Review Article

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Integrative literature review of the reported uses of serological tests in leprosy management

***Angélica da Conceição Oliveira Coelho Fabri*[1],[2], *Ana Paula Mendes Carvalho*[2], *Nayara Figueiredo Vieira*[2], *Isabela de Caux Bueno*[3], *Rayssa Nogueira Rodrigues*[2], *Thayenne Barrozo Mota Monteiro*[4], *Rodrigo Correa-Oliveira*[2],[5],[6],[7], *Malcolm S. Duthie*[8] *and Francisco Carlos Félix Lana*[2],[9]**

[1]. Departamento de Enfermagem Básica, Faculdade de Enfermagem, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brasil. [2]. Programa de Pós-graduação em Enfermagem, Escola de Enfermagem, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil. [3]. Escola de Enfermagem, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil. [4]. Faculdade de Enfermagem, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brasil. [5]. Laboratório de Imunologia Celular e Molecular, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brasil. [6]. Laboratório de Imunologia, Núcleo de Pesquisas em Ciências Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brasil. [7]. Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais, Brasil. [8]. Infectious Disease Research Institute, Seattle WA, USA. [9]. Departamento de Enfermagem Materno Infantil e Saúde Pública, Escola de Enfermagem, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.

ABSTRACT

An integrative literature review was conducted to synthesize available publications regarding the potential use of serological tests in leprosy programs. We searched the databases *Literatura Latino-Americana e do Caribe em Ciências da Saúde*, *Índice Bibliográfico Espanhol em Ciências da Saúde*, *Acervo da Biblioteca da Organização Pan-Americana da Saúde*, Medical Literature Analysis and Retrieval System Online, *Hanseníase*, National Library of Medicine, Scopus, Ovid, Cinahl, and Web of Science for articles investigating the use of serological tests for antibodies against phenolic glycolipid-I (PGL-I), ML0405, ML2331, leprosy IDRI diagnostic-1 (LID-1), and natural disaccharide octyl-leprosy IDRI diagnostic-1 (NDO-LID). From an initial pool of 3.514 articles, 40 full-length articles fulfilled our inclusion criteria. Based on these papers, we concluded that these antibodies can be used to assist in diagnosing leprosy, detecting neuritis, monitoring therapeutic efficacy, and monitoring household contacts or at-risk populations in leprosy-endemic areas. Thus, available data suggest that serological tests could contribute substantially to leprosy management.

Keywords: Serologic tests. Recombinant proteins. Glycolipids. *Mycobacterium leprae*. Leprosy.

INTRODUCTION

Leprosy persists as a significant public health issue in many countries, especially those that are socially and economically underdeveloped. In 2013, the World Health Organization (WHO) was notified of 215.656 new cases in 102 countries, with India and Brazil accounting for 58.9% and 14.4% of new cases, respectively[1]. Among the goals set by the WHO to achieve by end of 2015 was to reduce, by at least 35% compared to 2010, new cases among children under 15 years of age, as well new cases with grade 2 disability. These goals were intended to stimulate activities aimed at achieving early diagnosis and providing timely treatment with multidrug therapy, thereby helping to both reduce new cases and minimize transmission of *Mycobacterium leprae*[2].

At present, diagnosis is predominantly based on clinical assessment, and therefore depends on recognition of characteristic symptoms. However, investment in new technologies may be necessary to facilitate early diagnosis and to achieve WHO goals. Indeed, while tests such as skin slit smear, Mitsuda test, and histological analyses can accelerate diagnosis, none are 100% sensitive or specific[3]. In addition, a sensitive and specific test to classify leprosy as either paucibacillary or multibacillary is critical in selecting the most appropriate treatment regimen. Thus, the current diagnostic challenge is to identify or develop a point-of-care test that can contribute to or facilitate early diagnosis and classification. Toward this end, immunological tests are being evaluated. Among the most advanced tools in development are tests to detect antibodies against phenolic glycolipid-I (PGL-I), or its di- and trisaccharide analogs NDO and NTP, respectively. Additional diagnostic markers include antibodies against Leprosy IDRI Diagnostic-1 (LID-1), which is a fusion of the ml0405 and ml2331 gene products[4], as well as antibodies against NDO-LID, a conjugate of natural disaccharide octyl (NDO) and LID[5]. In this study, we mined the available peer-reviewed scientific literature to assess the value of serological tests in leprosy control programs.

Corresponding author: Dra. Angélica da Conceição Oliveira Coelho Fabri.
e-mail: angelicacoelho8@gmail.com
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INTEGRATIVE LITERATURE REVIEW

In integrative literature review, relevant publications are collected and summarized in a systematic manner, with the intent of consolidating present knowledge of the subject of interest[6]. To guide the review, we asked the following question: "How have serology tests for antibodies against PGL-I, ML0405, ML2331, LID-1, and NDO-LID been used to manage leprosy?"

We searched for articles in the databases *Literatura Latino-Americana e do Caribe em Ciências da Saúde* (LILACS), National Library of Medicine (PUBMED), Medical Literature Analysis and Retrieval System Online (MEDLINE), Cumulative Index to Nursing and Allied Health Literature (CINAHL), *Índice Bibliográfico Espanhol em Ciências da Saúde* (IBECS), *Acervo da Biblioteca da Organização Pan-Americana da Saúde* (PAHO), *Hanseníase* (HANSEN), SCOPUS, OVID, and Web of Science.

To search in LILACS, IBECS, and PAHO of the *Descritores em Ciência da Saúde*, the following descriptors were used: "LID-1", "NDO-LID", "PGL-I", "PGL-1", "ML0405", "ML2331", "Recombinant Proteins", "Recombinant Fusion Proteins", "Fusion Proteins", "Glycolipids", "Antigens, Bacterial", "Immunoglobulin G", "Immunoglobulin M", "gamma-Globulins", "Immunoglobulin", "Antibodies, Bacterial", "IgM", "IgG", "ELISA", "Serologic Tests", "Serodiagnosis", "Agglutination Tests", "Enzyme-Linked Immunosorbent Assay", and "*Mycobacterium leprae*". The descriptors were used in English, Portuguese, and Spanish, alone or in combination.

In PUBMED, MEDLINE, and CINAHL the following search terms from Medical Subject Headings were used: "Recombinant Proteins", "Recombinant Fusion Proteins", "Glycolipids", "Antigens, Bacterial", "LID-1", "NDO-LID", "PGL-I", "PGL-1", "Recombinant Proteins", "ML0405", "ML2331", "Immunoglobulin G", "Immunoglobulin M", "Antibodies, Bacterial", "Gamma Globulin", "IgM", "IgG", "Antibody", "Serologic Tests", "Agglutination Tests", "Enzyme-Linked Immunosorbent Assay", "ELISA", "ML FLOW", "*Mycobacterium leprae*", "Leprosy", and "Hansen's Disease".

To be considered for review, papers should have been published between January 2002 and January 2015 in Portuguese, English, or Spanish; should be available as full-text in electronic format; and should contain at least one of the following terms in the title in any of the three languages: "serology", "seroprevalence", "seropositivity", "subclinical infection", "antibody", "antigen", "immunological", "ML Flow", "laboratory test", "PGL-I", "PGL-1", "LID-1", "NDO-LID", "protein", "ML0405", and "ML 2331". In addition, we considered only those articles with level of evidence 1, 2, 3, and 4, as defined in the classification of evidence hierarchical system[7]. Article ID, methodological characteristics, methodological rigor, and soundness of results were collected from all candidate articles by four researchers, following an instrument adapted from Ursi[8]. Articles were formally and finally selected by consensus based on suitability and preliminary data from all four researchers. Descriptive analysis was then performed to summarize the main findings of each article with regard to the following themes: "use of serological assays in diagnosis", "surveillance of household contacts and at-risk populations", "value of serological tools in assessing multidrug therapy", and "potential relationship between serum antibodies and *M. leprae* transmission".

ARTICLE SELECTION

Based on the question "How have serology tests detecting anti-PGL-I, anti-ML0405, anti-ML2331, anti-LID-1 and anti-NDO-LID antibodies been used in leprosy?", we identified a total of 3.514 articles, of which 2.554 were duplicated among databases. Duplicates were excluded, along with a further 856 articles excluded based on title, 33 based on abstract, and 31 based on full text, either because the articles did not meet inclusion criteria, or were otherwise unsuitable for the objectives of the review. In the end, 40 articles were considered for final integrative review (**Figure 1**).

USE OF SEROLOGICAL ASSAYS IN DIAGNOSIS OF LEPROSY

Testing for anti-PGL-I can help classify leprosy[9] [10] [11] [12] [13] [14] [15] into paucibacillary and multibacillary forms[5] [15] [16] [17] [18] [19] [20] [21] [22] and facilitate differential diagnosis [23] (**Table 1**). When used in Brazil as an additional tool for classification, a rapid anti-PGL-I test called ML Flow reduced the number of cases treated as multibacillary, thus reducing the use of anti-mycobacterial drugs, as well as the amount of case management required. Hence, the test provided a direct benefit during treatment[24].

Testing for antibodies against recombinant ML0405, ML2331, and LID-1 antigens can also assist in diagnosis and classification[5] [17] [25] [26] (**Table 1**). In particular, antibodies against LID-1 can be used as an immunological marker for leprosy[27], more specifically of the multibacillary presentation[17]. Indeed, reports indicate that anti-LID-1 can detect leprosy 6-8 months before clinical symptoms manifest[4]. Thus, screening for anti-LID-1 in the general population or in populations at greatest risk of *M. leprae* infection could potentially accelerate treatment, and reduce transmission by effectively reducing the number of individuals with high bacterial load[4].

Tests for antibodies against the NDO-LID conjugate are also essentially tests for antibodies against both PGL-I and LID-1. Thus, such tests can increase the sensitivity and specificity of diagnosis over levels achieved by serological tests for anti-PGL-I only. Indeed, tests based on NDO-LID assist in rapid and consistent detection and monitoring of multibacillary leprosy[5] [28] [29] (**Table 1**).

Antibodies in the sera may be measured by techniques such as enzyme linked immunosorbent assay (ELISA)[4] [5] [16] [17] [18] [28] [29], and lateral flow tests such as ML Flow[9] [10] [11] [12] [13] [16] [18] [19] [20] [24] and NDO-LID® rapid test[28]. Indeed, laboratory-based ELISA has been used to analyze seroreactivity against PGL-I[4] [5] [17], LID-1[4] [5] [17], ML0405, ML2331[4] [17], and NDO-LID[5] [29]. On the other hand, ML Flow is a simple, fast[9], and reliable tool to detect anti-PGL-I[10] using NTP as antigen, a semi-synthetic trisaccharide analog of PGL-I that is chemically linked to either bovine or human serum albumin[11]. Finally, the NDO-LID® rapid test is used to detect antibodies against both the LID-1 chimeric fusion protein and NDO, a disaccharide analog of PGL-I[28]. Importantly, ML Flow and NDO-LID®

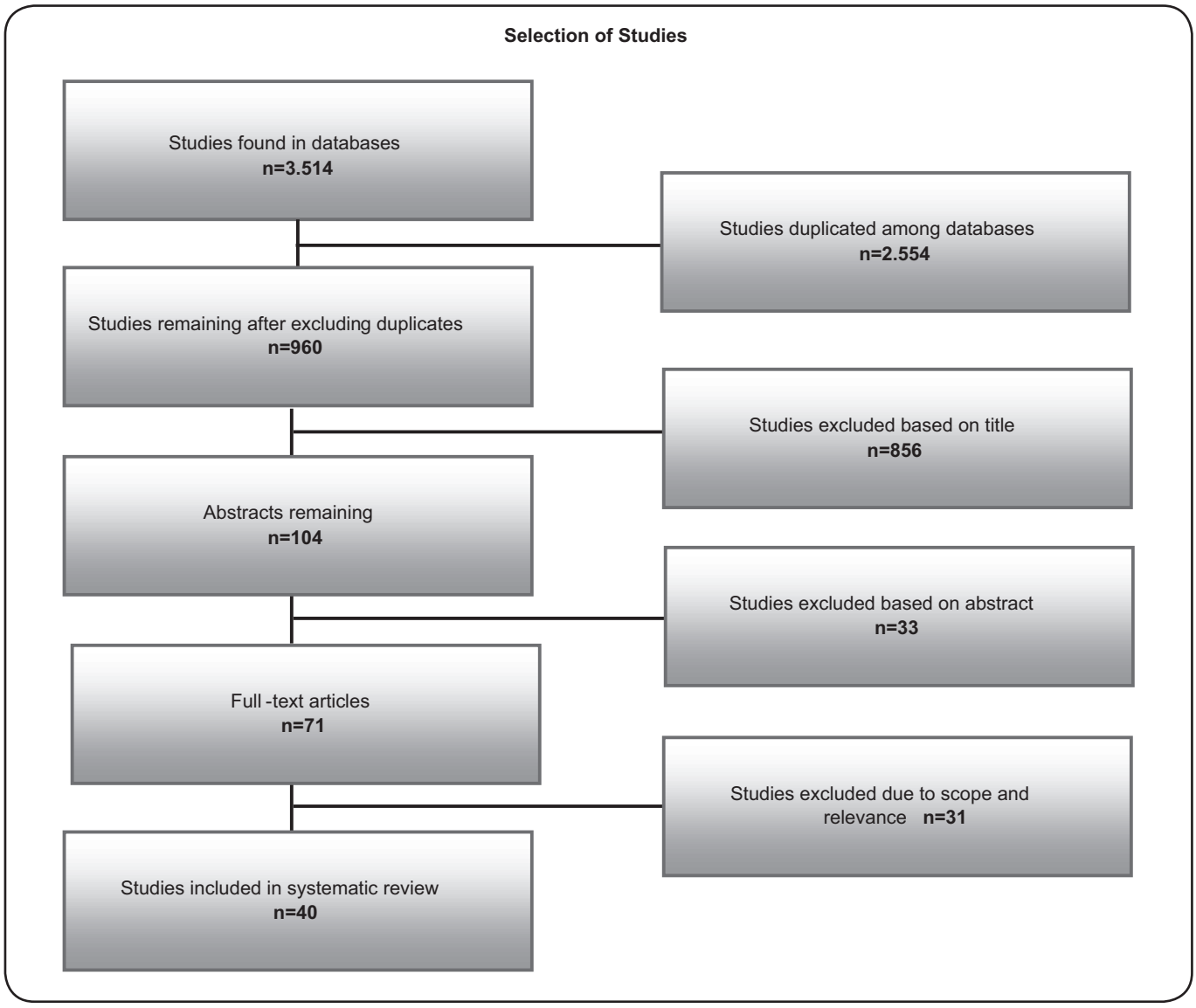

FIGURE 1 - Selection of studies for integrative review.

rapid test are simple serological tests that can readily be used by healthcare professionals providing primary care, and, unlike ELISA, are not dependent upon laboratory analysis.

SURVEILLANCE OF HOUSEHOLD CONTACTS AND AT-RISK POPULATIONS

Measurement of immunoglobulin M (IgM) antibodies against PGL-I may be useful to assess exposure to *M. leprae*. As anti-PGL-I is a marker for those at a higher risk of developing leprosy, screening could be used for early diagnosis in household contacts[12,21,30,31], and for detection of individuals with subclinical, asymptomatic *M. leprae* infection[18,21,32] (**Table 2**). Indeed, household contacts who test positive for anti-PGL-I are at increased risk of developing either form of leprosy[33,34], especially in households with an index case of multibacillary leprosy[21]. In particular, the estimated risk of developing multibacillary leprosy is 34.4 times higher when antibodies against PGL-I are detected[33]. Similarly, evaluation of IgM and immunoglobulin G (IgG) antibodies against the NDO-LID conjugate allows detection of a significant number of *M. leprae*-infected individuals in early stages of disease development[29]. Further, accurate measurement of antibody titers may also be useful, because increased titers of anti-PGL-I (NDO-BSA) and anti-LID-1 could potentially identify household contacts that require careful monitoring or clinical examination[35] (**Table 2**). On balance, the data suggest that simultaneous assessment of multiple antibodies to detect possible *M. leprae* infection may identify contacts at greatest risk of becoming ill[36].

People at risk of developing leprosy should be carefully monitored[34,37] (**Table 2**) or, to prevent new cases, subjected to intervention strategies such as post-exposure prophylaxis[21,37].

TABLE 1 - Use of serological tests as an auxiliary tool to diagnose leprosy.

Antigen	Major findings/comments	References
PGL-I native and/or mimetic	Helps detect early-stage leprosy	39
	Helps classify patients correctly	9, 10, 12, 13, 14, 15, 24
	Assists in differentiating between MB and PB forms	5, 15, 16, 17, 18, 19, 20, 21, 22, 24, 44
	Assists in differential diagnosis	23
	Assists in identifying patients with high bacterial load	11
	Helps diagnose MB leprosy	25, 43
ML0405	Improves performance of anti-PGL-I tests	25
ML2331	Assists in diagnosis and classification	5, 17, 26
LID-1	Assists in diagnosis	27
	Assists in diagnosis, specifically of MB leprosy	17, 25
	Assists in detecting early-stage leprosy	4
	Improves performance of anti-PGL-I tests	25
NDO-LID	Assists in rapid and consistent detection of MB leprosy	5, 28
	Assists in patient monitoring	29
	Increases sensitivity and specificity of anti-PGL-I tests	29

PGL-I: phenolic glycolipid-I**; ML:** *Mycobacterium leprae***; LID-1:** leprosy IDRI diagnostic-1**; NDO-LID:** natural disaccharide octyl-leprosy IDRI diagnostic-1**; MB:** multibacillary; **PB:** paucibacillary.

TABLE 2 - Use of serological tests in surveillance programs.

Antigen	Major findings/comments	References
PGL-I native and/or mimetic	Used to evaluate exposure to antigen	30
	Assists in identifying individuals with subclinical infection	18, 21, 32
	Assists in early diagnosis among household contacts	30, 43
	Can identify contacts at high risk of developing leprosy	11, 14, 21, 31, 33, 34, 36
	Can identify broad groups most at risk of becoming ill	37
	Can assist in monitoring household contacts	35
	Can indicate need for clinical examination	35
	Assists in identifying school-age children with higher risk of developing leprosy	38
	Assists in estimating potential of *M. leprae* transmission	37
ML0405 **ML2331**	Enhances PGL-I tests in identifying contacts at greater risk of developing leprosy	40
LID-1	Can be used to detect *M. leprae* infection	41
	Assists in identifying household contacts that require careful surveillance	35
	Can indicate need for clinical examination	35
NDO-LID	Allows detection of early-stage infection	29
	Can be used to monitor suspected cases of *M. leprae* infection	29

PGL-I: phenolic glycolipid-I**; ML:** *Mycobacterium leprae***; LID-1:** leprosy IDRI diagnostic-1**; NDO-LID:** natural disaccharide octyl-leprosy IDRI diagnostic-1**; *M.*:** *Mycobacterim.*

Indeed, household contacts with subclinical *M. leprae* infection could actively transmit *M. leprae* to susceptible individuals[21], and are thus a concern. Detection of antibodies against PGL-I can identify school-age children with increased risk of developing leprosy[38] (**Table 2**). Indeed, seroepidemiology studies of anti-PGL-I and the prevalence of previously undetected leprosy among household contacts and school children suggest that active *M. leprae* infection and sustained circulation persist in many regions. Notably, clinical and serological surveys among students in hyperendemic areas help identify patients at an earlier stage of disease than otherwise would be achieved. Fortunately, diagnoses are typically provided before physical disabilities develop, while treatment and education help prevent further infections in the community[39].

In addition, detection of antibodies against recombinantly expressed *M. leprae* proteins enhances PGL-I-based identification of household contacts at greater risk of developing leprosy[40] (**Table 2**). Indeed, the LID-1 antigen may be used as a screening tool[41], while evaluation of antibodies against NDO-LID can improve surveillance, facilitate referral to an expert[29], and help develop new interventions and treatments.

We note that in hyperendemic areas, leprosy control activities should extend beyond household contacts and into the general population, which might also be considered at high risk[31]. The possibility and feasibility of carrying out large-scale screening campaigns to detect antibodies against PGL-I, LID-1, and NDO-LID should therefore be investigated as a means to identify *M. leprae*-infected individuals[5]. Regular and sustained monitoring of suspected cases is also suggested[29].

Since successive evaluation of antibodies may contribute to early diagnosis, rapid tests based on PGL-I, LID-1, and NDO-LID antigens should be incorporated into primary health care services in order to detect household contacts and other individuals at risk of becoming sick. It also appears prudent to extend these assays into clinical surveys in schools and the general population in leprosy endemic areas.

SEROLOGICAL TOOLS IN EVALUATING MULTIDRUG THERAPY

As noted, serological tests can help classify leprosy patients, and thus help determine the most appropriate multidrug therapy regimen[3] [10] [18] [42] (**Table 3**). As serological evaluations correlate well with bacterial index[3] [42] [43], these tests can inform treatment-related decisions when bacilloscopy is not available[13] [44]. Indeed, serological assays could reduce the risk of undertreatment and supplant bacilloscopy, a more invasive and risky procedures[13]. When used as basis for selecting the multidrug therapy regimen, serological anti-PGL-I tests reduce nerve damage and associated physical disabilities[18]. Corollarily, as patients with impaired nerve function typically have high levels of anti-PGL-I IgM and IgG, serological evaluation may also assist in identifying individuals with nerve damage (**Table 3**). The association between high antibody titers and nerve damage highlights the need to detect and treat serologically positive individuals as soon as possible[45].

Serum antibodies can also be a useful measure of the effectiveness of treatments[4] [26] [27] (**Table 3**). Indeed, antibody titers decline after multidrug therapy[4] [18] [27] [29], with IgG against LID-1 diminishing faster than IgM against PGL-I or NDO-BSA[26]. Hence, serological assays could be used to indirectly assess bacterial load after completion of treatment, and may detect persistent bacterial multiplication, which could result in reactions or relapse[9]. Re-emergence or increase of IgM and IgG titers can also indicate reinfection, and persistent seropositivity may indicate high risk of symptoms appearing even years later[26] (**Table 3**). Further, measurement of anti-PGL-I in patients with reactions after treatment helps to identify individuals that should undergo further treatment, prevention, or monitoring. Leprosy control personnel should thus evaluate the possibility of using serological assays in patients who are at risk for developing reactions after multidrug therapy, because these tools can support new strategies for prevention and disease control[9].

SERUM ANTIBODIES AS MEASURES OF *MYCOBACTERIUM LEPRAE* TRANSMISSION POTENTIAL

Detection of serum anti-PGL-I enhances diagnostic accuracy, guides treatment, and thereby reduces transmission of *M. leprae* from patients[10] [36]. Anti-PGL-I screening to identify, monitor, and treat contacts or other people most at risk of developing leprosy may also reduce *M. leprae* transmission[12]. Indeed, individuals living in proximity to patients with anti-PGL-I

TABLE 3 - Use of serological tests in therapy and neuritis.

Antigens	Major finding/comments	References
PGL-I native and/or mimetic	Assists in selecting the most appropriate multidrug therapy	3, 10, 18, 24, 42
	Can be considered as marker of re-infection and indicate long-term high risk of leprosy	26
	Allows evaluation of multidrug therapy	4, 26
	Enables assessment of bacterial load after treatment	9
	Helps detect nerve damage	45
ML0405	Allows evaluation of multidrug therapy	
ML2331	Indicates long-term high risk of leprosy	4, 26, 27
LID-1	Can be considered as markers of re-infection	26

PGL-I: phenolic glycolipid-I**; ML:** *Mycobacterium leprae***; LID-1:** leprosy IDRI diagnostic-1**.**

have increased likelihood of themselves producing antibodies to *M. leprae*. Thus, assessing serological status with simple tests such as ML flow can provide a more reliable assessment of transmission potential, as well as broaden the definition of contact[37] (**Table 2**). Detection of antibodies against LID-1, or against both PGL-I and LID-1, has similar benefits[5] [28]. In the end, simple serological tools could enable monitoring at a greater frequency than can be achieved with clinical exams, and this is an important consideration for the success of any leprosy control program.

In summary, the published literature indicates that serological tests for antibodies against PGL-I, ML0405, and ML2331 (in the form of conjugated antigens LID-1 or NDO-LID) can assist in the diagnosis and classification of leprosy. Furthermore, assessment of these antibodies may also help determine the choice of multidrug therapy, as well as monitor its effectiveness. Moreover, anti-PGL-I testing may assist in identifying individuals with nerve damage, as well as those more likely to be affected by reaction episodes after bacteriological clearance.

Most people infected with *M. leprae* do not develop clinical symptoms. Therefore, a major challenge for leprosy control and elimination is to identify individuals who will become ill, and to take steps to prevent illness[38]. Fortunately, detection of antibodies against LID-1 and NDO-LID can facilitate early diagnosis of multibacillary leprosy. Indeed, serological tests for antibodies against PGL-I, ML0405, ML2331, LID-1, and NDO-LID can contribute to surveillance of both household contacts and the general population.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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