, sensitivity, and specificity, Related to Figures 1-3 and Table 2*

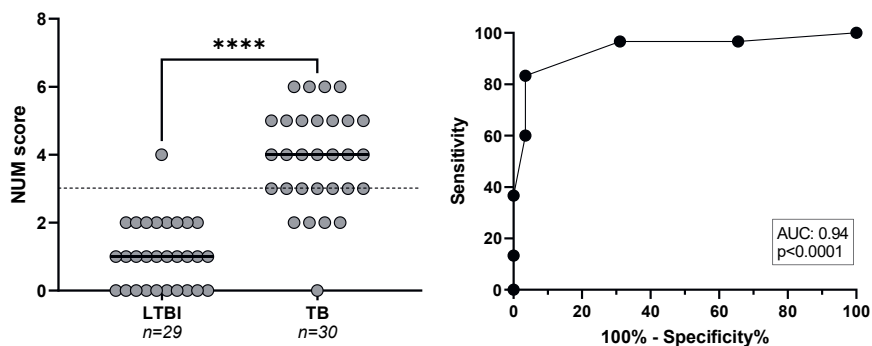
| Cohort comparison             | # of markers used in NUM score | Markers used in NUM score                           | AUC  | Cut-off | Sn/Sp    |
|-------------------------------|--------------------------------|-----------------------------------------------------|------|---------|----------|
| TB vs. LTBI                   | 6                              | ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2          | 0.94 | ≥3      | 83%/97%  |
|                               | 5                              | CRP, ferritin, IL-6, IP-10, SAA1/A2                 | 0.93 | ≥2      | 87%/86%  |
|                               | 4                              | CRP, IL-6, IP-10, SAA1/A2                           | 0.93 | ≥2      | 87%/86%  |
|                               | 3                              | CRP, IL-6, SAA1/A2                                  | 0.94 | ≥2      | 80%/97%  |
|                               | 2                              | CRP, SAA1/A2                                        | 0.91 | ≥1      | 83%/97%  |
|                               | 1                              | CRP*                                                | 0.87 | ≥1      | 77%/97%  |
|                               | 1                              | SAA1/A2*                                            | 0.87 | ≥1      | 100%/77% |
| COVID-19 vs. healthy controls | 7                              | ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, S100A12 | 0.99 | ≥4      | 93%/100% |
|                               | 6                              | ApoA1, CRP, ferritin, IP-10, SAA1/A2, S100A12       | 1.00 | ≥3      | 98%/100% |
|                               | 5                              | ApoA1, CRP, ferritin, SAA1/A2, S100A12              | 1.00 | ≥3      | 95%/100% |
|                               | 4                              | CRP, ferritin, SAA1/A2, S100A12                     | 0.99 | ≥2      | 96%/97%  |
|                               | 3                              | CRP, ferritin, SAA1/A2                              | 0.99 | ≥2      | 95%/97%  |
|                               | 2                              | ferritin, SAA1/A2                                   | 0.98 | ≥1      | 99%/92%  |
|                               | 1                              | SAA1/A2                                             | 0.98 | ≥1      | 92%/97%  |
| COVID-19 vs. TB               | 7                              | ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, S100A12 | 0.95 | ≥4      | 91%/87%  |
|                               | 6                              | ApoA1, CRP, ferritin, IL-6, SAA1/A2, S100A12        | 0.95 | ≥3      | 98%/83%  |
|                               | 5                              | ApoA1, CRP, ferritin, SAA1/A2, S100A12              | 0.94 | ≥3      | 93%/83%  |
|                               | 4                              | ApoA1, CRP, SAA1/A2, S100A12                        | 0.95 | ≥3      | 88%/89%  |
|                               | 3                              | CRP, SAA1/A2, S100A12                               | 0.94 | ≥2      | 90%/87%  |
|                               | 2                              | CRP, SAA1/A2                                        | 0.93 | ≥1      | 94%/80%  |
|                               | 1                              | CRP                                                 | 0.93 | ≥1      | 78%/96%  |

Overview of the different NUM scores per cohort comparison with corresponding AUC, sensitivity, and specificity. A cut-off ratio for positivity for each biomarker was determined by calculating the maximal Youden's index. Using the cut-offs for positivity, a NUM score was calculated; the number of biomarkers that scored above the threshold of positivity. Three comparisons were made: TB vs. LTBI, COVID-19 vs. healthy controls, and COVID-19 vs. TB. Biomarkers were deleted from the NUM score based on their contribution (AUC); the biomarker with the lowest AUC was removed first, ending up with the most discriminatory marker for that comparison. \*Since CRP and SAA1/A2 both showed an AUC of 0.87 in the comparison of TB vs. LTBI, both options as a final marker remaining are shown. AUC: area under the curve; COVID-19: coronavirus disease 2019; LTBI: latent tuberculosis infection; Sn/Sp: sensitivity/specificity; TB: tuberculosis.

**B**

## 170



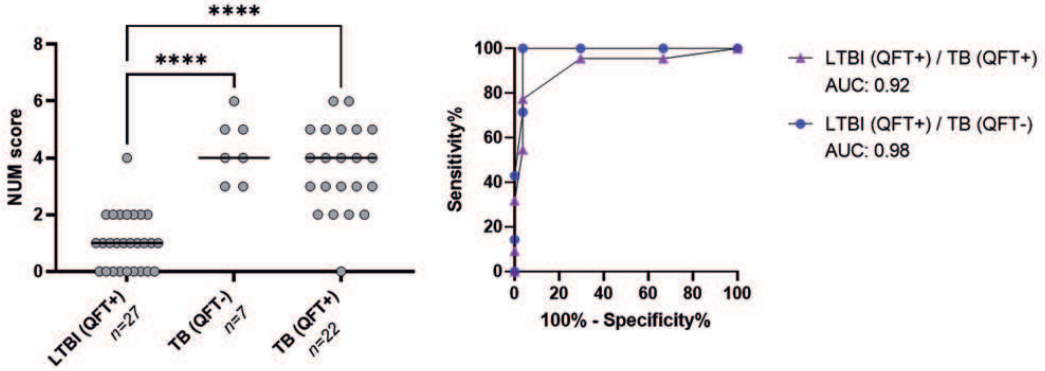


| Cut-off    | Sensitivity (%) | 95% CI                | Specificity (%) | 95% CI                |
|------------|-----------------|-----------------------|-----------------|-----------------------|
| ≥ 1        | 97              | 83.3% to 99.8%        | 34              | 19.9% to 52.7%        |
| ≥ 2        | 97              | 83.3% to 99.8%        | 69              | 50.8% to 82.7%        |
| <b>≥ 3</b> | <b>83</b>       | <b>66.4% to 92.7%</b> | <b>97</b>       | <b>82.8% to 99.8%</b> |
| ≥ 4        | 60              | 42.3% to 75.4%        | 97              | 82.8% to 99.8%        |
| ≥ 5        | 37              | 21.9% to 54.5%        | 100             | 88.3% to 100%         |
| ≥ 6        | 13              | 5.3% to 29.7%         | 100             | 88.3% to 100%         |

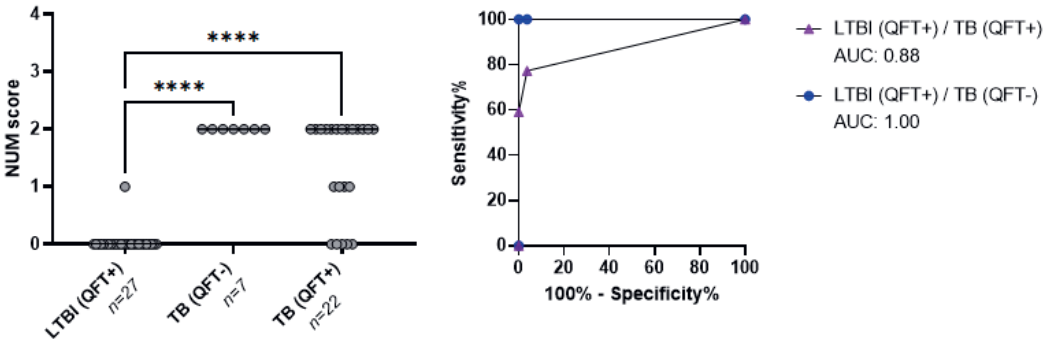
**SUPPLEMENTARY FIGURE S2: NUM score evaluation of six biomarkers for TB and LTBI in European cohort, Related to Figure 1**

ApoA1, CRP, ferritin, IL-6, IP-10, and SAA1/A2 were measured by singleplex strips in serum samples of TB patients (n=30) and LTBI (n=29) from Europe. The NUM score (the number of positive biomarkers) is indicated on the y-axis. Median values for each group are indicated by horizontal bars. A Mann-Whitney U test was performed to determine the statistical significance between groups (p-values: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001, \*\*\*\*p≤0.0001). The cut-off with optimal sensitivity and specificity is written in bold and depicted as a dotted horizontal line in the graph. AUC: area under the curve; CI: confidence interval; LTBI: latent tuberculosis infection; TB: tuberculosis.

## A 6-marker NUM score

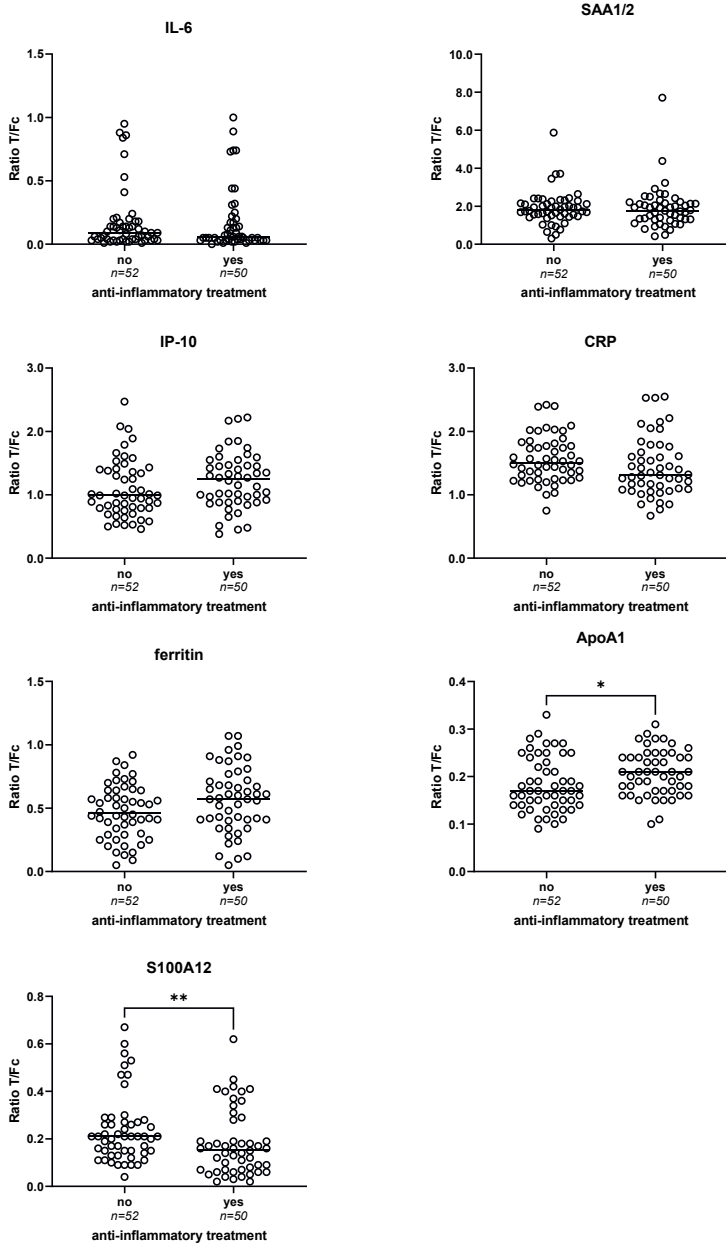


## B 2-marker NUM score



**SUPPLEMENTARY FIGURE S3: Comparison of QuantiFERON and NUM score results for TB and LTBI patients, Related to Figure 1**

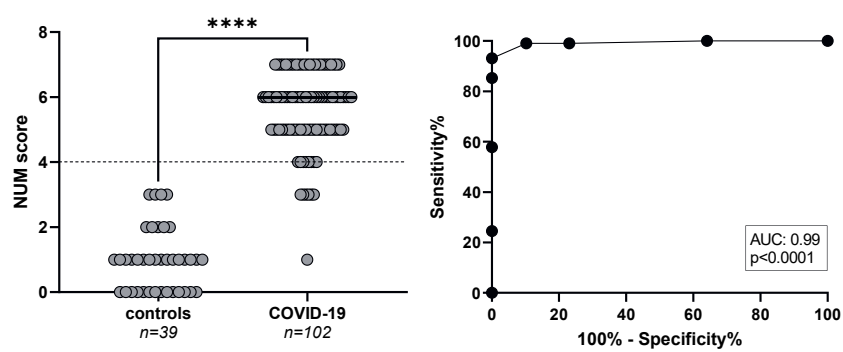
ApoA1, CRP, ferritin, IL-6, IP-10, and SAA1/A2 were measured by singleplex strips in serum samples of: QuantiFERON-negative TB patients (n=7) and QuantiFERON-positive LTBI (n=27) and TB (n=22) patients from Europe. The NUM score (the number of positive biomarkers) is indicated on the y-axis; ApoA1, CRP, ferritin, IL-6, IP-10, and SAA1/A2 were combined into a 6-marker NUM score (A) and CRP and SAA1/A2 were combined into a 2-marker NUM score (B). Median values for each group are indicated by horizontal bars. A Kruskal-Wallis test was performed to determine the statistical significance between groups (p-values: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ ). AUC: area under the curve; LTBI: latent tuberculosis infection; QFT: QuantiFERON; TB: tuberculosis.



**SUPPLEMENTARY FIGURE S4: Effect of anti-inflammatory treatment on seven host proteins in COVID-19 patients, Related to Figure 2**

ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 were measured by singleplex strips in serum samples at hospital admission of COVID-19 patients which had already received anti-inflammatory treatment before the first sample collection (n=50) and those who had not (n=52). Anti-inflammatory treatment included betamethasone, dexamethasone, hydrocortisone, methylprednisolone or prednisolone. Median values for each group are indicated by horizontal bars. A Mann-Whitney U test was performed to determine the statistical significance between groups (p-values: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001, \*\*\*\*p≤0.0001). Fc; flow control line; T; test line.

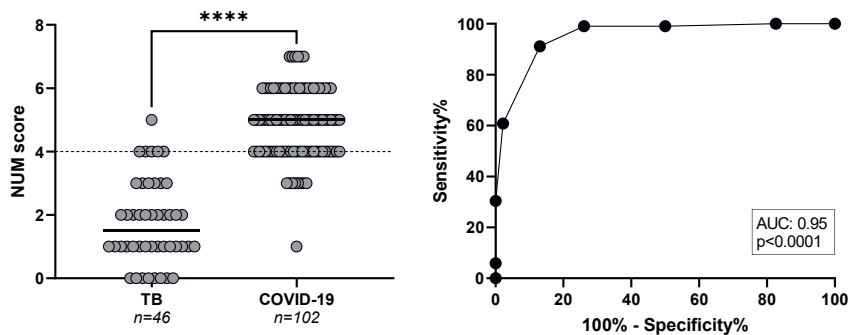




| Cut-off    | Sensitivity (%) | 95% CI                | Specificity (%) | 95% CI               |
|------------|-----------------|-----------------------|-----------------|----------------------|
| ≥ 1        | 100             | 96·4% to 100%         | 36              | 22·7% to 51·6%       |
| ≥ 2        | 99              | 94·7% to 100%         | 77              | 61·7% to 87·4%       |
| ≥ 3        | 99              | 94·7% to 100%         | 90              | 76·4% to 95·9%       |
| <b>≥ 4</b> | <b>93</b>       | <b>86·5% to 96·6%</b> | <b>100</b>      | <b>91·0% to 100%</b> |
| ≥ 5        | 85              | 77·2% to 90·9%        | 100             | 91·0% to 100%        |
| ≥ 6        | 58              | 48·2% to 67·0%        | 100             | 91·0% to 100%        |
| ≥ 7        | 25              | 17·2% to 33·7%        | 100             | 91·0% to 100%        |

**SUPPLEMENTARY FIGURE S5: NUM score evaluation of seven biomarkers for healthy controls and COVID-19 patients from the Netherlands, Related to Figure 2**

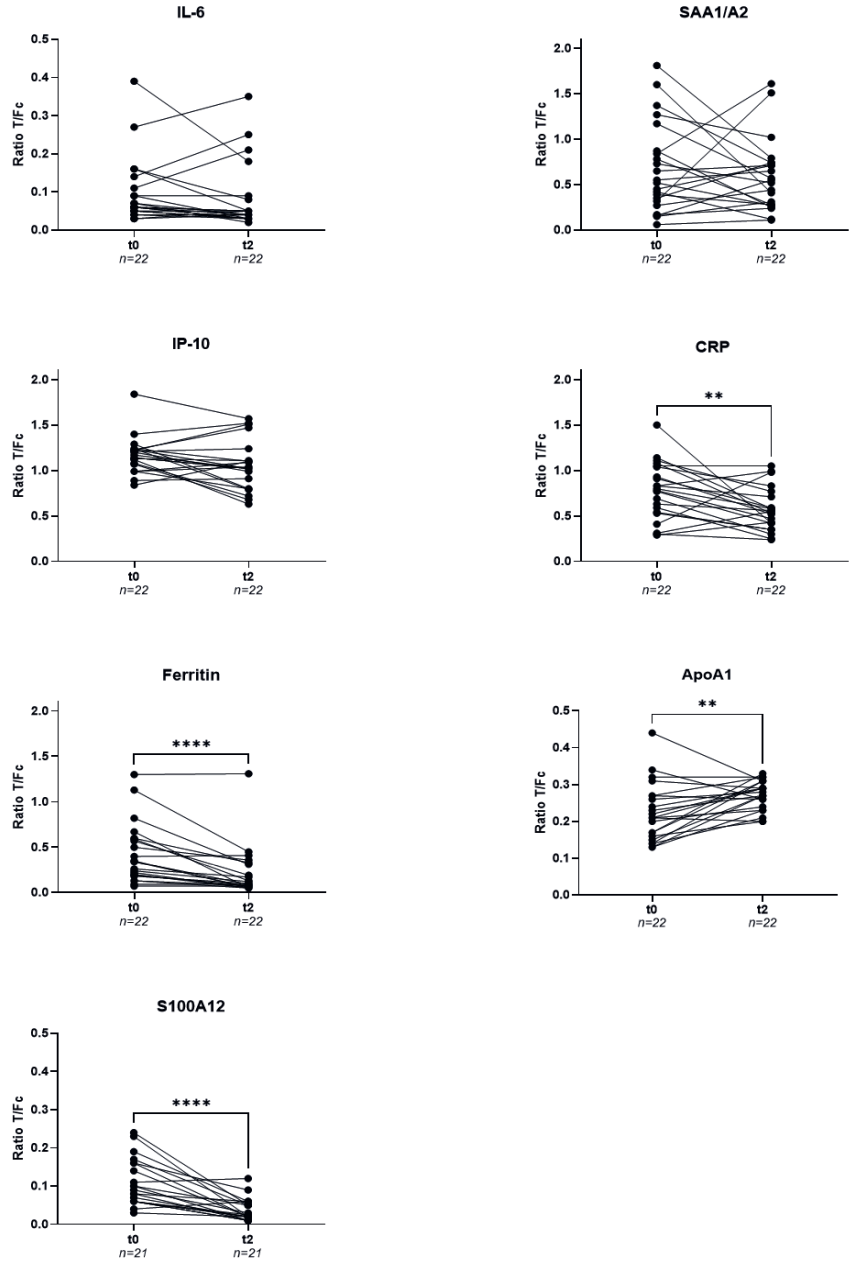
ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 were measured by singleplex strips in serum samples of controls (n=39) and COVID-19 patients (n=102) from the Netherlands. The NUM score (the number of positive biomarkers) is indicated on the y-axis. Median values for each group are indicated by horizontal bars. A Mann-Whitney U test was performed to determine the statistical significance between groups (p-values: \*p≤0·05, \*\*p≤0·01, \*\*\*p≤0·001, \*\*\*\*p≤0·0001). The cut-off with optimal sensitivity and specificity is written in bold and depicted as a dotted horizontal line in the graph. AUC: area under the curve; CI: confidence interval; COVID-19: coronavirus disease 2019.



| Cut-off    | Sensitivity (%) | 95% CI                | Specificity (%) | 95% CI                |
|------------|-----------------|-----------------------|-----------------|-----------------------|
| ≥ 1        | 100             | 96.4% to 100%         | 17              | 9.1% to 30.7%         |
| ≥ 2        | 99              | 94.7% to 100%         | 50              | 36.1% to 63.9%        |
| ≥ 3        | 99              | 94.7% to 100%         | 74              | 59.7% to 84.4%        |
| <b>≥ 4</b> | <b>91</b>       | <b>84.1% to 95.3%</b> | <b>87</b>       | <b>74.3% to 93.9%</b> |
| ≥ 5        | 61              | 51.1% to 69.7%        | 98              | 88.7% to 99.9%        |
| ≥ 6        | 30              | 22.3% to 39.9%        | 100             | 92.3% to 100%         |
| ≥ 7        | 6               | 2.7% to 12.2%         | 100             | 92.3% to 100%         |

**SUPPLEMENTARY FIGURE S6: NUM score evaluation of seven biomarkers for TB and COVID-19 patients, Related to Figure 3**

ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 were measured by singleplex strips in serum samples of TB patients (n=46) from Europe and COVID-19 patients (n=102) from the Netherlands. The NUM score (the number of positive biomarkers) is indicated on the y-axis. Median values for each group are indicated by horizontal bars. A Mann-Whitney U test was performed to determine the statistical significance between groups (p-values: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001, \*\*\*\*p≤0.0001). The cut-off with optimal sensitivity and specificity is written in bold and depicted as a dotted horizontal line in the graph. AUC: area under the curve; CI: confidence interval; COVID-19: coronavirus disease 2019; TB: tuberculosis.



**SUPPLEMENTARY FIGURE S7: Paired visualization of treatment monitoring for TB, Related to Figure 4**  
Treatment monitoring of TB patients (n=22) over time. S100A12 data were missing for one patient. Levels of IL-6, IP-10, ferritin, SAA1/A2, CRP, ApoA1, and S100A12 were measured by UCP-LFA in serum samples of pulmonary TB patients before treatment (t<sub>0</sub>), and months 5 – 9 (t<sub>2</sub>). Wilcoxon matched pairs signed rank tests were performed to determine the statistical significance between timepoints (p-values: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001, \*\*\*\*p≤0.0001). Fc: flow control line; T: test line; TB: tuberculosis.





# 8

## General discussion

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## The ongoing challenge of leprosy and TB control: why new tools are needed

Leprosy and TB both remain significant global health challenges, particularly in resource-limited settings where healthcare infrastructure is often inadequate [1, 2]. Despite being ancient diseases with well-known causative agents and effective treatments, persistent transmission highlights critical gaps in current diagnostics and control strategies [3-5].

For leprosy, diagnosis largely depends on recognizing clinical symptoms and is frequently delayed, increasing the risk of irreversible nerve damage and disability [6, 7]. The disease has a notoriously long incubation period during which asymptomatic infected individuals can go unnoticed and contribute to transmission [4]. Moreover, clinical expertise has declined in many leprosy endemic areas due to reduced case numbers and diminished focus within health systems, further contributing to missed or late diagnoses [8]. Although significant progress has been made towards leprosy elimination, the current standard approach of passive case detection followed by MDT appears insufficient to fully interrupt transmission [9]. Moreover, tools to monitor the impact of public health interventions on transmission remain inadequate. At present, new child cases are used as a proxy indicator for recent transmission, but this method does not capture asymptomatic cases.

TB diagnosis faces different but related challenges, relying heavily on sputum-based tests such as microscopy, culture, and molecular assays. These methods require laboratory infrastructure, skilled personnel, and often take time, limiting their accessibility, especially for children [10] and for people living with HIV, in whom disease is frequently paucibacillary and sputum samples may be difficult to obtain or yield low bacillary loads [11]. Additionally, the COVID-19 pandemic severely disrupted TB diagnostic services worldwide, further exacerbating delays in case detection and increasing community transmission [12, 13].

These diagnostic limitations hinder early treatment initiation and effective disease control for both leprosy and TB. There is thus an urgent need for low-complexity, rapid, and field-friendly diagnostic tools that can enhance early detection, transmission monitoring, and control strategies for leprosy and TB. This thesis set out to address critical gaps in the detection and monitoring of those poverty-associated infectious diseases by developing and validating novel diagnostic tools based on the UCP-LFA platform. The key aims across the chapters were:

- To review the available literature reporting serological data on anti-*M. leprae* antibodies in children, evaluating their potential as proxy for recent transmission (**Chapter 2**);
- To validate the PGL-I QURapid, the final version of a quantitative LFA for detection of anti-PGL-I IgM in serum and FSB, demonstrating its accuracy and field applicability (**Chapter 3**);
- To apply the quantitative anti-PGL-I UCP-LFA in large-scale serosurveys among children in India and Indonesia, assessing seroprevalence as an indicator of ongoing transmission and exploring factors such as socioeconomic status and helminth infections (**Chapters 4-5**);
- To evaluate the use of longitudinal serological monitoring at large scale to assess the impact of active case finding combined with SDDR-PEP on *M. leprae* infection rates in the Comoros and Madagascar (**Chapter 6**);

- To explore quantitative LFAs measuring non-sputum based host protein biomarkers for active TB triage and individual treatment monitoring (**Chapter 7**).

### The need for a standardized quantitative test to detect *M. leprae* infection

Eliminating leprosy requires interruption of *M. leprae* transmission [14]. Assessment of transmission is crucial for evaluating the effectiveness of interventions and guiding strategies to achieve this goal. However, current methods relying on disease incidence are inherently delayed and do not take into account asymptomatic infection [4]. Although seropositivity for anti-PGL-I IgM cannot discriminate between past or present infection, measuring seroprevalence in young children per definition reflects recent transmission, as the infection cannot predate their age [15].

From the systematic literature review presented in **Chapter 2**, it became clear that current serological data on anti-PGL-I IgM antibodies in children offer valuable insights into recent *M. leprae* transmission, but also reveal significant limitations. Serological studies conducted in diverse endemic settings, including multiple regions in Brazil, India, and Indonesia, consistently reported anti-PGL-I IgM seropositivity rates among children ranging from approximately 10 to 19%. Interestingly, seroprevalence did not significantly differ between household contacts and non-contacts in highly endemic areas, suggesting broader community transmission beyond direct contacts. Notably, higher seropositivity rates have been consistently observed in younger age groups, suggesting that children may serve as a sentinel for detecting recent *M. leprae* infection in a population.

Despite promising correlations between anti-PGL-I IgM seropositivity and endemicity levels - further supported by findings from a similar literature review conducted in China [15] - comparisons across studies remained challenging due to significant variability in serological assays and cut-off determinations. Differences in sample types, antigen preparations, antibody isotypes targeted, and operator-dependent qualitative readouts further complicated comparison of serological data, stressing the need for test standardization.

A standardized, quantitative serological test that provides reproducible, objective measurements of anti-PGL-I IgM antibody levels would allow for measurement of changes in transmission over time, and reliable comparison between different geographic regions. Moreover, field-friendly formats that are rapid and easy to deploy in resource-limited environments are essential for wide-scale application [16].

### PGL-I QURapid and its potential use cases

Building on the identified need for such a test, **Chapter 3** described the development and validation of the PGL-I QURapid - the final (horizontal, cassette-based) version of a rapid, low-invasive, quantitative lateral flow assay for measuring anti-PGL-I IgM antibodies in blood (Figure 1). The test demonstrated excellent diagnostic performance and operational feasibility. A major advantage of the quantitative format over a qualitative rapid test is its versatility: the PGL-I QURapid test can support multiple key use cases in leprosy control:



### 1. *Adjunct diagnostic for individual diagnosis and treatment monitoring*

The PGL-I QURapid showed strong correlation between antibody levels and bacterial load, with a sensitivity of 91.5% for MB leprosy cases and a specificity of 99.8%, including negative results in patients with leishmaniasis or other mycobacterial infections (**Chapter 3**). The PGL-I QURapid is well-suited for field deployment, requiring only a FSB sample and delivering accurate quantitative results within minutes, meeting the optimal time frame recommended by the WHO target product profile [16]. A previous version of the assay demonstrated similarly strong diagnostic performance under field conditions in the Comoros and Madagascar (**Chapter 6**): 92.5% of BI-positive MB patients tested seropositive using the anti-PGL-I UCP-LFA. The quantitative output allows differentiation between MB and PB cases, although detection of PB cases may require complementary biomarkers (see below). These findings align with previous research [17-19] and support the use of the PGL-I QURapid as a valuable adjunct to clinical assessment - both for diagnosing MB leprosy and for monitoring treatment response over time [20, 21].

### 2. *Monitoring transmission through serosurveys in children*

**Chapters 4 and 5** applied the PGL-I QURapid in large-scale serosurveys among children in India and Indonesia, showing its utility as a proxy for recent *M. leprae* transmission. Seroprevalence rates of 11.6% and 12.2%, respectively, were consistent with other endemic regions, and the observed increase in seropositivity with age underscores the sensitivity of this approach for detecting ongoing transmission. The assay's quantitative capacity enables detection of subtle changes over time and identification of hidden transmission hotspots, such as the high seroprevalence observed in rural, low socioeconomic settings in Indonesia. Associations with helminth infections further highlight the complex factors influencing transmission dynamics. Furthermore, the study in India (**Chapter 4**) highlighted the benefit of community seroscreening, with seropositive children representing a group at increased risk for developing clinical disease, underscoring the potential for early case detection as well as identifying hotspots of transmission which can be targeted for PEP.

### 3. *Evaluating intervention impact on transmission*

In **Chapter 6**, the PGL-I UCP-LFA (a UCP-LFA format prior to the QURapid) was used in a longitudinal study involving over 33,000 tests in the Comoros and Madagascar to assess the impact of active case finding combined with SDDR-PEP. The study conducted within the PEOPLE trial demonstrated the value of quantitative serology in assessing changes in *M. leprae* infection over time. Importantly, reductions in anti-PGL-I IgM seroprevalence were observed in two of the three study sites (Mohéli and Madagascar) following the implementation of repeated door-to-door screening and SDDR-PEP, while trends on Anjouan were more modest. These site-specific differences may reflect underlying variations in baseline endemicity, population density, and programmatic implementation. Nonetheless, across all sites, longitudinal data from paired samples showed decreasing anti-PGL-I levels among seropositive individuals, suggesting a population-wide decline in infection burden. Crucially, this reduction was observed regardless of whether individuals had received PEP,

highlighting the synergistic role of active case finding and prompt treatment in interrupting transmission.

#### 4. One Health surveillance

Although not covered extensively in this thesis, the PGL-I QURapid also holds promise for One Health applications. Detection of *M. leprae* infection in animals and environmental samples could augment understanding of zoonotic and environmental transmission [22-25], particularly in regions where human cases alone do not fully explain transmission patterns (see below).



**Figure 1. PGL-I QURapid: quantitative UCP-LFA for detection of *M. leprae* infection.**

#### **Test integration with public health strategies**

The development of the PGL-I QURapid offers significant opportunities for integration into comprehensive public health strategies aimed at leprosy control and elimination. Its quantitative, field-friendly design supports applications at both the individual and population level, enhancing early diagnosis, transmission monitoring, and guiding prophylactic interventions in diverse epidemiological contexts.

*Serological screening at the individual and population level*

While passive case detection has proven insufficient for achieving early leprosy diagnosis at scale, timely and accurate identification remains critical when individuals do present at health facilities. In non-endemic countries, leprosy is often overlooked due to its rarity and limited clinician familiarity [26]. Yet, even in highly endemic areas, leprosy cases may go undiagnosed due to nonspecific symptoms and low clinical suspicion [27]. Anti-PGL-I serology offers a valuable, minimally invasive tool to support earlier (MB) diagnosis, especially where bacilloscopy is unavailable. PB leprosy is characterized by a very low bacterial load, which is typically detectable only through invasive biopsies [6]. Since the quantitative results from the PGL-I QURapid correlate with bacterial load [17, 18], this minimally invasive test could serve as a useful tool to differentiate MB from PB leprosy in settings where biopsies and their analysis are not readily accessible. Quantitative tests like the PGL-I QURapid are well-suited for use in decentralized settings, with strong diagnostic performance and rapid results. Integrating serology into routine workflows - particularly among specialists likely to encounter atypical cases - can help close diagnostic gaps and support timely treatment [27].

The effectiveness of leprosy control programs likely depends on a multifaceted approach combining early diagnosis, timely treatment, and prophylaxis. The WHO recommends ACF with contact tracing and single-dose rifampicin post-exposure prophylaxis (SDR-PEP), targeting high-risk groups such as household contacts [2]. However, the dynamics of transmission differ markedly between high- and low-endemic settings, influencing optimal screening strategies [28].

In highly endemic settings such as parts of Indonesia, Brazil, and Bangladesh, transmission likely extends beyond immediate contacts, necessitating broader screening efforts. Seroprevalence data from these regions demonstrated similar infection rates between household contacts and the wider community (**Chapter 2**), underscoring the value of serological tools in guiding ACF strategies. This phenomenon was also observed in the Prata, a former leprosy colony in Brazil (data not shown), where virtually the entire population can be considered at risk due to pervasive exposure [29]. The Prata site was part of the LRI BraBo study on point-of-care (POC) tests for leprosy in South America, alongside a site in Bolivia. Although the results from the respective study are not presented in this thesis, fieldwork at both locations offered valuable insights into the operational differences between low- and high-endemic settings, particularly in the context of POC diagnostics.

In contrast, low-endemic settings like Bolivia face a different set of challenges [30]. Although overall case numbers are low and incidence is gradually declining, the detection of new cases - particularly among children - indicates ongoing transmission. Bolivia exemplifies a low-endemic country grappling with barriers such as stigma, centralized health services, and a shortage of trained personnel. A recent phenomenological study by Messa Carmona et al. (2025) emphasized the critical need to prioritize ACF efforts despite declining incidence, noting that centralized diagnosis and treatment services hinder accessibility and adherence, while stigma continues to impede effective case finding [30]. In these contexts, more

targeted ACF combined with sensitive diagnostic tools is essential to uncover hidden cases and interrupt transmission chains.

Integrating serological screening with ACF could further enhance early detection efforts. Although anti-PGL-I seropositivity does not necessarily indicate active disease, it identifies individuals with present or past infection who may benefit from closer clinical follow-up (as also shown in **Chapter 4**). Monitoring these seropositive individuals can allow timely detection of disease progression and facilitates targeted intervention. This approach also underpins the potential for serology-guided administration of PEP, optimizing prophylactic efforts by focusing on those at higher risk of developing clinical disease.

Serological screening at the community level also offers a powerful tool to detect hidden transmission hotspots. In Southwest Sumba, Indonesia, official case notifications were low, suggesting minimal transmission. Yet, a serosurvey using the PGL-I QURapid revealed a striking 31% seroprevalence among children, uncovering substantial ongoing transmission (**Chapter 5**). This disparity highlights limitations of relying solely on new case detection rates, particularly where control programs are weak or underperforming [31]. This early warning capability is especially important in remote or resource-limited settings, allowing national programs and partners to prioritize areas for intensified case-finding, contact tracing, and preventive strategies. Given the baseline data provided in **Chapter 5**, using serosurveillance, Sumba could serve as an example site for evaluating the effects of integrated control strategies.

As low-SES, undernutrition, rural areas and helminthiasis have been associated with increased anti-PGL-I seroprevalence and risk of leprosy [32-35], targeted intensified screening of high-risk populations is essential to identify subclinical infections and interrupt transmission chains early. Deploying field-friendly assays like the PGL-I QURapid enhances screening efficiency, enabling timely prophylaxis and treatment, thereby reducing disease burden and moving closer to elimination goals. In this context, Integrated public health strategies addressing nutrition, water, sanitation, and hygiene (WASH), alongside helminth control, are critical to optimize intervention outcomes and sustainably curb transmission (also see below) [36, 37].

### Assessing the impact of interventions

Monitoring anti-PGL-I IgM seropositivity in children has been recommended by the WHO Task Force for criteria for elimination of leprosy as a key indicator to evaluate interruption of transmission and elimination progress [14]. Unlike the proportion of child cases, which reflects detected clinical disease, seroprevalence among young children may offer a more sensitive indication of recent infection and ongoing transmission, including asymptomatic infection.

Repeated cross-sectional serosurveys in the same age groups within a geographic area can track changes over time, reflecting the impact of interventions such as ACF and SDR-PEP. For example, follow-up assessments 5 to 10 years post-intervention in regions like Bihar or Southwest Sumba would provide critical insights into transmission trends. The quantitative nature of the PGL-I QURapid allows detection of subtle shifts in antibody levels, enabling

early identification of declining transmission even before case numbers decline. Integrating serological surveillance into national leprosy programs could strengthen control efforts and accelerate progress toward sustainable transmission interruption.

### *Continued serological screening in the post-elimination phase*

As some countries move towards or have already achieved leprosy elimination - based on the decline in new child cases [14, 38-40] - the need for vigilant surveillance remains critical [41]. Even in settings officially declared leprosy-free, such as the Maldives, ongoing serological screening remains crucial in identifying silent transmission [42].

Clinical cases represent only the visible part of the disease burden; subclinical infections often go undetected. Yet, these asymptomatic individuals can maintain hidden transmission chains, posing a risk for new cases to emerge if surveillance efforts lapse. Serological tools such as the PGL-I QURapid provide a sensitive and specific means of detecting recent or ongoing *M. leprae* infection in young children, offering a more comprehensive view of endemicity than case reporting alone. This is further supported by data from **Chapter 4** and unpublished findings: sera from 70 children in a non-endemic region (the Netherlands) all showed R-values of zero, highlighting the test's specificity.

Routine serological monitoring in post-elimination contexts enables early identification of potential recrudescence or emerging hotspots, allowing for timely public health intervention. As such, it fits well within the WHO Leprosy Elimination Framework [38, 43], supporting the third phase focused on sustained post-elimination surveillance and preventing re-emergence.

### *Importance of cut-off values in immunodiagnostic testing*

A critical aspect of any diagnostic test, including immunodiagnostic assays like the UCP-LFA, is the determination of the cut-off value that defines a positive result [44]. These thresholds are essential for distinguishing between positive and negative, but no universally accepted cut-off exists for anti-PGL-I IgM levels. As a result, cut-off selection must be tailored to the intended application and epidemiological context.

In settings where high sensitivity is prioritized - for example, identifying individuals eligible for PEP - a lower cut-off may be selected to detect subclinical infection. Conversely, in settings where only higher levels of infection are of practical relevance - avoiding unnecessary treatment or social stigma - a higher cut-off value might be preferred.

To add further nuance and improve diagnostic accuracy, this thesis discussed the concept of an indeterminate range (**Chapter 3**), a buffer zone between clearly positive and negative results. Samples falling within this range could be flagged for retesting or further clinical evaluation rather than being classified definitively. A similar approach is already applied in TB diagnostics, where borderline or indeterminate zones in tests such as QuantiFERON-TB are used [45].

To improve consistency and reliability across different settings and over time, a humanized recombinant anti-PGL-I IgM control was developed (**Chapter 3**) to serve as an internal standard. This control helps address variability in cut-off determination and enables more reproducible and comparable results. The humanized, recombinant anti-PGL-I IgM control introduced in this study as part of the PGL-I QURapid test kit, provides an essential tool to indicate thresholds for specific applications (sample type and use case). It offers high flexibility for manufacturing as test thresholds can be evaluated with a control sample of known IgM concentration.

By integrating robust quality controls, adaptable cut-off strategies, and the use of an indeterminate range, immunodiagnostic tools like the PGL-I QURapid can be effectively customized for a range of public health applications - from widespread serosurveillance to targeted clinical diagnostics - thereby maximizing their contribution to leprosy control efforts.

## **Test implementation in practice: operational and ethical considerations**

### *Integrating immunodiagnostic testing into existing health structures*

Successful implementation of immunodiagnostic tools like the PGL-I QURapid relies not only on technical performance, but also on strategic integration into existing health infrastructure and social systems. Leveraging trusted networks could improve logistical feasibility, enhance acceptance, and reduce stigma.

In India, for instance, a pre-existing Health and Demographic Surveillance System (HDSS) originally established for visceral leishmaniasis was expanded to include anti-PGL-I IgM testing (**Chapter 4**) [46, 47]. Embedding leprosy serology within this established system allowed for smooth implementation and minimized resistance by drawing on already trusted relationships between health workers and the community.

Similarly, in Bolivia, the LRI BraBo study conducted community-based skin camps that provided screening for various skin conditions - not just leprosy. This broadened scope helped reduce the stigma of being singled out for a "leprosy test" and framed participation as part of a general health initiative [48, 49]. As a result, uptake improved and health workers had the opportunity to build broader awareness around skin health and leprosy detection.

These examples underscore the value of integrating new diagnostic strategies into broader, locally relevant health programs. Aligning with existing health priorities and delivery platforms reduces costs, builds on community familiarity, and enhances sustainability.

Governments and national leprosy programs play a critical role in enabling this integration [50]. Their support is essential to ensure alignment with public health goals, appropriate allocation of resources, and effective follow-up for individuals with positive test results.

### *Community trust, ethical communication, and stakeholder engagement*

Introducing a new diagnostic tool - particularly in populations with historical stigma around leprosy [51, 52] - requires careful attention to community trust, ethics, and stakeholder involvement. The act of screening, especially among healthy children, might raise questions

about perceived benefit, interpretation of results, and the potential for unintended consequences.

While the PGL-I QURapid test is minimally invasive, the implications of a positive result may not be straightforward. Anti-PGL-I IgM seropositivity does not indicate active disease but rather past or present infection [4]. This distinction must be clearly communicated to prevent misinterpretation and reduce the risk of social stigma or anxiety.

Informed, transparent communication is therefore essential - both before and after testing. This includes clear messaging for parents and guardians, appropriate training for healthcare workers, and meaningful involvement of local leaders. Community-wide serosurveys, rather than individual-based testing, can further minimize stigma by framing participation as a collective public health activity.

The ethical concerns around screening are not separate from stakeholder acceptance, they are deeply intertwined. In Bolivia, embedding serological testing in broader health camps not only addressed stigma but also fostered community trust. Participants perceived the initiative as a general health service rather than a disease-specific program, improving turnout and reducing fear.

Healthcare workers must also be confident in administering and interpreting the test. Proper training, clear guidelines, and support from national health authorities are key to fostering professional engagement [4, 53]. Likewise, engaging policymakers from the outset ensures that implementation strategies are realistic, ethical, and aligned with long-term elimination goals.

Taken together, these factors demonstrate that ethical, effective, and equitable implementation depends on holistic engagement - with communities, health systems, and governments - tailored to local realities and informed by ongoing dialogue.

## **Expanding the potential of immunodiagnostics**

### **Technical progress and multiplexing**

During the PEOPLE trial (**Chapter 6**), an earlier vertical flow version of the anti-PGL-I UCP-LFA was used. Since then, the LUMC immunodiagnostic research group developed the PGL-I QURapid (**Chapter 3**), a more user-friendly horizontal cassette format. This eliminates the need for 96-well plates, allowing diluted fingerstick blood samples to be applied directly to the test, greatly simplifying use in the field. To support more sustainable diagnostics, the PGL-I QURapid was also tested with plant-based biodegradable cassettes (Okos Diagnostics, the Netherlands; data not shown). In addition, pre-printed barcode labels can minimize errors in participant ID entry (unpublished data).

Standardization efforts, including the use of a humanized anti-PGL-I IgM standard with preset cut-off R-values, help ensure assay reliability and comparability across studies. In the future, UCP-LF readers could be calibrated to output actual concentrations instead of relative units, facilitating comparisons across sites and time points. These improvements

support broader use and consistent interpretation of test results, tailored to specific sample type and use case.

Another key advancement is the potential for multiplexing: detecting multiple biomarkers in a single test. Given the limited sensitivity of the PGL-I QURapid for PB leprosy (10.5-23.7%, **Chapters 3 and 6**), combining anti-PGL-I with additional host biomarkers could significantly improve detection. The UCP-LF platform is well-suited for such integration, as demonstrated in studies from Bangladesh, Brazil, China, and Ethiopia [54, 55], paving the way for promising multi-biomarker tests (MBTs) [56]. A MBT combining six markers (anti-PGL-I IgM, IP-10, CRP, S100A12, ApoA1, and CCL4) was successfully implemented in a single test strip. This allowed quantitative detection of all six serum proteins simultaneously and enabled discrimination of both MB and PB leprosy patients from controls, in both high- and low-endemic settings. As research continues to uncover novel and more predictive biomarkers, the modular nature of the UCP-LF platform allows for easy adaptation, enabling the continuous refinement of the test to further improve diagnostic performance across diverse populations and clinical presentations.

#### Host biomarker-based UCP-LFAs for TB triage

Since no specific and sensitive antibody has yet been identified for *M. tuberculosis* infection [57, 58], serological diagnosis based on pathogen-specific antibodies - as successfully applied in leprosy - is currently not feasible for TB and has limited use for bovine TB [87]. This underscores the importance of alternative host-response markers, such as inflammatory or acute-phase proteins, which can indicate active disease even in the absence of direct pathogen detection [59]. Interestingly, several of the host biomarkers explored in the context of leprosy immunodiagnosics also show relevance for TB. **Chapter 7** of this thesis therefore investigated whether combinations of UCP-LFAs measuring serum host biomarkers could support triage testing for active disease. In this study, quantitative UCP-LF assays were applied to detect seven host proteins - ApoA1, CRP, ferritin, IL-6, IP-10, SAA1/A2, and S100A12 - in sera from individuals with active TB, those latently infected with *M. tuberculosis* (LTBI), COVID-19 patients, and healthy controls. Several of these proteins were discriminatory in TB patients compared to LTBI. The resulting biomarker combinations achieved high diagnostic performance (AUC up to 0.94) and allowed treatment monitoring through quantitative changes during therapy. Importantly, this demonstrates that host-response markers measured by UCP-LFA can identify individuals with probable active disease, even in the absence of sputum samples. Notably, a three-biomarker signature - CRP, IP-10, and SAA1 applied to the UCP-LFA format - was recently validated by the TrENDx-TB consortium, meeting the WHO TPP for a TB triage test [60], supporting its further development [61, 62].

For implementation as a TB triage tool, such tests could be deployed at primary healthcare or community level to rapidly screen individuals presenting with respiratory symptoms. Those with positive biomarker signatures could then be referred for confirmatory testing using molecular assays such as GeneXpert or culture. The use of FSB, short assay time, and portable readers makes the test particularly suitable for decentralized settings where laboratory infrastructure is limited.



Integration of these host-biomarker UCP-LFAs into existing TB control programs would thus facilitate earlier detection, rational use of confirmatory diagnostics, and improved monitoring of treatment response. Ultimately, this approach supports a shift from purely pathogen-based diagnostics toward rapid, multiplexed, host-response-based triage that can be implemented in resource-limited and high-burden settings and tested in low-invasive samples.

### Integrated disease surveillance

This growing versatility of the UCP-LFA platform - demonstrated through its application to both TB and leprosy - highlights its potential role in broader, integrated disease surveillance strategies. Integrated disease surveillance is particularly relevant in regions where multiple neglected tropical diseases (NTDs) are co-endemic. In Bihar, for example, leprosy and visceral leishmaniasis (VL) both circulate in the same communities [46, 63], creating opportunities for coordinated screening, research, and public health interventions. While leishmaniasis is a differential diagnosis of leprosy [64], the two diseases are rarely addressed jointly. In **Chapters 3 and 4**, serum samples from individuals with confirmed *Leishmania donovani* infection were tested using the PGL-I QURapid primarily to assess the specificity of the test for *M. leprae* infection. These findings not only supported the test's specificity but also led to the idea of applying the same UCP-LFA platform across diseases.

This illustrates the broader potential of combining diagnostics and surveillance for multiple diseases within shared infrastructure. In settings where leprosy overlaps with TB, helminth infections (as described in **Chapter 5**), schistosomiasis, or even COVID-19, integrated approaches can minimize duplication, optimize resources, and generate more holistic data for guiding interventions [38]. The versatility of the UCP-LFA platform supports this vision: beyond leprosy, assays have been/are being developed or adapted for TB (host biomarkers; **Chapter 7**), leishmaniasis (via rK39 antigen [65]), and schistosomiasis (via circulating anodic antigen, CAA [66-69]).

Beyond logistical efficiency, integrated surveillance aligns closely with several Sustainable Development Goals (SDGs), notably SDG 3 (Good Health and Well-being), SDG 1 (No Poverty), and SDG 6 (Clean Water and Sanitation) [70]. NTDs like leprosy, leishmaniasis and schistosomiasis are deeply tied to poverty, poor hygiene, and limited access to water and sanitation - factors also targeted by WASH (Water, Sanitation and Hygiene) programs [71-73]. Integrating diagnostic tools and disease control strategies with broader development agendas can therefore amplify impact and support long-term health improvements in affected communities.

As diagnostic technologies like the UCP-LFA evolve to support multiplexing and simplified field deployment, their role in cross-cutting public health strategies becomes increasingly valuable. Rather than operating in vertical silos, NTD programs can benefit from shared platforms, personnel, and surveillance systems. This shift toward integrated, SDG-aligned diagnostics has the potential to strengthen health systems, improve detection of co-infections or hidden hotspots, and accelerate progress toward elimination goals across diseases.

### One Health: detecting infection beyond humans

Although not a primary focus of the main chapters, the PGL-I QURapid also shows promise within One Health surveillance strategies [22, 85, 87]. While human-to-human transmission remains the dominant route for *M. leprae* spread, increasing evidence points to zoonotic and environmental reservoirs. Notably, *M. leprae* DNA has been detected in soil [23, 74], water [75], and animals such as nine-banded armadillos (Americas) [25, 76-80] and Eurasian red squirrels (UK) [24, 81-84], suggesting these non-human sources may contribute to ongoing transmission or perpetuating of the pathogen in the environment in certain settings, particularly if human leprosy is virtually non-existent.

Originally developed for human diagnostics, the anti-PGL-I UCP-LFA has also proven adaptable for serological use in animals. This opens new avenues for monitoring of infection in wildlife and potentially livestock, supporting ecological risk mapping where human case data alone may not explain transmission dynamics. The PGL-I QURapid has been successfully applied cross-species, to armadillos and red squirrels in earlier studies [22, 85, 86], and more recently to earprick samples from live, experimentally infected armadillos, demonstrating strong correlation with infection (in collaboration with the National Hansen's Disease Program, USA; unpublished data). Furthermore, in line with ELISA-based findings by Avanzi et al. [24], the PGL-I QURapid detected anti-PGL-I antibodies in *M. lepromatosis*-infected squirrels [85], and in a confirmed human case with this infection (Avanzi, Geluk, unpublished data).

Moreover, UCP-LFAs have also been adapted for use in bovine TB diagnostics, enabling detection of both anti-*M. bovis* PGL antibodies [87] and relevant host protein biomarkers [88] in cattle. These findings underline the broader applicability of the UCP-LFA format in One Health surveillance, offering a valuable tool in identifying hidden reservoirs and better understanding transmission routes.

## **Concluding remarks**

### In summary

This thesis underscores the critical need for improved diagnostic tools to support the global control and elimination of leprosy and TB; two poverty-associated diseases that continue to challenge public health systems, especially in low-resource settings. Despite longstanding treatment protocols, both diseases suffer from delayed detection and incomplete surveillance. For leprosy, the long incubation period and reliance on clinical diagnosis hinder early case detection and contribute to ongoing transmission. The development of the quantitative PGL-I QURapid addresses a major gap by enabling standardized, field-friendly serological testing for *M. leprae* exposure. Its application ranges from supporting individual diagnosis to population-level surveillance and impact monitoring. Findings from field studies in India, Indonesia, and the Comoros show that the QURapid can uncover hidden hotspots of transmission and provide early indicators of program effectiveness. Importantly, seropositivity can guide targeted follow-up and PEP administration, especially in high-risk groups. The thesis also highlights the ethical, operational, and social considerations of implementing serological tools, advocating for integration with broader health outreach and

community engagement to reduce stigma. Looking ahead, the potential of multiplexed diagnostics - allowing simultaneous detection of multiple host biomarkers or pathogens - combined with One Health surveillance and integrated disease programs suggests a path towards more holistic, efficient, and responsive disease control strategies. Together, these innovations position immunodiagnostics as a cornerstone of future elimination efforts.

### *Using what we have: progress over perfection in the path toward elimination*

While no diagnostic test is perfect, waiting for an ideal solution may delay urgently needed action. Tools like the PGL-I QURapid may not capture every leprosy case or answer every diagnostic question, but they offer valuable, actionable data - especially in low-resource and high-transmission settings where other options are limited.

Importantly, a test does not need to function as a standalone diagnostic to be impactful. It can serve as an adjunct to clinical diagnosis or as a triage tool, helping to prioritize individuals for further evaluation, follow-up, or preventive treatment.

At present, diagnostic tools are not yet widely used in leprosy programs, and most cases are still diagnosed clinically. However, as the global incidence of leprosy continues to decline, and clinical expertise becomes less readily available - particularly in low-endemic or post-elimination settings - reliable diagnostic tools will become increasingly critical. In this final phase toward Zero Leprosy, investing in and applying the tools we do have is not only pragmatic, but necessary. Even if not perfect, such tools can help detect hidden transmission, support targeted interventions, and prevent resurgence.

Rather than letting the perfect become the enemy of the good, we must embrace and optimize the tools that are available now, refining them as we go, and integrating them smartly into surveillance and elimination strategies.

## References

1. World Health Organization. Tuberculosis. 2025; Available from: <https://www.who.int/news-room/fact-sheets/detail/tuberculosis>.
2. World Health Organization. Leprosy. Fact sheets 2025; Available from: <https://www.who.int/news-room/fact-sheets/detail/leprosy>.
3. Scollard, D.M., et al., The continuing challenges of leprosy. Clin Microbiol Rev, 2006. 19(2): p. 338–81.
4. Grijzen, M.L., et al., Leprosy. Nat Rev Dis Primers, 2024. 10(1): p. 90.
5. Bartolomeu-Goncalves, G., et al., Tuberculosis Diagnosis: Current, Ongoing, and Future Approaches. Diseases, 2024. 12(9).
6. Chen, K.H., et al., Leprosy: A Review of Epidemiology, Clinical Diagnosis, and Management. J Trop Med, 2022. 2022: p. 8652062.
7. Dharmawan, Y., et al., Delayed detection of leprosy cases: A systematic review of healthcare-related factors. PLoS Negl Trop Dis, 2022. 16(9): p. e0010756.
8. Henry, M., et al., Factors Contributing to the Delay in Diagnosis and Continued Transmission of Leprosy in Brazil--An Explorative, Quantitative, Questionnaire Based Study. PLoS Negl Trop Dis, 2016. 10(3): p. e0004542.
9. Batista, G., et al., Feasibility of post-exposure-prophylaxis with single-dose rifampicin and identification of high prevalent clusters in villages' hyperendemic for leprosy in Senegal. PLoS Negl Trop Dis, 2024. 18(2): p. e0011755.

10. Brent, A.J., et al., Author Correction: Bacteriological diagnosis of childhood TB: a prospective observational study. *Sci Rep*, 2018. 8(1): p. 7223.
11. Johnson, J.L., et al., Impact of human immunodeficiency virus type-1 infection on the initial bacteriologic and radiographic manifestations of pulmonary tuberculosis in Uganda. Makerere University-Case Western Reserve University Research Collaboration. *Int J Tuberc Lung Dis*, 1998. 2(5): p. 397–404.
12. Migliori, G.B., et al., Gauging the impact of the COVID-19 pandemic on tuberculosis services: a global study. *Eur Respir J*, 2021. 58(5).
13. Visca, D., et al., Tuberculosis and COVID-19 interaction: A review of biological, clinical and public health effects. *Pulmonology*, 2021. 27(2): p. 151–165.
14. WHO, Interruption of transmission and elimination of leprosy disease - Technical guidance. . 2023: India.
15. Zhou, Z., Pierneef, L., van Hooij, A., Geluk, A., Detection of anti-*M. leprae* antibodies in healthy children in China: A systematic review of Chinese literature. *Frontiers in Tropical Diseases*, 2022. 3(963674).
16. World Health Organization, TPP: For a diagnostic test to detect *Mycobacterium leprae* infection among asymptomatic household and familial contacts of leprosy patients. 2023, World Health Organization.
17. van Hooij, A., et al., Field-friendly serological tests for determination of *M. leprae*-specific antibodies. *Sci Rep*, 2017. 7(1): p. 8868.
18. Tio-Coma, M., et al., Genomic Characterization of *Mycobacterium leprae* to Explore Transmission Patterns Identifies New Subtype in Bangladesh. *Front Microbiol*, 2020. 11: p. 1220.
19. Zenha, E.M., M.A. Ferreira, and N.T. Foss, Use of anti-PGL-1 antibodies to monitor therapy regimes in leprosy patients. *Braz J Med Biol Res*, 2009. 42(10): p. 968–72.
20. Corstjens, P., et al., Field-Friendly Test for Monitoring Multiple Immune Response Markers during Onset and Treatment of Exacerbated Immunity in Leprosy. *Clin Vaccine Immunol*, 2016. 23(6): p. 515–519.
21. Khadge, S., et al., Longitudinal immune profiles in type 1 leprosy reactions in Bangladesh, Brazil, Ethiopia and Nepal. *BMC Infect Dis*, 2015. 15: p. 477.
22. Zhou, Z., et al., Detection and Monitoring of *Mycobacterium leprae* Infection in Nine Banded Armadillos (*Dasypus novemcinctus*) Using a Quantitative Rapid Test. *Front Microbiol*, 2021. 12: p. 763289.
23. Tio-Coma, M., et al., Detection of *Mycobacterium leprae* DNA in soil: multiple needles in the haystack. *Sci Rep*, 2019. 9(1): p. 3165.
24. Avanzi, C., et al., Red squirrels in the British Isles are infected with leprosy bacilli. *Science*, 2016. 354(6313): p. 744–747.
25. Truman, R.W., et al., Probable Zoonotic Leprosy in the Southern United States. *New England Journal of Medicine*, 2011. 364(17): p. 1626–1633.
26. Lockwood, D.N., et al., Diagnosing and treating leprosy in a non-endemic setting in a national centre, London, United Kingdom 1995–2018. *PLoS Negl Trop Dis*, 2022. 16(10): p. e0010799.
27. Nobre, M.L., et al., Anti-PGL-I serology as a strategy for Hansen's Disease control in an endemic area in Brazil. *Leprosy Review*, 2025. 96(1).
28. Hasker, E., et al., Interrupting transmission of synthesis of new evidence and research recommendations. *Leprosy Review*, 2025. 96(2).
29. de Oliveira Mackert, C.C., et al., Insights into leprosy epidemiology from an isolated population located in the Brazilian Amazon. *Sci Rep*, 2025. 15(1): p. 6103.
30. Messa Carmona, P., et al., Barriers to leprosy elimination in Bolivia: Exploring perspectives and experiences of medical professionals and leprosy patients-A phenomenological study. *PLoS Negl Trop Dis*, 2025. 19(8): p. e0013345.
31. Siregar, G.O., et al., Leprosy identified in Sumba Island, eastern Indonesia: elimination targets under threat. *Lancet Reg Health Southeast Asia*, 2024. 26: p. 100409.
32. Oktaria, S., et al., Dietary diversity and poverty as risk factors for leprosy in Indonesia: A case-control study. *PLoS Negl Trop Dis*, 2018. 12(3): p. e0006317.
33. Nery, J.S., et al., Socioeconomic determinants of leprosy new case detection in the 100 Million Brazilian Cohort: a population-based linkage study. *Lancet Glob Health*, 2019. 7(9): p. e1226–e1236.
34. Anantharam, P., et al., Undernutrition, food insecurity, and leprosy in North Gondar Zone, Ethiopia: A case-control study to identify infection risk factors associated with poverty. *PLoS Negl Trop Dis*, 2021. 15(6): p. e0009456.
35. Dwivedi, V.P., et al., Diet and nutrition: An important risk factor in leprosy. *Microb Pathog*, 2019. 137: p. 103714.
36. Emerson, L.E., et al., Poor WASH (Water, Sanitation, and Hygiene) Conditions Are Associated with Leprosy in North Gondar, Ethiopia. *Int J Environ Res Public Health*, 2020. 17(17).
37. Hagge, D.A., et al., Opening a Can of Worms: Leprosy Reactions and Complicit Soil-Transmitted Helminths. *EBioMedicine*, 2017. 23: p. 119–124.
38. van Brakel, W.H., et al., Strategies for leprosy services in low-endemic areas and countries. *Leprosy Review*, 2024. 95(3).

39. Fastenau, A., et al., Roadmap towards zero leprosy, Pakistan. *Bull World Health Organ*, 2025. 103(8): p. 507–514.
40. Iqbal, M., et al., Pakistan on the Road to Zero Leprosy, an analysis of routine data for the period 1980-2022: a retrospective cohort study. *Lancet Glob Health*, 2025. 13(8): p. e1458–e1465.
41. WHO, Jordan becomes first country to receive WHO verification for eliminating leprosy. 2024.
42. World Health Organization. World Health Organization honors Maldives for achieving five consecutive years of zero child Leprosy case detection and Interruption of transmission. 2023.
43. World Health Organization, Leprosy Elimination Monitoring Tool: The LEMT is a tool accompanying the Technical guidance on interruption of transmission and elimination of leprosy. 2023, World Health Organization: New Delhi.
44. Pugh, S., et al., Estimating cutoff values for diagnostic tests to achieve target specificity using extreme value theory. *BMC Med Res Methodol*, 2024. 24(1): p. 30.
45. Wikell, A., et al., The Impact of Borderline Quantiferon-TB Gold Plus Results for Latent Tuberculosis Screening under Routine Conditions in a Low-Endemicity Setting. *Journal of Clinical Microbiology*, 2021. 59(12).
46. Malaviya, P., et al., Health & Demographic Surveillance System profile: the Muzaffarpur-TMRC Health and Demographic Surveillance System. *Int J Epidemiol*, 2014. 43(5): p. 1450–7.
47. Hasker, E., et al., Post kala azar dermal leishmaniasis and leprosy prevalence and distribution in the Muzaffarpur health and demographic surveillance site. *PLoS Negl Trop Dis*, 2019. 13(10): p. e0007798.
48. Hambridge, T., et al., The Epidemiological Impact of Community-Based Skin Camps on Leprosy Control in East Hararge Zone, Ethiopia: a Modelling Study. *J Epidemiol Glob Health*, 2025. 15(1): p. 34.
49. Schoenmakers, A., et al., PEP4LEP study protocol: integrated skin screening and SDR-PEP administration for leprosy prevention: comparing the effectiveness and feasibility of a community-based intervention to a health centre-based intervention in Ethiopia, Mozambique and Tanzania. *BMJ Open*, 2021. 11(8): p. e046125.
50. Fastenau, A., et al., Integrating Community Engagement in Zero Leprosy Efforts: A Pathway to Sustainable Early Detection, Control and Elimination. *Trop Med Infect Dis*, 2024. 9(12).
51. Willis, M., et al., Interventions to reduce leprosy related stigma: A systematic review. *PLOS Glob Public Health*, 2024. 4(8): p. e0003440.
52. Arudchelvam, U., et al., Stigma of People Affected by Leprosy and its determinants: a cross-sectional study in Sri Lanka. *BMC Infect Dis*, 2025. 25(1): p. 148.
53. Zafirah, T.N., et al., Knowledge and Skills of Leprosy Among Healthcare Workers and Its Associated Factors in Primary Healthcare. *Inquiry*, 2025. 62: p. 469580241312059.
54. van Hooij, A., et al., Evaluation of Immunodiagnostic Tests for Leprosy in Brazil, China and Ethiopia. *Sci Rep*, 2018. 8(1): p. 17920.
55. van Hooij, A., et al., Application of new host biomarker profiles in quantitative point-of-care tests facilitates leprosy diagnosis in the field. *EBioMedicine*, 2019. 47: p. 301–308.
56. van Hooij, A., et al., Prototype multi-biomarker test for point-of-care leprosy diagnostics. *iScience*, 2021. 24(1): p. 102006.
57. Rijnink, W.F., T.H.M. Ottenhoff, and S.A. Joosten, B-Cells and Antibodies as Contributors to Effector Immune Responses in Tuberculosis. *Front Immunol*, 2021. 12: p. 640168.
58. Melkie, S.T., et al., The role of antibodies in tuberculosis diagnosis, prophylaxis and therapy: a review from the ESGMYC study group. *Eur Respir Rev*, 2022. 31(163).
59. Chegou, N.N., et al., Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB. *Thorax*, 2016. 71(9): p. 785–94.
60. Denking, C.M., et al., Defining the needs for next generation assays for tuberculosis. *J Infect Dis*, 2015. 211 Suppl 2(Suppl 2): p. S29–38.
61. Sutherland, J.S., et al., Performance of 2 Finger-Stick Blood Tests to Triage Adults With Symptoms of Pulmonary Tuberculosis: A Prospective Multisite Diagnostic Accuracy Study. *Clin Infect Dis*, 2025.
62. Richardson, T.R., et al., Field evaluation of a point-of-care triage test for active tuberculosis (TriageTB). *BMC Infect Dis*, 2023. 23(1): p. 447.
63. Kumar, V., et al., Kala-azar elimination in a highly-endemic district of Bihar, India: A success story. *PLoS Negl Trop Dis*, 2020. 14(5): p. e0008254.
64. Trindade, M.A., et al., Post-kala-azar dermal leishmaniasis and leprosy: case report and literature review. *BMC Infect Dis*, 2015. 15: p. 543.
65. Singh, O.P., et al., Xenodiagnosis to evaluate the infectiousness of humans to sandflies in an area endemic for visceral leishmaniasis in Bihar, India: a transmission-dynamics study. *Lancet Microbe*, 2021. 2(1): p. e23–e31.
66. Corstjens, P.L., et al., Up-converting phosphor technology-based lateral flow assay for detection of *Schistosoma* circulating anodic antigen in serum. *J Clin Microbiol*, 2008. 46(1): p. 171–6.

67. Corstjens, P.L., et al., Tools for diagnosis, monitoring and screening of *Schistosoma* infections utilizing lateral-flow based assays and upconverting phosphor labels. *Parasitology*, 2014. 141(14): p. 1841–55.
68. Hoekstra, P.T., et al., Early diagnosis and follow-up of acute schistosomiasis in a cluster of infected Belgian travellers by detection of antibodies and circulating anodic antigen (CAA): A diagnostic evaluation study. *Travel Med Infect Dis*, 2021. 41: p. 102053.
69. de Jong, D., et al., Flow-S: A Field-Deployable Device with Minimal Hands-On Effort to Concentrate and Quantify *Schistosoma* Circulating Anodic Antigen (CAA) from Large Urine Volumes. *Diagnostics (Basel)*, 2024. 14(8).
70. United Nations Department of Economic and Social Affairs, The Sustainable Development Goals Report 2025. 2025: New York.
71. Boisson, S., et al., Water, sanitation and hygiene for accelerating and sustaining progress on neglected tropical diseases: a new Global Strategy 2015–20. *Int Health*, 2016. 8 Suppl 1(Suppl 1): p. i19–21.
72. Grimes, J.E., et al., The roles of water, sanitation and hygiene in reducing schistosomiasis: a review. *Parasit Vectors*, 2015. 8: p. 156.
73. Addisu, A., et al., Neglected tropical diseases and the sustainable development goals: an urgent call for action from the front line. *BMJ Glob Health*, 2019. 4(1): p. e001334.
74. Lavania, M., et al., Detection of *Mycobacterium leprae* DNA from soil samples by PCR targeting RLEP sequences. *J Commun Dis*, 2006. 38(3): p. 269–73.
75. Turankar, R.P., et al., Survival of *Mycobacterium leprae* and association with *Acanthamoeba* from environmental samples in the inhabitant areas of active leprosy cases: A cross sectional study from endemic pockets of Purulia, West Bengal. *Infect Genet Evol*, 2019. 72: p. 199–204.
76. Amezcua, M.E., et al., Wild Mexican Armadillo with Leprosy-Like Infection. *International Journal of Leprosy and Other Mycobacterial Diseases*, 1984. 52(2): p. 254–255.
77. da Silva, M.B., et al., Evidence of zoonotic leprosy in Para A, Brazilian Amazon, and risks associated with human contact or consumption of armadillos. *Plos Neglected Tropical Diseases*, 2018. 12(6).
78. Loughry, W.J., et al., Is Leprosy Spreading among Nine-Banded Armadillos in the Southeastern United States? *Journal of Wildlife Diseases*, 2009. 45(1): p. 144–152.
79. Stefani, M.M.A., et al., Leprosy survey among rural communities and wild armadillos from Amazonas state, Northern Brazil. *Plos One*, 2019. 14(1).
80. Walsh, G.P., et al., Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*): recent epizootologic findings. *J Reticuloendothel Soc*, 1977. 22(4): p. 363–7.
81. Butler, H.M., et al., Further evidence of leprosy in Isle of Wight red squirrels. *Veterinary Record*, 2017. 180(16): p. 407–407.
82. Meredith, A., et al., WILDLIFE Leprosy in red squirrels in Scotland. *Veterinary Record*, 2014. 175(11).
83. Schilling, A.K., et al., British Red Squirrels Remain the Only Known Wild Rodent Host for Leprosy Bacilli. *Frontiers in Veterinary Science*, 2019. 6.
84. Simpson, V., et al., Leprosy in red squirrels on the Isle of Wight and Brownsea Island. *Veterinary Record*, 2015. 177(8).
85. Zhou, Z., et al., Molecular and Serological Surveillance for *Mycobacterium leprae* and *Mycobacterium lepromatosis* in Wild Red Squirrels (*Sciurus vulgaris*) from Scotland and Northern England. *Animals (Basel)*, 2024. 14(13).
86. Schilling, A.K., et al., Diagnosing and Categorizing Leprosy in Live Eurasian Red Squirrels (*Sciurus vulgaris*) for Management, Surveillance, and Translocation Purposes. *J Zoo Wildl Med*, 2021. 52(2): p. 648–659.
87. Zhou, Z., et al., Anti-phenolic glycolipid antibodies in *Mycobacterium bovis* infected cattle. *One Health*, 2025. 20: p. 100982.
88. Khalid, H., et al., Development of lateral flow assays to detect host proteins in cattle for improved diagnosis of bovine tuberculosis. *Front Vet Sci*, 2023. 10: p. 1193332.

**List of abbreviations**

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## List of abbreviations

|                        |                                                                                          |
|------------------------|------------------------------------------------------------------------------------------|
| ApoA1                  | Apolipoprotein A1                                                                        |
| AUC                    | Area under the curve                                                                     |
| BCG                    | Bacillus Calmette-Guérin                                                                 |
| BI                     | Bacteriological index                                                                    |
| BU                     | Buruli ulcer                                                                             |
| COVID-19               | Coronavirus disease 2019                                                                 |
| CRP                    | C-reactive protein                                                                       |
| EC                     | Endemic control                                                                          |
| ELISA                  | Enzyme-linked immunosorbent assay                                                        |
| FC line                | Flow control line                                                                        |
| FSB                    | Fingerstick blood                                                                        |
| IgG                    | Immunoglobulin G                                                                         |
| IgM                    | Immunoglobulin M                                                                         |
| IL-6                   | Interleukin 6                                                                            |
| IP-10                  | Interferon gamma-induced protein 10                                                      |
| <i>L. donovani</i>     | <i>Leishmania donovani</i>                                                               |
| LFA                    | Lateral flow assay                                                                       |
| LMIC                   | Low- and middle-income country                                                           |
| LTBI                   | Latent tuberculosis infection                                                            |
| MB                     | Multibacillary                                                                           |
| MDT                    | Multidrug therapy                                                                        |
| <i>M. leprae</i>       | <i>Mycobacterium leprae</i>                                                              |
| <i>M. lepromatosis</i> | <i>Mycobacterium lepromatosis</i>                                                        |
| <i>Mtb</i>             | <i>Mycobacterium tuberculosis</i>                                                        |
| NCDR                   | New case detection rate                                                                  |
| ND-O-HSA               | Natural disaccharide-octyl human serum albumin                                           |
| NEC                    | Non-endemic control                                                                      |
| NPT-H-BSA              | Phenolic trisaccharide functionalized with a hexanoic acid linker for conjugation to BSA |
| NTD                    | Neglected tropical disease                                                               |
| NTM                    | Non-tuberculous mycobacteria                                                             |
| PB                     | Paucibacillary                                                                           |
| PEOPLE                 | Post-ExpOsure Prophylaxis for LEprosy                                                    |
| PEP                    | Post-exposure prophylaxis                                                                |
| PGL-I                  | Phenolic glycolipid-I                                                                    |
| PKDL                   | Post-kala-azar dermal leishmaniasis                                                      |
| POC                    | Point-of-care/point-of-contact                                                           |
| QURapid                | Quantitative UCP-based rapid test                                                        |
| R                      | Ratio                                                                                    |
| ROC                    | Receiver operating characteristic                                                        |
| SAA                    | Serum amyloid A                                                                          |
| SARS-CoV-2             | Severe acute respiratory syndrome coronavirus 2                                          |
| S(D)DR                 | Single (double) dose rifampicin                                                          |
| SES                    | Socioeconomic status                                                                     |
| Sn/Sp                  | Sensitivity/specificity                                                                  |
| SSS                    | Slit-skin smears                                                                         |
| STH                    | Soil-transmitted helminths                                                               |
| S100A12                | S100 calcium-binding protein A12                                                         |
| TB                     | Tuberculosis                                                                             |
| Th1                    | T-helper 1                                                                               |
| Th2                    | T-helper 2                                                                               |
| T line                 | Test line                                                                                |
| TPP                    | Target product profile                                                                   |
| UCP                    | Up-converting phosphor                                                                   |
| VL                     | Visceral leishmaniasis                                                                   |
| WBA                    | Whole blood assay                                                                        |
| WCS                    | Whole cell sonicate                                                                      |
| WHO                    | World Health Organization                                                                |



## Nederlandse samenvatting

Lepra en tuberculose (TB) blijven belangrijke volksgezondheidsuitdagingen in lage- en middeninkomenslanden, waar beperkte toegang tot snelle en betrouwbare diagnostiek leidt tot vertraagde detectie en voortdurende transmissie. Bij lepra is diagnose grotendeels afhankelijk van klinische herkenning, waardoor infecties vaak laat worden vastgesteld en asymptomatische transmissie onopgemerkt kan blijven. TB diagnostiek is daarentegen sterk afhankelijk van sputum-gebaseerde laboratoriumtesten, die niet altijd toepasbaar zijn in kwetsbare populaties en afhankelijk zijn van een beperkte en soms kwetsbare diagnostische infrastructuur. Gezamenlijk onderstrepen deze beperkingen de behoefte aan snelle, gebruiksvriendelijke diagnostische tools die geschikt zijn voor gebruik in het veld en die zowel vroege detectie van infectie als het monitoren van transmissie op populatieniveau mogelijk maken.

Dit proefschrift onderzoekt de ontwikkeling, validatie en implementatie van in het veld toepasbare immunodiagnostische hulpmiddelen op basis van het UCP-LFA-platform voor de detectie en monitoring van infectieziekten die samenhangen met armoede, met een primaire focus op lepra en aanvullende toepassingen voor TB en COVID-19. Door technologische innovatie van diagnostische testen te combineren met grootschalige veldstudies beoogt dit onderzoek bij te dragen aan effectievere strategieën voor ziektebestrijding en surveillance in omgevingen met beperkte middelen en infrastructuur.

De eliminatie van lepra is gericht op het onderbreken van de transmissie van *Mycobacterium leprae* (*M. leprae*), die zich niet beperkt tot huishoudens van patiënten. Omdat in veel landen de onderbreking van transmissie nog niet bereikt is, is er behoefte aan een test die geïnfecteerde asymptomatische individuen kan identificeren die de transmissie in stand kunnen houden. Om de eliminatie van lepra te monitoren, is het essentieel om geschikte indicatoren te identificeren die de transmissie binnen een populatie weerspiegelen. Antistoffen tegen *M. leprae* antigenen wijzen op infectie, maar kunnen geen onderscheid maken tussen een recente of eerdere infectie. In dit kader beschrijft **Hoofdstuk 2** een systematische review van de beschikbare literatuur naar serologische testen voor anti-*M. leprae*-antilichamen bij kinderen zonder klinische lepra die in endemische gebieden wonen. Aangezien antilichaamresponsen bij jonge kinderen per definitie wijzen op recente infectie, werd in dit hoofdstuk onderzocht of anti-PGL-I seroprevalentie gebruikt kan worden als maat voor de huidige transmissie van *M. leprae*. Er werd literatuuronderzoek uitgevoerd in PubMed, Infolep, Web of Science en de Virtual Health Library. Dertig artikelen uit tien landen voldeden aan de inclusie criteria. In alle studies werden antistoffen tegen phenolic glycolipid-I (PGL-I) of synthetische varianten daarvan gemeten, zowel kwantitatief (ELISA of UCP-LFA) als kwalitatief (ML-flow of NDO-LID sneltesten). De mediane seroprevalentie bij kinderen in endemische gebieden was 14.9% en bleef stabiel over de tijd wanneer de incidentie van de ziekte niet veranderde. Hoewel kwantitatieve anti-PGL-I serologie onder jonge kinderen daarmee veelbelovend leek als proxy voor transmissie, bleek vergelijking tussen gebieden beperkt mogelijk door verschillen in gebruikte testen en afkapwaarden.

In **Hoofdstuk 3** wordt de definitieve versie van een kwantitatieve sneltest voor de detectie van *M. leprae* infectie geëvalueerd, de PGL-I QURapid. Deze test, gebaseerd op UCP-LFA-technologie, meet anti-PGL-I IgM antilichamen in vingerprikbloed en serum. In dit hoofdstuk

worden de diagnostische prestaties, reproduceerbaarheid en toepasbaarheid van de test in veldomstandigheden onderzocht. Er wordt aangetoond dat de test multibacillaire leprapatiënten met hoge sensitiviteit en specificiteit kan identificeren, in zowel vingerprikbloed en serum, terwijl resultaten al binnen enkele minuten beschikbaar zijn. Ook wordt de inzet van een gehumaniseerde recombinante anti-PGL-I IgM controle beschreven, waarmee testprestaties kunnen worden gemonitord en gestandaardiseerde afkapwaarden kunnen worden vastgesteld. De resultaten laten zien dat de PGL-I QURapid voldoet aan de door de WHO opgestelde criteria voor point-of-care diagnostiek en geschikt is voor implementatie binnen publieke gezondheidsprogramma's in endemische gebieden.

In **Hoofdstuk 4** wordt een veldstudie beschreven die is uitgevoerd in de deelstaat Bihar in India. In deze studie werd met behulp van de kwantitatieve UCP-LFA-test de seroprevalentie van anti-PGL-I IgM-antilichamen bepaald bij 1.857 kinderen in de leeftijd van drie tot elf jaar, afkomstig uit twaalf endemische dorpen. In dit hoofdstuk wordt onderzocht in hoeverre seropositiviteit bij jonge kinderen kan dienen als indicator voor recente *M. leprae* transmissie in endemische gebieden. De resultaten laten zien dat 11.58% van de kinderen seropositief was voor anti-PGL-I IgM, een niveau dat vergelijkbaar is met eerdere studies in andere endemische regio's zonder profylactische interventies. Daarnaast wordt beschreven dat de UCP-LFA een laagdrempelig en praktisch toepasbaar instrument is, dat goed integreert in grootschalige serosurveys en goed werd geaccepteerd door zowel lokaal zorgpersoneel als de betrokken gemeenschappen. De bevindingen ondersteunen het potentieel van anti-PGL-I serologie bij kinderen als hulpmiddel om recente transmissie van *M. leprae* te monitoren in de context van lepra eliminatieprogramma's.

**Hoofdstuk 5** beschrijft een tweede sero-epidemiologische studie in rurale en stedelijke gebieden in Indonesië. In deze studie werd bij 637 schoolgaande kinderen (6–15 jaar) de seroprevalentie van anti-PGL-I IgM-antilichamen bepaald met behulp van de voor gebruik in het veld toepasbare kwantitatieve UCP-LFA-test (PGL-I QURapid), waarbij tevens sociaaleconomische factoren en de aanwezigheid van worminfecties werden meegenomen. De resultaten laten zien dat de totale seroprevalentie 12.2% bedroeg, met duidelijke verschillen tussen regio's: in rurale gebieden werden aanzienlijk hogere percentages gevonden (tot 31.0% in Zuidwest Sumba) dan in stedelijke gebieden (3.1–3.8%). Daarnaast was de seroprevalentie significant hoger onder kinderen uit lage sociaaleconomische scholen (13.7%) vergeleken met hoge SES-scholen (4.1%). Ook werd een sterke associatie gevonden tussen worminfecties en verhoogde anti-PGL-I IgM-niveaus; kinderen met een worminfectie waren vaker seropositief dan kinderen zonder infectie. Deze bevindingen wijzen op verhoogde *M. leprae* transmissie in rurale, sociaaleconomisch kwetsbare en worm-geïnfecteerde populaties. Het hoofdstuk benadrukt daarmee het belang van geïntegreerde infectieziektebestrijding, waarbij zowel lepra als worminfecties worden meegenomen. Daarnaast wordt bevestigd dat de PGL-I QURapid een effectief instrument is voor serologische monitoring en het identificeren van hotspots met verhoogde transmissie, waarmee gerichte interventies in endemische gebieden kunnen worden ondersteund.

In **Hoofdstuk 6** wordt de implementatie van grootschalige serologische monitoring beschreven binnen de Post-ExpOsure Prophylaxis for LEprosy (PEOPLE)-studie in de

Comoren en Madagaskar (2019–2023). In deze studie werd de toediening van verschillende manieren van post-exposure profylaxe met rifampicine aan contacten van leprapatiënten geëvalueerd, in combinatie met actieve opsporing van nieuwe gevallen. Daarnaast werd in 16 van de 64 deelnemende dorpen gedurende drie opeenvolgende jaren de seroprevalentie van anti-PGL-I IgM gemeten bij nieuw gediagnosticeerde leprapatiënten en bij meer dan 17.000 huishoudelijke en buurtcontacten. Vingerprikbloed werd ter plaatse door lokaal personeel geanalyseerd met behulp van de veldtoepasbare, kwantitatieve UCP-LFA-test. De resultaten laten zien dat 92.5% van de multibacillaire patiënten met een bacteriële index van  $\geq 1$  seropositief was, wat de hoge sensitiviteit van de test in veldomstandigheden bevestigt. Voorafgaand aan de interventies varieerde de seroprevalentie onder contacten tussen 21.9% en 27.4% tussen de verschillende locaties. Na twee jaar actieve opsporing van gevallen en toediening van profylaxe was de seroprevalentie in alle studiegebieden significant gedaald tot 11.6%–22.8%. Daarnaast werd een significante daling in anti-PGL-I IgM-niveaus op individueel niveau waargenomen bij aanvankelijk seropositieve personen. Deze daling werd gezien ongeacht het al dan niet ontvangen van profylaxe. De bevindingen suggereren dat de combinatie van actieve case finding en profylaxe leidt tot een afname van *M. leprae* infectieniveaus in de populatie. Dit hoofdstuk laat daarmee zien dat de anti-PGL-I UCP-LFA geschikt is voor grootschalige serologische surveillance en het monitoren van de impact van leprabestrijdingsinterventies op populatieniveau.

In **Hoofdstuk 7** wordt de reikwijdte van het proefschrift verbreed door de toepassing van host-biomarker-gebaseerde UCP-LFAs te onderzoeken voor een andere mycobacteriële ziekte: TB. In dit hoofdstuk worden de diagnostische prestaties van deze assays voor het onderscheiden van actieve en latente TB onderzocht, evenals COVID-19-patiënten en gezonde controles, en wordt de bruikbaarheid van deze testen voor triage-doeleinden besproken. De resultaten laten zien dat de onderzochte biomarkers een hoge mate van diagnostische nauwkeurigheid bieden, onder andere voor het onderscheiden van latente infectie en actieve TB (AUC 0.94), en voor het differentiëren tussen gezonde individuen en COVID-19-patiënten (AUC 0.99). Daarnaast blijkt dat deze markers ook bruikbaar zijn voor het monitoren van behandeling bij zowel TB als COVID-19. Dit hoofdstuk laat daarmee zien dat niet-sputum gebaseerde, kwantitatieve LFAs een veelbelovend alternatief kunnen vormen als aanvullende diagnostische en monitoringstool voor respiratoire infectieziekten, en onderstreept de bredere toepasbaarheid van het UCP-LFA-platform.

Samenvattend laat dit proefschrift zien dat het UCP-LFA platform een veelzijdig en schaalbaar diagnostisch systeem vormt voor de detectie en monitoring van infectieziekten in uiteenlopende epidemiologische contexten. Voor lepra blijkt kwantitatieve anti-PGL-I IgM-serologie een waardevolle indicator van infectie en transmissie, met toepassingen variërend van individuele diagnostiek tot grootschalige serosurveillance en evaluatie van volksgezondheidsinterventies. De resultaten uit veldstudies in India, Indonesië, de Comoren en Madagaskar tonen aan dat deze benadering niet alleen geschikt is voor het identificeren van geïnfecteerde individuen, maar ook voor het opsporen van verborgen transmissie hotspots en het volgen van veranderingen in infectie niveaus over tijd. Daarnaast benadrukken de bevindingen het belang van geïntegreerde strategieën, waarin diagnostiek, behandeling en preventie gezamenlijk worden ingezet en worden afgestemd op lokale epidemiologische en sociaaleconomische omstandigheden. De resultaten voor TB en

COVID-19 illustreren de brede toepasbaarheid van het UCP-LFA-platform voor verschillende infectieziekten. Host-biomarker-gebaseerde UCP-LFAs bieden perspectief als snelle, niet-invasieve en tools die geschikt zijn voor het veld voor zowel triage als monitoring van respiratoire infectieziekten, vooral in settings waar toegang tot laboratoriumdiagnostiek beperkt is. Tot slot onderstreept dit proefschrift dat de grootste impact niet alleen ligt in het ontwikkelen van perfecte diagnostische tests, maar in het slim inzetten van beschikbare tools binnen realistische publieke gezondheidsstrategieën. Zelfs wanneer diagnostische methoden niet volledig alle klinische of epidemiologische vragen beantwoorden, kunnen zij toch cruciale informatie leveren om transmissie te detecteren, interventies te sturen en eliminatiedoelen dichterbij te brengen.

## Dankwoord

Ik wil graag iedereen bedanken die, op welke manier dan ook, heeft meegeholpen om dit proefschrift mogelijk te maken.

Annemieke, dankzij jouw vertrouwen en begeleiding kijk ik nu met trots terug op dit bijzondere promotietraject. Bedankt voor alles wat je me hebt geleerd en de inspirerende/gezellige reizen!

Paul, bedankt dat je mijn co-promotor wilde zijn en dat ik me altijd welkom heb gevoeld bij CCB. Ik waardeer de waardevolle feedback die je me gedurende het traject hebt gegeven.

Anouk, wat zou de groep zonder jou moeten? Jij weet je altijd alles te herinneren - of het nu gaat om een experiment van jaren geleden of een klein detail in een project. Jouw scherpste en kennis zijn bewonderenswaardig. Maar bovenal ben je ook een ontzettend fijn persoon om mee samen te werken. Ik ben dankbaar dat ik met jou dit traject heb mogen delen.

Danielle en Elisa, heel erg bedankt voor al het werk wat jullie hebben gedaan voor de projecten die onderdeel zijn van dit proefschrift en wat jullie me hebben geleerd op het lab rondom de UCP-LFA. Maar minstens zo belangrijk: dank voor alle gezelligheid en fijne gesprekken, zowel op kantoor als daarbuiten. Jij natuurlijk ook, Annelies! Jullie hebben het werk nog leuker gemaakt.

Gaby, ik ben heel blij dat jij bij de groep bent gekomen. Els, ook jou wil ik heel erg bedanken voor alles wat je hebt gedaan. Beiden bedankt voor al het harde werken aan de verschillende projecten. Ik heb onze samenwerking altijd als heel prettig en leuk ervaren.

Alle andere INZI-collega's en mede-PhD's - bedankt voor de fijne werksfeer op zowel E5 als K5, en natuurlijk voor de gezellige borrels, etentjes en labuitjes (shout-out naar de FECO!). Krista en Annelies, bedankt voor alles wat jullie doen om het lab op rolletjes te laten lopen.

De rest van de CCB-collega's die ik nog niet genoemd heb: Pauline, Sherif, Karien, Julia, Lennard, Maarten, Claudia, Stan, Willem, Romina - bedankt voor alle gezellige gesprekken tijdens de lunch, de Sinterklaasavonden en alle andere leuke uitjes die we de afgelopen jaren samen hebben meegemaakt.

To all the international colleagues I've had the pleasure of working with - Zijie, Luana, Matheus, Antoniella, Hamza, Hana and Luca: thank you for the great collaboration and good times. It was a real pleasure getting to know you all!

De studenten die ik heb mogen begeleiden - Lotte, Nadine en Sanne, maar ook alle studenten van de halfminor in Indonesië: bedankt voor jullie inzet en waardevolle bijdrage aan ons onderzoek. To the Indonesian students and colleagues - terima kasih! Rike, I really enjoyed our collaboration and was very happy to share this unique experience with you.

I would also like to extend my sincere thanks to all the (international) collaborators from Bangladesh, Belgium, Bolivia, Brazil, the Comoros, India, Indonesia, Italy, Madagascar and the Netherlands whose valuable contributions have been instrumental to the research presented in this thesis. It was a great privilege to experience the fieldwork side of the research, and I am especially grateful to the local health workers, nurses, doctors, researchers and participants - thank you all for your dedication and support.

En dan natuurlijk mijn familie. Lieve pap en mam, jullie zijn altijd mijn grootste steun en voorbeeld geweest - niet alleen tijdens mijn promotietraject, maar in alles wat ik doe. Jullie onvoorwaardelijke liefde, geduld en vertrouwen hebben me altijd het gevoel gegeven dat ik alles kon bereiken, ook op momenten dat ik zelf twijfelde. Jullie hebben me laten zien hoe belangrijk het is om door te zetten, maar ook om met beide benen op de grond te blijven staan. Ik ben ontzettend dankbaar dat ik zulke ouders heb. Di en Den, bedankt voor jullie steun, humor en nuchterheid. Jullie hebben me altijd geholpen om te relativiseren en te blijven lachen, ook in de drukste tijden. Ik ben heel erg blij met zo'n hechte broer-zussenband. Ook wil ik de rest van mijn lieve (schoon)familie en vrienden bedanken. Room, jij in het bijzonder!

Als laatste, Ricardo, dankjewel voor jouw steun, je begrip en je liefde. Je was er op de momenten dat ik het nodig had en bleef altijd geloven in mij. Met jou voelt alles mogelijk.

## Curriculum vitae

Louise Pierneef was born on the 14<sup>th</sup> of May 1994, in Heemskerk, the Netherlands. From 2006 to 2012, she attended Jac. P. Thijssen College in Castricum, where she completed her pre-university education (VWO) with a Nature and Health profile. In 2012, she enrolled in the Bachelor's program in Health and Life Sciences at the Vrije Universiteit (VU) in Amsterdam. During her Bachelor studies, she gained hands-on research experience through an internship at the Department of Pathology at the Leiden University Medical Center (LUMC), where she focused on diabetic nephropathy. She continued her academic journey by pursuing a Master's degree in Biomedical Sciences (VU Amsterdam). In the first year of the program, she specialized in Neurobiology. As part of her training, she undertook an internship at the Netherlands Brain Institute, where she contributed to a project investigating demyelination and inflammation mediated by microglia and macrophages in multiple sclerosis. In the second year of her Master's studies, Louise shifted her focus to Immunology and completed an internship at the Department of Infectious Diseases at the LUMC, within the research group of Prof. Dr. Annemieke Geluk. Her work during this period centered on the transmission of *Mycobacterium leprae* and the early diagnosis of leprosy reactions. After completing her Master's in Biomedical Sciences in 2018, Louise pursued a second Master's degree in International Public Health (IPH; VU Amsterdam). During this program, she continued her work on leprosy, conducting qualitative research in Nepal for four months. Her research, carried out in collaboration with the Netherlands Leprosy Relief, investigated factors influencing the mental wellbeing of individuals affected by leprosy. Upon completing her Master's in IPH in 2019, Louise returned to the research group of Prof. Dr. Annemieke Geluk at the LUMC, where she was appointed as a junior researcher. In August 2021, she began her PhD research, focusing on field-friendly immunodiagnosics for poverty-associated infectious diseases. This work was conducted in close collaboration with the research group of Dr. Ir. Paul Corstjens at the Department of Cell and Chemical Biology. The results of her doctoral research are presented and discussed in this thesis and have been published in international scientific journals.

## List of publications

1. Tió-Coma M, Wijnands T, **Pierneef L**, Schilling AK, Alam K, Roy JC, Faber WR, Menke H, Pieters T, Stevenson K, Richardus JH, Geluk A. Detection of *Mycobacterium leprae* DNA in soil: multiple needles in the haystack. *Sci Rep*. 2019 Feb 28;9(1):3165.
2. Tió-Coma M, Avanzi C, Verhard EM, **Pierneef L**, van Hooij A, Benjak A, Roy JC, Khatun M, Alam K, Corstjens P, Cole ST, Richardus JH, Geluk A. Genomic Characterization of *Mycobacterium leprae* to Explore Transmission Patterns Identifies New Subtype in Bangladesh. *Front Microbiol*. 2020 Jun 16;11:1220.
3. **Pierneef L**, van Hooij A, Taal A, Rumbaut R, Nobre ML, van Brakel WH, Geluk A. Detection of anti-*M. leprae* antibodies in children in leprosy-endemic areas: A systematic review. *PLoS Negl Trop Dis*. 2021 Aug 27;15(8):e0009667.
4. Zhou Z, Pena M, van Hooij A, **Pierneef L**, de Jong D, Stevenson R, Walley R, Corstjens PLAM, Truman R, Adams L, Geluk A. Detection and Monitoring of *Mycobacterium leprae* infection in Nine Banded Armadillos (*Dasypus novemcinctus*) Using a Quantitative Rapid Test. *Front Microbiol*. 2021 Oct 28;12:763289.
5. **Pierneef L**, van 't Noordende AT, Bista M, Singh U, Scheltema MB, Joshi AK, Pant M, Shakya L, Waltz MM, van Brakel WH. Factors influencing the mental wellbeing of persons affected by leprosy in Far-Western Nepal. *Lep Rev*. 2022 March 1;93;1:12-25.
6. Zhou Z, **Pierneef L**, van Hooij A, Geluk A. Detection of anti-*M. leprae* antibodies in healthy children in China: A systematic review of Chinese literature. *Front Trop Dis*. 2022 Oct 7;3:963674.
7. **Pierneef L**, van Hooij A, de Jong D, Tjon Kon Fat EM, van Meijgaarden KE, Petruccioli E, Vanini V, Roukens AHE, Goletti D, Corstjens PLAM, Joosten SA, Geluk A; BEAT-COVID study group. Host biomarker-based quantitative rapid tests for detection and treatment monitoring of tuberculosis and COVID-19. *iScience*. 2023 Jan 20;26(1):105873.
8. Khalid H\*, **Pierneef L\***, van Hooij A, Zhou Z, de Jong D, Tjon Kon Fat E, Connelley TK, Hope JC, Corstjens PLAM, Geluk A. Development of lateral flow assays to detect host proteins in cattle for improved diagnosis of bovine tuberculosis. *Front Vet Sci*. 2023 Aug 15;10:1193332.
9. **Pierneef L**, Malaviya P, van Hooij A, Sundar S, Singh AK, Kumar R, de Jong D, Meuldijk M, Kumar A, Zhou Z, Cloots K, Corstjens PLAM, Hasker E, Geluk A. Field-friendly anti-PGL-I serosurvey in children to monitor *Mycobacterium leprae* transmission in Bihar, India. *Front Med*. 2023 Sep 27;10:1260375.
10. **Pierneef L**, van Hooij A, de Jong D, Wassenaar G, Verhard E, Tjon Kon Fat E, Engel N, Khatun M, Soren S, Chowdhury AS, van Hees C, Corstjens PLAM, Geluk A. Rapid test for *Mycobacterium leprae* infection: a practical tool for leprosy. *Infect Dis Poverty*. 2024 Dec 2;13(1):88.
11. Zhou Z, van Hooij A, van Dijk JHM, Musch N, **Pierneef L**, Khalid H, Franken K, Holder T, Watt N, Michel AL, Codée JDC, Vordermeier M, Corstjens PLAM, van der Heijden EMDL, Hope JC, Geluk A. Anti-phenolic glycolipid antibodies in *Mycobacterium bovis* infected cattle. *One Health*. 2025 Jan 28;20:100982.
12. **Pierneef L**, Braet SM\*, de Jong D\*, Ramboarina S, van Hooij A, Abdou MW, Ortuno-Gutierrez N, Snijders R, Maharavo L, Grillone SH, Andriamira R, Andrianarivelo R, Rasamoelina T, Hasker E, Corstjens PLAM, de Jong BC, Cauchois B, Assoumani Y, Geluk A. Large-scale field application of a fingerstick blood test for *Mycobacterium leprae* infection: Monitoring population-wide effects of case finding and post-exposure prophylaxis on transmission in the Comoros and Madagascar. *PLOS Glob Pub Health*. 2025 Dec 2;5(12):e0005270.
13. **Pierneef L**, Muhammad M, Wassenaar G, Edbert D, Krismawati H, Wahyuni S, Ali S, Kaisar MMM, Corstjens PLAM, Geluk A. Seroprevalence of anti-*Mycobacterium leprae* PGL-I antibodies among school children in rural and urban Indonesia: a cross-sectional study (2023). *The Lancet Reg Health SEA*. 2026 Jun; 49:100779.

\*Contributed equally



## Portfolio

During my PhD, I was part of the Leiden University Center for Infectious Diseases department, working under the supervision of Prof. Dr. A. Geluk (primary advisor) and Dr. Ir. P.L.A.M. Corstjens (co-advisor). My thesis, titled "*Field-deployable immunodiagnostics for poverty-associated infectious diseases: development and use cases in public health*", focuses on the development and evaluation of diagnostic tools for diseases such as tuberculosis and leprosy. This chapter outlines the academic training, dissemination activities, and teaching experiences I undertook during my PhD trajectory.

My training started in 2020 with the course *Basic Methods and Reasoning in Biostatistics*, which provided a strong foundation in data analysis. In 2021, I completed the *Leiden University Onboarding Programme* and attended the *LAP PhD Day*, introducing me to the broader academic environment and connecting me with other early-stage researchers.

In 2022, I deepened my scientific knowledge by participating in the *LIFI course* and attending multiple national and international conferences, including the *LRI congress 2022*, *ILC 2022*, and *NVVI 2022*. I also was able to engage in field work and provide a lab training in Brazil as part of the LRI BraBo project (**Chapter 8**), and later that year, at the National Institute for Infectious Diseases "L. Spallanzani", IRCCS, in Rome, Italy (**Chapter 7**). Additionally, in 2022, I supervised a BSc student (Biomedical sciences) during her internship, providing guidance and support throughout her research project. These experiences contributed to my development as a collaborative and globally engaged researcher.

The year 2023 was marked by an expansion in both research dissemination and teaching activities. I attended the *Leprosy Research Initiative (LRI) congress 2023* in Eindhoven and the *Global Research on Worldwide challenges (GROW) congress 2023* in Soesterberg. I also presented during a *LU-CID* session and delivered lab trainings to project collaborators in Bolivia and Indonesia. A major highlight was my supervision of the half minor *Infections in Health and Disease* during a field trip in October 2023 in South Sulawesi, Indonesia (**Chapter 5**). I was one of the supervisors to a group of medical students from the Netherlands and Indonesia - supporting them in conducting field research, laboratory work, data analysis and presentation and report writing. This role involved coordinating activities, troubleshooting challenges in real time, and fostering cross-cultural scientific collaboration. In addition to this, I supervised an MSc student (Biomedical sciences) during her internship (acquiring data for **Chapter 3**) and assessed students during the *FOS* journal club course. Furthermore, I supported a PhD student from Edinburgh during his exchange in Leiden by assisting in the setup and development of assays for his project on bovine TB. Beyond this, I provided guidance and assistance to three other international PhD students visiting Leiden for exchanges or internships, helping them with various aspects of their research projects. These experiences sharpened my mentorship skills and strengthened my ability to communicate scientific concepts across different academic levels.

In 2024, I participated in and presented at the *Workshop on Research on Tuberculosis and Leprosy* and the *Theme Infection Meeting*. I also completed the *Responsible Research* training, including a *Workshop on Scientific Conduct for PhDs*, both of which reinforced the importance of ethics and integrity in science. I also supported two international PhD students and contributed to their research progress as part of a collaborative research team.

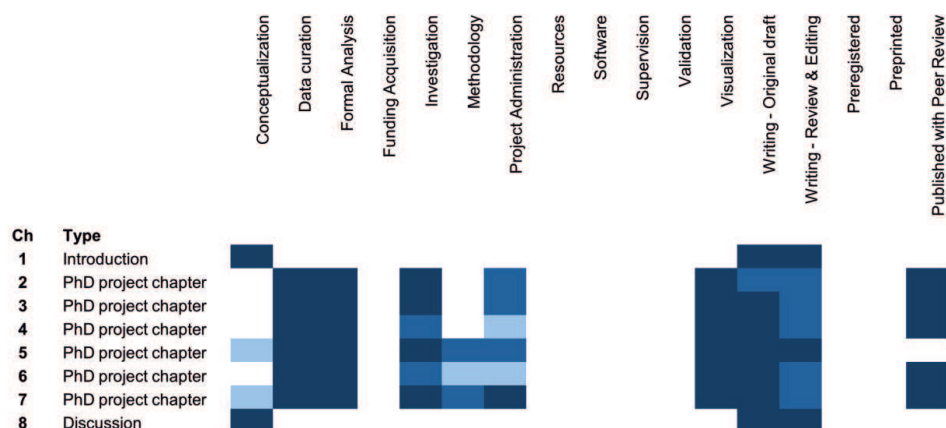
Additionally, I gave a presentation at the *LiTBC meeting*, contributing to broader institutional knowledge exchange.

The final year of my PhD, 2025, included attendance at the *LRI congress 2025* and the *ILC 2025*, where I shared the final outcomes of my work. To support my attendance and presentation at ILC 2025, I successfully applied for and received a travel grant from the *Leiden University Fund (LUF)*. The ILC conference was a significant milestone in my PhD journey, providing an excellent platform not only to present my research findings but also to engage in a wide range of scientific and political discussions. Additionally, I participated in numerous networking dinners and meetings, which gave me valuable insights into the broader impact of immunodiagnostics and infectious disease research on global health policy. This conference truly marked a meaningful culmination of my PhD trajectory, integrating scientific communication, interdisciplinary collaboration, and career development.

Across my PhD trajectory, I regularly attended theme meetings, internal lectures, and LUMC presentations, which allowed me to stay connected to ongoing developments and to contribute to interdisciplinary discussions. In addition, my training and dissemination activities included diverse teaching responsibilities, active participation in national and international scientific meetings, and the delivery of hands-on laboratory training to collaborators in Brazil, Bolivia and Indonesia. These international training activities were particularly formative, as they enabled me to apply my scientific expertise in collaborative, real-world settings and to contribute to capacity building among research partners.

Overall, my PhD journey has been shaped not only by research, but also by teaching, extensive global collaboration, and scientific exchange. Successfully obtaining external funding to present my work at a major international conference, alongside supervising students and supporting collaborative research and training activities, reflects my development into an independent and proactive researcher.

### CRedit statement for the Chapters in this thesis



### Dissemination table

|                                                                                                                                                                                                                                                        |                                                                                                   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| <b>1. Pierneef et al. Detection of anti-M. leprae antibodies in children in leprosy-endemic areas: a systematic review</b>                                                                                                                             |                                                                                                   |
| Chapter in this thesis                                                                                                                                                                                                                                 | 2                                                                                                 |
| Publication in peer-reviewed journal                                                                                                                                                                                                                   | <a href="https://pubmed.ncbi.nlm.nih.gov/34449763/">https://pubmed.ncbi.nlm.nih.gov/34449763/</a> |
| <b>2. Pierneef et al. Host biomarker-based quantitative rapid tests for detection and treatment monitoring of tuberculosis and COVID-19</b>                                                                                                            |                                                                                                   |
| Chapter in this thesis                                                                                                                                                                                                                                 | 7                                                                                                 |
| Publication in peer-reviewed journal                                                                                                                                                                                                                   | <a href="https://pubmed.ncbi.nlm.nih.gov/36590898/">https://pubmed.ncbi.nlm.nih.gov/36590898/</a> |
| <b>3. Pierneef et al. Field-friendly anti-PGL-I serosurvey in children to monitor Mycobacterium leprae transmission in Bihar, India</b>                                                                                                                |                                                                                                   |
| Chapter in this thesis                                                                                                                                                                                                                                 | 4                                                                                                 |
| Publication in peer-reviewed journal                                                                                                                                                                                                                   | <a href="https://pubmed.ncbi.nlm.nih.gov/37828950/">https://pubmed.ncbi.nlm.nih.gov/37828950/</a> |
| <b>4. Pierneef et al. Rapid test for Mycobacterium leprae infection: a practical tool for leprosy</b>                                                                                                                                                  |                                                                                                   |
| Chapter in this thesis                                                                                                                                                                                                                                 | 3                                                                                                 |
| Publication in peer-reviewed journal                                                                                                                                                                                                                   | <a href="https://pubmed.ncbi.nlm.nih.gov/39617937/">https://pubmed.ncbi.nlm.nih.gov/39617937/</a> |
| <b>5. Pierneef et al. Large-scale field application of a fingerstick blood test for Mycobacterium leprae infection: Monitoring population-wide effects of case finding and post-exposure prophylaxis on transmission in the Comoros and Madagascar</b> |                                                                                                   |
| Chapter in this thesis                                                                                                                                                                                                                                 | 6                                                                                                 |
| Publication in peer-reviewed journal                                                                                                                                                                                                                   | <a href="https://pubmed.ncbi.nlm.nih.gov/41329688/">https://pubmed.ncbi.nlm.nih.gov/41329688/</a> |
| <b>6. Pierneef et al. Seroprevalence of anti-Mycobacterium leprae PGL-I antibodies among school children in rural and urban Indonesia: a cross-sectional study (2023)</b>                                                                              |                                                                                                   |
| Chapter in this thesis                                                                                                                                                                                                                                 | 5                                                                                                 |
| Publication in peer-reviewed journal                                                                                                                                                                                                                   | <a href="https://pubmed.ncbi.nlm.nih.gov/42256252/">https://pubmed.ncbi.nlm.nih.gov/42256252/</a> |

## Overview of completed courses and other training

| <b>Completed courses and other training</b>                                             |                                                                 |            |
|-----------------------------------------------------------------------------------------|-----------------------------------------------------------------|------------|
| <b>Mandatory activities</b>                                                             |                                                                 |            |
| Year                                                                                    | Title                                                           | EC / hours |
| 2020                                                                                    | Basic Methods and Reasoning in Biostatistics                    | 42         |
| 2021                                                                                    | Leiden University Onboarding Inform & Connect (2 activities)    | 5          |
| 2024                                                                                    | Responsible Research (2 activities)                             | 20         |
| <b>Scientific courses, workshops and other training activities</b>                      |                                                                 |            |
| 2021                                                                                    | LAP PhD day                                                     | 6          |
| 2022                                                                                    | LIFI course                                                     | 45         |
| 2025                                                                                    | Symposium "A Dutch perspective on mycobacterial infections"     | 8          |
| 2025                                                                                    | Symposium "Past, present and future of immunology in Leiden"    | 8          |
| <b>Dissemination, acknowledgement, esteem, and other relevant scientific activities</b> |                                                                 |            |
| 2021                                                                                    | LRI Congress 2021                                               | 8          |
| 2022                                                                                    | NVVI (poster presentation)                                      | 24         |
| 2022                                                                                    | LRI Congress 2022 (oral presentation)                           | 16         |
| 2022                                                                                    | ILC Conference 2022 (oral presentation)                         | 24         |
| 2023                                                                                    | LRI Congress 2023 (oral presentation)                           | 24         |
| 2023                                                                                    | GROW Congress 2023                                              | 16         |
| 2023                                                                                    | Presentation at LU-CID meeting                                  | 5          |
| 2024                                                                                    | Presentation at Workshop 'Research on Tuberculosis and leprosy' | 4          |
| 2024                                                                                    | LRI Congress 2024 (oral presentation)                           | 24         |
| 2024                                                                                    | Presentation at Theme Infection meeting                         | 8          |
| 2024                                                                                    | Pitch presentation at WINNER                                    | 4          |
| 2024                                                                                    | Presentation at LiTBC meeting                                   | 4          |
| 2025                                                                                    | LRI Congress 2025 (oral presentation)                           | 12         |
| 2025                                                                                    | ILC Conference 2025 (oral presentation)                         | 30         |
| <b>Other activities related to this thesis</b>                                          |                                                                 |            |
| 2022                                                                                    | Lab training in Brazil to co-workers LRI BraBo project          | 4          |
| 2022                                                                                    | Lab training in Rome to co-workers                              | 4          |
| 2022                                                                                    | Supervision of internship BSc student Biomedical Sciences       | 28         |
| 2022                                                                                    | Supervision of international PhD student                        | 28         |
| 2023                                                                                    | Lab training in Bolivia to co-workers LRI BraBo project         | 4          |
| 2023                                                                                    | Lab training in Indonesia to co-workers                         | 16         |
| 2023                                                                                    | Supervision of internship MSc student Biomedical Sciences       | 28         |
| 2023                                                                                    | Journal club assessment of students during FOS course           | 2          |
| 2023                                                                                    | Supervision of halfminor "Infections in Health and Disease"     | 84         |
| 2024                                                                                    | Supervision of international PhD student                        | 28         |
| 2024                                                                                    | Supervision of International PhD student                        | 28         |
| <b>Total number of hours</b>                                                            |                                                                 | <b>591</b> |







