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

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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, enhances acceptance, and reduces 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 immunodiagnostics 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. Grijsen, 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'sDisease 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 Hararghe 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. Denkinger, 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 epizootiologic 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.