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HOW SUITABLE ARE NDO-LID AND PGL-I IN RAPID DIAGNOSTIC TESTS FOR SCREENING OF LEPROSY? A REVIEW OF DIAGNOSTIC STUDIES

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ABSTRACT

Background: Indonesia has the third highest prevalence of leprosy, a neglected tropical disease caused by the "Mycobacterium leprae complex which causes neuropathy and hypopigmented skin lesions with hypoesthesia. The main problem in eradicating leprosy is due to its negative social stigma and prevalence in lower socioeconomic populations, as the gold standard for leprosy diagnosis is histopathology of skin smears. Hence, cost and time effective screening tools are needed. PGL-I and NDO-LID are antigens that have been developed as biomarkers used in serological assays, one of them being rapid lateral flow tests. Therefore, a rapid test utilizing a combination of PGL-I and NDO-LID as its marker is potentially a suitable tool for leprosy screening in areas with limited access to healthcare. **Objective:** To evaluate the diagnostic potential of PGL-I and NDO-LID in rapid lateral flow tests for leprosy. **Methods:** This literature review was done by using the search engines PubMed, Science Direct, and SpringerLink. **Results:** Diagnostic potential studies for PGL-I rapid tests using UCP-LFA and Gold-LFA showed high overall sensitivity and specificity for leprosy patients, with more positive results in MB and PB patients. NDO-LID rapid test diagnostic studies which used the also showed similar results. However, these tests are not as accurate as ELISA and histopathology, but modifications such as additional biomarkers can be used to improve its utility. **Conclusion:** PGL-I and NDO-LID have good sensitivity and specificity from the results of various diagnostic studies, thus are potentially suitable for field screening of leprosy.

BACKGROUND

In 2023, Indonesia reported 14,376 new cases of leprosy, making it the third highest in the world. 8.2% of those cases occurred in children, and 6% resulted in significant disability.[1] Leprosy, also known as Morbus Hansen or Hansen's Disease, is a chronic granulomatous infection caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis*, which make up the "*Mycobacterium leprae* complex". These bacteria primarily affect peripheral nerves and skin, resulting in neuropathy and skin lesions that lead to disability among patients.[2] Although the global burden of leprosy has decreased ever since the implementation of multi-drug therapy by WHO in the 1980s, the annual new case detection rate has remained nearly static. The distribution of leprosy is also highest in India, Brazil, and Indonesia.[3] This disease is often found in lower socioeconomic populations, where it is associated negative social stigma as patients are considered "cursed".[4] Hence, leprosy is considered a neglected tropical disease (NTD), increasing its burden in developing countries in which it is endemic.

Transmission of leprosy is not fully understood, but most cases have been found to be linked with transmission through the upper respiratory tract as well as broken skin. Risk factors including close contact with leprosy patients, armadillo exposure, old age, genetics, and immunosuppression also increase the likelihood of contracting leprosy. In the body, *M. leprae* attacks mostly peripheral neurons, more specifically often its Schwann cells, hence resulting in demyelination and loss of conduction within axons. *M. leprae* also infiltrates the dermis and activates immune

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cells. This along with neuron damage results in hypopigmented skin lesions with hypoesthesia.[2]

According to the WHO, severity of leprosy is classified into paucibacillary (PB), where 5 or less skin lesions present or in other words low bacterial load, and multibacillary (MB), also known as high bacterial load and patients present with 5 or more skin lesions. The Ridley-Jopling classification is also often used, in which five subtypes are present: Tuberculoid Tuberculoid (TT), Borderline Tuberculoid (BT), Borderline Borderline (BB), Borderline Lepromatous (BL), and Lepromatous Lepromatous (LL). TT and BT forms fall into the PB category, which are less contagious, a BB, BL, and LL are classified into the MB group, hence are highly contagious.[5]

The main problem in eradicating leprosy is its difficulty in screening, as this disease is found mostly in lower socioeconomic populations in which access to health resources is limited.[6] Leprosy is also associated with negative social stigma, as in the past, patients were discriminated against society due to being regarded as "sinners".[4] This results in leprosy patients less likely to seek for medical help, worsening the symptoms experienced until it has developed into severe disability. Moreover, the gold standard for leprosy screening is histopathological skin biopsy or smears, with scoring according to their bacterial indeces.[7] This method is quite time consuming and has relatively low accessibility for lower socioeconomic populations.

Certain biomarkers, namely anti-phenolic glycolipid-I (PGL-I) antigen and anti-natural octyl disaccharide-leprosy IDRI diagnostic (NDO-LID), have been found to be effective in detecting the presence of leprosy.[8] High seropositivity of PGL-I were found in leprosy patients, with higher positivity in multibacillary than paucibacillary patients.[9] The same can be said for NDO-LID.[10] Being antigens, they are measured using serological assays, with a cost and time effective method being lateral flow test for *M. leprae* (ML Flow test). Hence, the combination of these two antigens have potential to be used for field screening of leprosy, especially in areas with limited access to healthcare.[11]

METHODS

This literature review was done by doing a literature using the following search engines: PubMed, ScienceDirect, SpringerLink, and ProQuest. The following keyword combination was used: ((PGL-I OR phenolic glycolipid I) OR NDO-LID) AND (Mycobacterium Leprae OR Leprosy) AND Rapid Test. Inclusion and exclusion criteria were applied when choosing the literature to be used.

- Inclusion criteria
  - Studies with in vivo, in vitro, and clinical trial research designs
  - Articles published within the last 10 years, that is 2014-2024
  - Articles written in English
- Exclusion criteria
  - Articles that do not have complete data or has not been published
  - Articles in which the keywords do not match with the title and abstract
  - Articles that cannot be fully accessed
  - Articles that do not discuss ROC analysis (sensitivity, specificity, positive predictive value, or negative predictive value) of the diagnostic test

Date was extracted and organized into two tables, for NDO-LID and PGL-I, based on author, name of test, sample, population, and diagnostic studies results (sensitivity, specificity, PPV, and NPV). Results were ordered based on publication year.

RESULTS

A total of seven articles were used in the final synthesis. Three articles discussed only the use of PGL-I rapid test, three articles discussed only the use of NDO-LID rapid test, whereas one article discussed both PGL-I and NDO-LID rapid test for diagnosis of leprosy. The countries in which the studies were conducted in included Brazil, Philippines, China and Ethiopia. The majority of the studies utilized serum as sample, while only one study used whole blood. The diagnostic study results for PGL-I rapid tests can be found in Table 1., while the results for NDO-LID are presented in Table 2.

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Table 1. Data extraction of included studies for PGL-I rapid test

Author	Test	Sample	Population	Results							
				Sensitivity		Specificity		PPV		NPV	
				PB	MB	PB	MB	PB	MB	PB	MB
Moura, et al. 2014	ML-Flow PGL-I rapid test	Serum	Brazil		BSA: 87.2% HSA: 81.1%		BSA: 98.8% HSA: 96.7%		BSA: 76.9% HAS: 76.4%		BSA: 88.3% HSA: 83.7%
Hooji et al. 2017	UCP-LFA (up-converting phosphor lateral flow assay)	Serum	Philippines	94%	94%	81%	100%		-	-	-
	Gold-LFA (immunogold lateral flow assay)			78%	94%	78%	94%	-	-	-	-
Hooji et al. 2018	UCP LFA	Blood	China	-	78%	-	94%	-	-	-	-
			Brazil	-	17%	-		-	-	-	-
			Ethiopia	-	55%	-		-	-	-	-
	UCP LFA (with additional cellular markers)		China	-	91%	-		-	-	-	-
			Brazil	-	97%	-		-	-	-	-
			Ethiopia	-	75%	-		-	-	-	-
Leturiondo et al. 2019	ML-Flow PGL-I rapid test	Serum	Brazil	32%	81%	-	75.9%	-	43.4%	-	94.6%

Table 2. Data extraction of included studies for NDO-LID rapid test

Author	Test	Sample	Population	Results							
				Sensitivity		Specificity		PPV		NPV	
				PB	MB	PB	MB	PB	MB	PB	MB
Duthie, et al. 2014	NDO-LID Test: LID-1	Serum	Philippines	32.3%	87%	97.4%		-	-	-	-
Duthie, et al. 2016	OnSite Leprosy Ab Rapid	Serum	Philippines	77.3%		96.4%					
		Blood	Philippines	83.3%							
	NDO-LID Test: LID-1	Serum	Philippines	95.5%		25%					
Leturiondo, et al. 2019	ML-Flow NDO-LID rapid test	Serum	Brazil	34%		73.6%		-		81.7%	
Frade et al. 2019	Lateral Flow NDO-LID	Blood	Brazil	Patient vs Healthy (PvN): 62.9% Contact vs Non-Contact (CvN): 51,6%		PvN: 42.01% CvN: 39.59%		CvN: 89.87% CvN: 68.31%		PvN: 12.11% CvN: 24.49%	

DISCUSSION

PGL-I as a Marker in Rapid Lateral Flow Test for *Mycobacterium leprae*

Phenolic glycolipid-I (PGL-I), a crucial component of the outer membrane of *Mycobacterium leprae*, plays a significant role in the bacterium's ability to infect Schwann cells by binding to laminin α2. This binding initiates a cascade of events that leads to infection, characterized by excessive production of nitric oxide in response to the interaction. The overproduction of nitric oxide is detrimental, as it causes damage to the mitochondria of axons, ultimately resulting in demyelination. In addition to its effects on Schwann cells, PGL-I also influences the immune response. It stimulates tissue-resident macrophages to first engulf the invading bacteria and subsequently produce CCL2, a chemokine that attracts permissive macrophages to the site of infection. This recruitment is critical as it allows mycobacteria to transfer from the initially activated

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microbicidal macrophages to newly recruited monocytes that are more conducive to bacterial growth.[12]

For decades, tests have been employed to detect the humoral immune response against PGL-I, which serves as an important biomarker for leprosy infection. These tests not only indicate the presence of infection but also help assess infection rates within populations and guide appropriate multidrug treatments for patients with either paucibacillary (PB) or multibacillary (MB) forms of leprosy. Furthermore, studies have shown that antibody levels targeting PGL-I are closely correlated with bacterial load and an increased risk of developing leprosy, highlighting its significance in understanding the disease's progression and potential treatment strategies.[13,14]

Rapid lateral flow tests for *M. leprae* have been developed for detecting PGL-I. Hooij et al. in 2017 used rapid tests with UCP-LFA (up-converting phosphor lateral flow assay) and Gold-LFA (immunogold lateral flow assay) in the Philippines with serum as the sample. In PB patients, UCP-LFA showed 94% sensitivity and 81% specificity, while Gold-LFA had lower sensitivity (78%) and higher specificity (94%).[11] A study done in Brazil also used an ML-Flow PGL-I rapid test, and results showed 32% sensitivity in PB patients.[15]

A study was done by Hooij et al. in 2017 for MB patients, and it showed that UCP-LFA had 94% sensitivity and 100% specificity, while Gold-LFA had 78% and 94% sensitivity. Hence, both LF-based tests identified the majority of MB patients (≥78%) and was able to distinguish MB patients from PB patients.[11] In 2018, Hooij, et al. tested UCP-LFA combined with additional cellular markers (IP-10) in China, Brazil, and Ethiopia, for MB patients, specifically LL/BL. In China, the test had 78% sensitivity on its own and 91% combined with cellular markers; in Brazil, 17% sensitivity without and 97% with cellular markers; and in Ethiopia, 55% without and 75% with additional cellular markers. Overall, the specificity of UCP-LFA had 89% specificity with IP-10 as the additional marker, with cut-off points >0.205 in Brazil, >0.61 in China, and >1.195 in Ethiopia.[16] Lastly, research by Leturiondo, et al. (2019) in Brazil showed that an ML-Flow PGL-I rapid test had in MB patients, the test had 81% sensitivity, 75.9% specificity, 43.4% positive predictive value (PPV), and 94.6% negative predictive value (NPV). The study concluded that this rapid test isn't very suitable in clinical diagnosis, but since it has high NPV, it is able to exclude leprosy cases, making it useful for screening in hyperendemic regions.[15]

Moura et al. (2014) tested a PGL-I ML-Flow test with the addition of human serum albumin (HSA) in comparison with bovine serum albumin (BSA) as antigen carriers. Results showed that ML Flow with BSA had 87.2% sensitivity, 98.8% specificity, 76.9% PPV, and 88.3% NPV; while the ML Flow with HSA had 81.1% sensitivity, 96.7% specificity, 76.4% PPV, and 83.7% NPV. These results show that the use of HSA did not enhance the performance of this serological test. However, ML Flow is still a useful auxiliary tool to identify leprosy patients with high bacterial load.[17]

NDO-LID as a Breakthrough in Rapid Lateral Flow Test for Detecting *Mycobacterium Leprae*

NDO-LID is an advanced serological test for leprosy that combines the natural disaccharide octyl (NDO) and the IDRI Diagnostic Leprosy Antigen (LID-1), enhancing the detection of antibodies against Mycobacterium leprae, the causative agent of leprosy. This test has shown significant promise in identifying individuals at risk of developing leprosy reactions (LRs) and neuritis, with elevated levels of anti-NDO-LID antibodies found in patients with a history of LRs compared to those without such a history. [18] The ability to detect these antibodies prior to the onset of clinical symptoms positions NDO-LID as a crucial tool for early diagnosis, enabling timely intervention that is essential for effective treatment and management of leprosy.[19]

Moreover, studies indicate that antibody levels against NDO-LID correlate strongly with bacterial load, suggesting that higher antibody levels are associated with more severe infections.[20] This correlation highlights the test's potential utility not only in diagnosing leprosy but also in monitoring disease progression and guiding treatment strategies. The integration of NDO-LID into routine diagnostic protocols could significantly improve surveillance efforts in leprosy-endemic regions, facilitating earlier detection and reducing transmission rates.[21] Overall, NDO-LID represents a significant advancement in leprosy diagnostics, offering a reliable method for

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identifying infections and assessing individual risks within affected population.[15, 22]

The NDO-LID lateral flow test has shown significant promise in the detection of leprosy, particularly in improving diagnostic accuracy. According to research, the NDO-LID test achieved a sensitivity of 87% and a specificity of 97.4%, outperforming the Standard Diagnostics leprosy test, which had a sensitivity of 81.7% and a specificity of 90.4%. Notably, the NDO-LID test was able to detect 32.3% of paucibacillary (PB) cases compared to only 6.5% by the alternative test. This advancement is crucial for early diagnosis and treatment, especially in areas with limited access to healthcare professionals capable of diagnosing leprosy accurately. The incorporation of a smartphone-based reader further enhances the reliability and accessibility of the NDO-LID test, making it a valuable tool in leprosy elimination efforts (Duthie et al., 2014). [22]

In a comparative evaluation of antibody detection tests for diagnosing multibacillary leprosy, Duthie et al. (2015) assessed the performance of several assays using serum samples. The NDO-LID® test demonstrated a sensitivity of 95.5% and a positive predictive value (PPV) of 25.0%. In contrast, the OnSite Leprosy Ab Rapid test (CTK Biotech) showed a sensitivity of 77.3% and a high specificity of 96.4%. Blood sample analysis yielded a sensitivity of 83.3%, with no significant difference compared to serum results, while an ELISA method exhibited an overall sensitivity of 80.3% and specificity of 93.7%. Due to its high sensitivity and specificity, the OnSite Leprosy Ab Rapid test is recommended for field screening, providing reliable results from both serum and blood samples.[23]

The study by Frade et al. (2017) assessed the diagnostic accuracy of Lateral Flow (NDO-LID) tests for leprosy in midwestern Brazil. Key findings for the Lateral Flow test included a Negative Predictive Value (NPV) of 89.87% for patient vs. healthy (PvH) and 68.31% for contact vs. non-contact (CvN), Positive Predictive Value (PPV) of 12.11% (PvH) and 24.49% (CvN), sensitivity of 62.79% (PvH) and 51.61% (CvN), and specificity of 42.01% (PvH) and 39.59% (CvN).[24]

In the study by Leturiondo et al. (2019), the NDO-LID rapid test was evaluated for its accuracy in diagnosing leprosy in Brazil. The test demonstrated a high negative predictive value (NPV) of 93.1%, making it effective for ruling out leprosy cases, particularly useful in high-endemic regions. The positive predictive value (PPV) was 47.9%, indicating that almost half of the positive results might not accurately reflect true leprosy cases. Sensitivity for multibacillary (MB) cases was 73.6% and 34.0% for paucibacillary (PB) cases, meaning it detects MB cases with moderate accuracy but is less reliable for PB cases. Specificity was 81.7%, which shows that the test is moderately effective in correctly identifying non-leprosy cases. These findings highlight that while the NDO-LID test is beneficial for screening, especially in excluding cases, its limitations in sensitivity and PPV reduce its effectiveness as a standalone diagnostic tool in the context of clinical practice.[15]

Limitations and Recommendations for Future Studies

Serological tests have an advantage over conventional tests, slit-skin smears and histopathology examinations, for leprosy as they are not invasive and consume less time. The results can also be considered more objective than histopathology as they do not require subjective interpretation of the reader.[25] However, positive serology results do not always correlate with the development of leprosy in individuals. The opposite is also true, as a negative serology result does not always mean the patient is free from the disease.[26] This is because anti-PGL-I serology has better sensitivity and lower specificity for MB. As a result, PGL-I tests are not suitable to be used for differentiation of MB with other types.[25] NDO-LID also has relatively low sensitivity and specificity as biomarkers, even though higher levels are found in confirmed leprosy patients rather in comparison with healthy individuals.[8]

The sample used in diagnostic studies for PGL-I and NDO-LID rapid tests mostly utilized serum.[11,15,16,23] When used for field screening, it is ideal to use blood for the sample, particularly fingerstick blood as it is the least invasive and the most time effective method.[14] Out of the studies that evaluated the diagnostic potential (sensitivity and specificity) of those tests, only two of those studies used blood as their sample.[23,24] Other previous studies that did use blood as their samples only measured seropositivity or other indicators, but did not analyze

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sensitivity, specificity, PPV nor NPV, which is important in understanding its potential for clinical application.[10,13,14,27–29] Even though research done by Duthie, et al. in 2015 showed that sensitivity and specificity of these tests were not significantly different when using blood rather than serum, more research should be done to ensure its efficacy if it were to be implemented on the field.

CONCLUSION

PGL-I is an antigen found in Mycobacterium leprae that is essential in the leprosy pathogenesis as it helps the bacterium invade Schwann cells. NDO-LID is a combination of natural disaccharide octyl and the IDRI Diagnostic Antigen. Antibodies against PGL-I and NDO-LID can be detected in serological tests, with rapid lateral flow test being a cost and time effective option. Studies showed that these biomarkers in ML flow tests had relatively good sensitivity and specificity, with better results in MB patients rather than PB as they have higher bacterial load. However, these tests are not as accurate when compared with ELISA and histopathology. Thus, these rapid tests are not suitable for clinical diagnosis, but potentially more fitting for field screening of leprosy as they are more cost and time effective.

ETHICAL APPROVAL

There is no ethical approval.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Author contributions as follows: conceptualization, GVPH, ADAK, and AHF; methodology, GVPH; software, GVPH; validation, GVPH, ADAK, and AHF; formal analysis, GVPH, ADAK, and AHF; investigation, GVPH, ADAK, and AHF; resources, GVPH, ADAK, and AHF; data curation, GVPH, ADAK, and AHF; writing—original draft preparation, GVPH, ADAK, and AHF; writing—review and editing, GVPH; visualization, GVPH, ADAK, and AHF; supervision, GVPH; project administration, GVPH.

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