Comparison of three immunological tests for leprosy diagnosis and detection of subclinical infection

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Summary
Objective: Our aim was to compare the performance of three serological assays in leprosy patients and their household contacts utilising two quantitative ELISA tests using native PGL-I (PGL-1 ELISA), synthetic ND-O-HSA (ND-O-HSA ELISA), and the semi-quantitative lateral flow test (ML Flow).

Methods: Comparisons among three immunological assays, PGL-1 ELISA, ND-O-HSA ELISA, and ML Flow were performed in 154 leprosy patients, 191 household contacts and 52 health subjects.

Results: The sensitivity results of the PGL-1, ND-O-HSA, and ML Flow were 68·83%, 63·84%, and 60·65%, respectively, with specificity of 98% for both ELISA assays. The native and synthetic PGL-1 ELISA assays detected antibodies in 22·73% and 31·82% of the paucibacillary (PB) patients, respectively and the ML Flow test did not detect antibodies in this group. The ML Flow test was able to discriminate patients into PB or multibacillary (MB) forms, while the native PGL-I and ND-O-HSA was correlated with the bacillary load and the Ridley-Jopling clinical forms.

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In household contacts, the native PGL-I, ND-O-HSA, and ML Flow assays detected seropositivity of 25%, 17%, and 10%, respectively.

**Conclusions:** The use of ELISA and ML Flow tests are thus recommended as additional tools in the diagnosis and classification of the clinical forms, aiding in prescribing the correct treatment regimen to prevent subsequent nerve damage and disability, and besides, the PGL-I ELISA may be used to detect subclinical infection in leprosy.

**Introduction**

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an obligate intracellular parasite that affects peripheral nerves, skin, and other organs. Despite the success of multidrug therapy (MDT) introduced in the mid-1980’s, the world prevalence of 211,903 cases registered at the beginning of 2010 still makes the disease a serious public health problem. If not properly diagnosed or treated, leprosy can result in permanent physical impairment. It is a disease associated with deformity and disability, generating social stigmas and discrimination against patients and their respective families.

Leprosy is a disease with a wide spectrum of clinical forms for which there is still no gold standard diagnostic test, and its detection is usually based on clinical signs and symptoms. Various clinical manifestations can present in leprosy, considering immunopathological parameters and bacterial load. According to the spectrum, leprosy patients are classified as tuberculoid (TT), borderline tuberculoid (BT), borderline-borderline (BB), borderline-lepromatous (BL), and lepromatous (LL). Currently, patients are classified based on the number of skin lesions, with the paucibacillary (PB) group presenting five lesions or less, and the multibacillary (MB) group with six or more lesions. However, the reliability of this classification criterion has been questioned, since patients with less than five lesions may be classified as MB based on an assessment of the Bacterial Index (BI) results.

In endemic areas of leprosy, sensitive and specific diagnostic laboratory tests would be of great use in the detection of leprosy patients in early stages of the disease. One of the first *M. leprae*-specific antigens to be isolated and characterised was the phenolic glycolipid (PGL-1). The PGL-I is an antigen found within the cell wall of the bacillus, which is able to induce the production of antibodies in the host. The presence of circulating IgM antibodies has been correlated with the clinical spectrum and bacterial index in leprosy patients. A significant inverse correlation has been shown between the antibody titer and the Mitsuda test, which evaluates the specific cellular immune response against Hansen’s bacilli.

The main antigenic determinant of PGL-I recognised is the trisaccharide portion of the molecule. Assays that detect PGL-I antibodies can utilise the native glycolipid, synthetic PGL-I di- or trisaccharides coupled to bovine or human serum albumin and a variety of neoglycoconjugates with up to 40 to 50 residues per molecule, with a sensitivity higher than that of the native PGL-I antigen. These soluble forms of PGL-1 can also be more readily incorporated into lateral flow devices for the rapid detection of antibodies in multibacillary patient sera.

Although PGL-I antibody levels in tuberculoid patients are low or negative, the use of the quantitative PGL-I assay to assess the titer plays an important role in detecting
subclinical infection, determining the proper duration and regimen of MDT, and in the prevention of relapses. 

Household contacts of leprosy patients that are positive for PGL-I display about a 7.2-fold higher risk of developing leprosy relative to PGL-I antibody-negative household contacts, and those who progress to disease predominantly develop the more severe MB form. Consequently, the identification of household contacts of leprosy patients who are positive for the PGL-I antibody could lead to an earlier detection of infection, more prompt diagnosis and treatment, and limit further lines of transmission.

This study was undertaken in order to develop a standardised protocol for a more sensitive and specific serological ELISA assay to detect the PGL-I antibody in patients and household contacts screened at Reference Centres. To this end, we have compared the performance of three serological assays in leprosy patients and their household contacts using two quantitative ELISA tests using native PGL-I, synthetic ND-O-HSA, and the semi-quantitative lateral flow test (ML Flow).

Material and Methods

SUBJECT OF STUDY AND SAMPLES

The study population was comprised of 191 serum samples of household contacts and 154 serum samples of newly diagnosed leprosy patients seen at the National Reference Center of Leprosy and Sanitary Dermatology of the Teaching Hospital – Federal University of Uberlândia (CREDESH/HC/UFU), Minas Gerais, Brazil. Household contacts were those individuals who reside or resided with the patient in the last 5 years. The patients were diagnosed according to dermato-neurological clinical exams and laboratory tests, as per Ridley-Jopling’s spectrum, into the five clinical forms: tuberculoid (TT), borderline-tuberculoid (BT), borderline-borderline (BB), borderline-lepromatous (BL), and lepromatous leprosy (LL). The number of skin lesions and the bacterial index of the skin smear were used to determine the operational classification (OC), considering as paucibacillary (PB) patients who had up to five skin lesions and a negative bacilloscopy; and multibacillary (MB), those with more than five lesions and/or positive bacilloscopy (WHO, 1988). Based on these criteria, 100% (14/14) of the TT patients and 51.67% (31/60) of the BT patients were considered PB. The rest of the BT (48.33% – 29/60) patients and 100% of the BB (28), BL (21), and LL (31) patients were classified as MB. As negative control of the ELISA tests we used 52 sera samples from healthy individuals who have no contact with leprosy patients. Of the 154 patients diagnosed with leprosy, 84 were followed during treatment, and the PGL-I ELISA was performed at the beginning and end of the MDT. The other two serology tests (ML Flow and ND-O-HSA ELISA) were not performed at the end of treatment due to the scarcity of these tests.

ETHICAL ASPECTS

The present study was approved by the Research Ethics Committee of the Federal University of Uberlândia (UFU) under protocol # 099/2003. Patients and household contacts who voluntarily agreed to participate in the study were enrolled after giving their Informed Consent.
Two antigens were used for ELISA antibody detection, the native PGL-I isolated by organic extraction of *M. leprae*-infected armadillo tissues from which the bacteria had been purified and utilised in PGL-I ELISA; the ND-O-HSA ELISA used the synthetic natural disaccharide octyl linked to human serum albumin (ND-O-HSA), obtained from Colorado State University through the NIH/NIAID Leprosy Contract N01 AI 25469. For the lateral flow assay, antigen NT-P-BSA was used, which is a natural trisaccharide antigen linked to bovine serum albumin. This is the antigen used in the ML Flow kit (KIT Biomedical Research, Amsterdam, The Netherlands).

**ANTIBODY TITER**

Briefly, for the PGL-I antibody detection ELISA assays, microtiter plates (Maxisorp®, NUNC) were coated with native PGL-I diluted in absolute ethyl alcohol, or synthetic ND-O-HSA in Phosphate Buffered Saline (PBS), at concentrations of 10 μg/ml and 0.2 μg/ml, respectively. Serum samples were added in duplicate using a dilution of 1:100 (native PGL-I) and 1:300 (ND-O-HSA) in PBS/BSA 1%, incubated for 1 hour at 37°C, followed by washing. The anti-human IgM-peroxidase conjugate (Sigma Chemical Co., St. Louis, MO) was added to the plates in the dilution of 1:10,000 (PGL-I ELISA) and 1:2,000 (ND-O-HSA). The substrate o-phenylenediamine dihydrochloride (OPD, Sigma) enzyme substrate was added to the plates and incubated at room temperature for 10 minutes in the dark. The reaction was stopped by the addition of H₂SO₄ 4N. The optical density (OD) was obtained in a microplate reader (THERMO PLATE, TP-READER, Rayto Life and Analytical Sciences C. Ltd, Germany) at 492 nm. Two positive and three negative controls were included in each plate. The antibody titers were expressed as the ELISA index (EI) according to the following formula: \( EI = \frac{OD_{\text{sample}}}{OD_{\text{cut-off}}} \), in which the cut-off point was determined by the average OD of the negative controls plus three standard deviations (ND-O-HSA ELISA) or four standard deviations (native PGL-I ELISA), as described previously.\(^{21}\) EI values above 1.1 were considered positive. We used two positive controls on each plate, one low positive, of low EI (E.I = 2.0), and a strong positive of high EI (E.I = 7.0). The ELISA specificity was calculated based on tests of 52 serum samples from healthy individuals. For the negative control, sera from three individuals mentioned above were selected and included in all test plates.

For the ML Flow (a lateral immunochromatographic flow test for anti-PGL-1 detection to investigate the *Mycobacterium leprae* immune response), 5 μl of whole blood was collected from the tip of the left index finger. The blood was placed in the test cartridge together with the buffer solution, and after 5 minutes the result was read by three independent readers. The absence of a line in the test zone indicated a negative result, while a positive result was graded in intensity from 1+ to 4+.\(^{15}\)

**BACTERIAL INDEX**

The bacterial load was calculated from the average of skin smears obtained from seven sites: the right and left earlobes, elbows, knees, and from one active lesion. The bacterial index (BI) was calculated according to Ridley’s Logarithmic Scale,\(^{22}\) based on the number of bacilli detected by field microscopy, using 100 X oil immersion lens, where: \( BI = 0 \) (no bacilli in
100 immersion fields); $\text{BI} = 1$ (average of 1 to 10 bacilli in 100 fields); $\text{BI} = 2$ (average of 1 to 10 bacilli in 10 fields); $\text{BI} = 3$ (1 to 10 bacilli in 1 field); $\text{BI} = 4$ (10 to 100 bacilli in 1 field); $\text{BI} = 5$ (100 to 1,000 bacilli in 1 field); and $\text{BI} = 6$ (>1,000 bacilli in 1 field).

**MITSUDA TEST**

The Mitsuda antigen is a heat-killed suspension of $6 \times 10^7$ bacilli/ml produced and donated by Dr. Maria Esther Nogueira Sales from the Lauro de Souza Lima Institute, Bauru, SP, Brazil (ILSL-Bauru, SP). An intradermal injection of 0.1 ml was applied at the upper third of the anterior surface of the right forearm of patients and household contacts, and the presence or absence of a granulomatous response on the 28th day following injection was determined. The diameter of the granulomatous lesion was measured in millimeters for quantitative and qualitative analyses. Patients and contacts were categorised into classes according to the World Health Organization (WHO) using the following criteria: negative – no reaction; doubtful – 0 to 3 mm; weakly positive – 4 to 7 mm; definitely positive – 8 to 10, and strongly positive – readings $>10$ mm or a granulomatous lesion of any diameter with ulceration.

**STATISTICAL METHODS**

Pearson’s coefficient was used to verify the degree of correlation among the tests and was applied to determine the degree of significance of the differences among the average of the serological and BI results. Student’s t test was used to analyse the differences between the average of the serological tests at the beginning and end of the MDT. The concordance between the laboratory tests was calculated by dividing the number of congruent cases by the total number of patients. The Kappa test was applied to evaluate the concordance results. Kappa values and their interpretations varied as follows: $<0$, no agreement; 0–0.19, poor agreement; 0.20–0.39, fair agreement; 0.40–0.59, moderate agreement; 0.60–0.79, substantial agreement; 0.80–1.00, almost perfect agreement. Differences between the groups were assessed by chi-square, when appropriate. Values of $P<0.05$ were considered statistically significant.

**Results**

Patients were classified into five clinical forms, represented in the following frequencies: 9.09% (14/154) were TT, 38.96% (60/154) were BT, 18.18% (28/154) were BB, 13.64% (21/154) were BL, and 20.13% (31/154) were LL.

**ANALYSIS OF MITSUDA TEST AND THE BACTERIAL INDEX**

A classification of the clinical forms using Ridley & Jopling criteria is shown in Table 1. A negative correlation was noted between the Mitsuda test results and the BI of the skin smear covering the spectrum of clinical forms. In patients with the TT form, the average Mitsuda intradermal test result was 11.15 mm and the BI was equal to zero (BI = 0), while at the other pole of the disease, patients with the LL form, presented a negative Mitsuda and a average value for skin smear BI of 5.16. Traversing the spectrum of the disease, from the TT pole to the LL pole, in patients in the borderline group, from
the BT to the BL form, a gradual decrease in the cellular immune response was observed, along with an increase in BI (Table 1).

ANALYSIS OF SEROLOGICAL TESTS IN PATIENTS WITH NO PRIOR TREATMENT

The sensitivity, specificity, and accuracy of the two ELISA tests were calculated, in which native PGL-1 ELISA and ND-O-HSA displayed, respectively, 68.83% and 63.84% for sensitivity; 98 and 98% for specificity; and 76 and 71% in accuracy.

The detection of IgM antibodies against PGL-1 in patient serum in the three tests analysed were 68.83% (106/154) for native PGL-1 ELISA, 63.64% (98/154) for ND-O-HSA ELISA, and 60.65% (74/122) for the ML Flow test. According to the operational classification (PB and MB), among the 44 PB patients, PGL-1 ELISA was reactive in 22.73% (10/44), ND-O-HSA ELISA in 31.82% (14/44), and the ML Flow test showed no reactivity in any of the PB patient sera samples (0/44). In the group of MB patients, the reactivity of the three tests was 86.36% (95/110) for PGL-1 ELISA, 76.36% (84/110) for ND-O-HSA ELISA, and 90% (99/110) for ML Flow.

In the classification of clinical forms, it was demonstrated that the seroreactivity for the three serum tests showed a gradual increase in positivity according to the spectrum of the disease in the direction of the LL pole (Table 1).

As to the dynamic range of the results of the three serologic tests, the two quantitative ELISA tests showed greater range that the semi-quantitative ML Flow. The ELISA test showed to the dynamic range of the ELISA Index (EI) wider, varying from 0·1 to 3·0 for the native PGL-1 and from 0·3 to 27·8 for the ND-O-HSA, differentiating the five clinical forms, with a gradual increase of EI accompanying the increase in BI towards the LL pole. The results of the ML Flow test varied from zero to four (0 to 4+) and did not differentiate between patients with clinical forms BT and BB, and between BL and LL (Table 1).

The PGL-1 ELISA displayed a significant positive correlation between positivity and clinical forms \( (r = 0.92; P < 0.05) \), and between EI average and clinical forms \( (r = 0.97 \).
for ND-O-HSA ELISA, the correlation was also significant \((r = 0.95; P < 0.05)\) and \((r = 0.93; P < 0.05)\), respectively. The ML Flow test positivity and average also showed a correlation per clinical form: \((r = 0.91; P < 0.05)\) and \((r = 0.96; P < 0.05)\), respectively.

As to the quantitative results of the serological tests and the BI of the skin smear of the patients, the quantification of IgM antibodies in the three tests, using the EI for the ELISA tests and the positivity intensity for the ML Flow test, a positive and significant correlation was demonstrated for PGL-1 ELISA \((r = 0.98; P = 0.001)\), signifying that the greater the number of bacilli, the greater the quantity of IgM anti-PGL-1 antibodies. There was a significant correlation for ND-O-HSA ELISA \((r = 0.88; P < 0.05)\), and for ML Flow \((r = 0.94; P < 0.05)\) (Figure 1) as well.

The concordance among the three serological tests was calculated using Kappa’s coefficient (Table 2).

Between PGL-1 ELISA and the ML Flow, the concordance was substantial \((k = 0.75; P < 0.0001)\). Between the PGL-1 ELISA and the ND-O-HSA ELISA, and between the ND-O-HSA ELISA and the ML Flow test, concordances were moderate and significant, with \(k = 0.48\) and \(k = 0.53\), respectively \((P < 0.001)\) (Table 2).

**Analysis of the PGL-1 ELISA Serum Test in Patients After Multidrug Therapy**

With the exception of the TT clinical form, which displayed reduced PGL-1 levels before treatment, all clinical forms showed a significant decline in PGL-1 upon MDT treatment (Figure 2).

**Analysis of the Cellular and Humoral Immune Response in Household Contacts**

Each of the household contacts was categorised according to their relationship with the index case. Accordingly, it was observed that 26% (49/191) were contacts of PB and 74% (142/191) were contacts of MB patients.

![Figure 1](image-url)

**Figure 1.** Average values for the native PGL-1 ELISA, ND-O-HSA ELISA and ML Flow assays, according to the bacterial index of leprosy patients.
The cellular immune response was evaluated by the Mitsuda test, which showed an average of 7.4 mm among household contacts.

Positivity of the three tests was not different in household contacts of MB patients when compared to contacts of PB patients, following the same positivity pattern found in patients, i.e., greater reactivity for PGL-1 ELISA with 25% (49/191) of the contacts followed by 17% (32/191) for the ND-O-HSA ELISA, and 10% (19/191) for the ML Flow, although with no statistical differences between the tests (Table 3).

**Discussion**

This present study proposed a comparison among three serological tests for the detection of specific anti-PGL-1 antibodies of *M. leprae* in treatment-naive leprosy patients and their household contacts, seeking to assess their use in diagnosis and/or in subclinical leprosy.

![Image](image-url)  
**Figure 2.** Average ELISA Index values for the native PGL-1 assays, according to clinical forms of the leprosy before and after MDT treatment.

### Table 2. Analysis of the Kappa agreement index among three serological assays in leprosy patients

<table>
<thead>
<tr>
<th></th>
<th>Positive (N)</th>
<th>Negative (N)</th>
<th>Total</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGL-1 ELISA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (N)</td>
<td>69</td>
<td>10</td>
<td>79</td>
<td>0.75*</td>
</tr>
<tr>
<td>Negative (N)</td>
<td>4</td>
<td>38</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>48</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td><strong>ND-O-HSA ELISA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (N)</td>
<td>62</td>
<td>17</td>
<td>79</td>
<td>0.48*</td>
</tr>
<tr>
<td>Negative (N)</td>
<td>12</td>
<td>30</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>47</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td><strong>ML FLOW</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (N)</td>
<td>60</td>
<td>14</td>
<td>74</td>
<td>0.53*</td>
</tr>
<tr>
<td>Negative (N)</td>
<td>13</td>
<td>34</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>48</td>
<td>121</td>
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</tr>
</tbody>
</table>

*P < 0.0001.
infections, as well as to monitor patient treatment, since there is considerable difficulty in leprosy in identifying the three reference points that are involved in the transmission of the disease, i.e., the onset points of exposure, infection, and disease. In order to identify these points involved in transmission, several markers for epidemiology and control of leprosy have been evaluated, including the use of molecular and immunological methods in the detection of *M. leprae*, in an effort to differentiate individuals with subclinical infections, as well as the early onset of the disease. In order to eliminate the bias of an incorrect clinical classification, analysis of the BI of the skin smear and Mitsu test demonstrated that the clinical forms of the patients are consistent with the classification of Ridley & Jopling, i.e., the cellular immune response and the bacillary load were inversely proportional. This differentiation of patients in the spectrum and the correct classification are essential for a work of this nature since it guarantees reliability of the serology results presented.

This is the first study comparing seropositivity of patients with Hansen’s disease with the PGL-1 ELISA test, the ND-O-HSA ELISA test, and the ML Flow test, according to the clinical forms of Ridley and Jopling. The native PGL-1 ELISA test showed greater seropositivity when compared to the other tests, with a growing EI accompanying the spectrum of the disease and it also showed amplitude of EI that was able to better differentiate between the five clinical forms of leprosy, although the difference between the tests showed no statistical differences.

The two ELISA tests showed a positive correlation with the bacterial load, and the average of the EIIs, as well as their amplitudes, increased in proportion to the BI, especially for the PGL-1 ELISA test. The lowest sensitivity observed with ND-O-HSA ELISA may be due to the use of the synthetic disaccharide antigen, since the antigenic determinant of PGL-1 is represented by the trisaccharide terminal, and among the synthetic antigens those that result in best reactivity with patient sera are those with di- (D-BSA) and trisaccharide (T-BSA) residues, which possess the non-reducing sugar 3, 6-dio-methyl B D-glycopyranoside. The two ELISA tests, with similar specificity, showed variation in the scale of amplitude of results, with a gradual increase of EI according to clinical forms of the disease. The native PGL-1 ELISA showed greater sensitivity and accuracy, making this test an important tool for the determination of the Ridley and Jopling forms, as well as for the control of therapeutic, as is demonstrated in the present paper. Nevertheless, since the ML Flow test is semi-quantitative, it has a small scale of variation ranging from 0 (negative) to 4+ (highly positive), which makes it difficult to precisely differentiate the levels of antibodies among the clinical forms and to monitor treatment (unpublished results).

<table>
<thead>
<tr>
<th>OC Index Case</th>
<th>Contacts (N)</th>
<th>Positivity in PGL-1 ELISA N (%)</th>
<th>Positivity in ND-O-HSA ELISA N (%)</th>
<th>Positivity in ML Flow N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td>142</td>
<td>39 (27.46)</td>
<td>25 (17)</td>
<td>14 (10)</td>
</tr>
<tr>
<td>PB</td>
<td>49</td>
<td>10 (20.41)</td>
<td>7 (14)</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
<td>49 (25.65)</td>
<td>32 (17)</td>
<td>19 (10)</td>
</tr>
</tbody>
</table>

MB: Multibacilary; PB: Paucibacilary.
The results of the ML Flow test showed an excellent correlation with the operational classification and the BI, as had already been reported in literature.\textsuperscript{18,33–35} The high positivity observed in MB patients to the ML Flow test may be due to the association of the anti-PGL\textsubscript{1} antibodies in the more bacillary forms of the disease with the synthetic trisaccharide antigen PGL-1 contained in the said test, demonstrating a positive correlation between the levels of antibodies and the bacterial load, such as is reported in literature.\textsuperscript{33–35}

The ML Flow is a limited test for the detection of anti-PGL-1 antibodies in sera of PB patients, as is shown in literature\textsuperscript{15,17,36} Nonetheless, in this study, the semi-quantitative ML Flow test demonstrated an excellent performance for discriminating patients as to the operational classification (PB and MB)\textsuperscript{15,17,18,36} which gives it the status of an important tool to be utilised by healthcare teams not specialised in leprosy in the care of field patients, seeking to classify the patient after diagnosis, considering that it is a simple and quick test to perform.\textsuperscript{17,35} Recently, studies performed with leprosy patients in Brazil, Nepal, and Nigeria with the ML Flow test, noted that the use of the test helped in the operational classification of the disease, reducing the risk of allocating patients to inadequate treatment schemes, which could favour sub-treatment and relapses.\textsuperscript{33,36}

The analysis of concordance among the three tests showed substantial agreement between PGL-I ELISA and the ML Flow, probably because the two antigens used in the tests are trisaccharides.\textsuperscript{32,37,38} As to the two ELISA tests displaying moderate concordance, this may be due to the difference in protocols and antigens. The ND-O-HSA is a terminal disaccharide epitope of the synthetic PGL-1, conjugated with a human serum albumin, containing only one fraction of the glycolipid, with a structure different from the native PGL-1 and from the synthetic trisaccharide antigen.\textsuperscript{12,37} New glycoconjugated antigens are correlated with the native PGL-1 in the detection of antibodies due to the presence of carbohydrate epitopes of PGL-1 that are critical in the antigen-antibody bond.\textsuperscript{39–41}

The decline of the EI in the PGL-1 ELISA test after MDT treatment was observed in patients of the borderline group (BT, BB, and BL) and of the clinical form, LL, showing that the quantitative test had good sensitivity to the decrease in antibodies that correlate with the drop in bacterial load promoted by the MDT. These serum monitoring in patients’ data corroborate other studies\textsuperscript{6,42–44} which have demonstrated a gradual reduction in IgM-anti PGL-1 antibodies in patients under treatment.

This present study demonstrated that there was persistence of IgM anti-PGL-1 antibodies at the end of treatment in multibacillary clinical forms. The persistence of these antibodies at high levels of MDT in MB patients may be due to the inefficiency of macrophages in cleaning out dead \textit{M. leprae} and their antigenic fragments from lesions.\textsuperscript{45,46} This fact may occur since the clearance of bacilli from the body is a slow process, and may represent a continual stimulus in antibody production,\textsuperscript{7} corroborating the determination of the epidemiology of \textit{M. leprae} as a slow growth bacillus.\textsuperscript{47} Moreover, the assumption of persistent bacilli present in patients already treated with MDT cannot be excluded.\textsuperscript{49} However, the origin or trigger of this immunological persistency must be determined.

In order to approach the problems with the point of first exposure and/or with the point of onset of infection, the present study evaluated the serum response of household contacts of patients with leprosy to the three anti-PGL-1 tests, considering that this is the group considered as having the greatest risk of becoming ill when compared to the general population of non-contacts.\textsuperscript{25,49–52} Among the three serology tests analysed in sera of household contacts, the native ELISA anti-PGL-1 displayed the greatest seropositivity. As the ELISA PGL-1 test showed greatest sensitivity and accuracy, as well as a greater correlation
with BI and clinical forms of the Ridley and Jopling spectrum, its indication for monitoring household contacts seems obvious. Seropositive contacts have demonstrated a risk 7-2 times higher of becoming ill when compared to seronegative contacts for ELISA anti-PGL-1. Another study also demonstrated that contacts with positive ML Flow, negative Mitsuda, and no BCG scar have a 24-26-fold higher risk of developing leprosy, as has been demonstrated by prior study of our group.53

There are already enough studies in literature that recommend the use of serological tests in the control of leprosy, and even as an aid in diagnosis and prognosis.53,58–60 The present work corroborates and advances knowledge as to prior study, demonstrating the novel pattern of anti-PGL-1 antibody detection in leprosy patients according to the clinical forms of Ridley-Jopling,3 determining the power of the PGL-1 ELISA test in discriminating titers of antibodies correlated to the bacterial index of patients, favouring the monitoring of treatment, as well as its performance for detection of subclinical infection in contacts.

Since the ELISA anti-PGL-1 test demands laboratorial structure and specialised human resources, it could be implanted in the routine of leprosy reference centres of endemic countries, while the ML Flow could be made available to the basic non-specialised healthcare services, favouring allocation of patients to treatment regimens for PB and MB, since these services do not have bacilloscopy test available for all patients, besides the fact that the reliability of these tests have been questioned in some control programmes.61

Briefly, the serological PGL-1 ELISA test could be offered by reference centres to patients referred by healthcare services, within a network of facilities between Basic Healthcare Units and Reference Centres, in order to accrue experience of their potential benefit for patients when used on a large scale in routines of leprosy control programmes, as well as to facilitate the identification and assessment of individuals with a higher risk of becoming ill for intervention with chemoprophylaxis.

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Immunological tests for leprosy


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