

EDITORIAL

PGL-I leprosy serology

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Mycobacterium leprae is the causative agent of leprosy, a disease that, despite having an effective cure with multidrug therapy (MDT), leaves millions of cured patients with sequelae as a consequence of the nerve damage that forms the hallmark of leprosy.

A number of diagnostic issues hamper the correct and timely diagnosis of leprosy and the correct classification of patients for treatment purposes: (i) bacterial examinations are often not used; (ii) there is a tendency to over-diagnose single lesion PB leprosy; and (iii) early (borderline) lepromatous often goes unnoticed due to the absence of sensibility loss and lesions. Approximately 70% of leprosy patients can be diagnosed on the basis of the single clinical sign of skin patches with sensory loss, but 30% of patients, including many MB patients, do not present this symptom¹⁶. The delayed detection of this latter group of patients may be a major cause of continued transmission.

Most mycobacteria have specific, highly antigenic glycolipids that can be used as tools for the serodiagnosis of distinct mycobacterial infections. As the mycobacterium enters the human body, the cell wall is the first barrier encountered by the immune system. The cell wall of *Mycobacterium leprae* consists mainly of lipids, including a considerable glycolipid fraction with a unique carbohydrate composition.

One of the first *Mycobacterium leprae*-specific antigens to be isolated and characterized was phenolic glycolipid-I (PGL-I)¹, the major antigenic glycolipid in the bacterium. The PGL-I molecule is composed of a unique trisaccharide, 3,6-di-*O*-methyl- β -D-glucopyranosyl-(1 \rightarrow 4)-2,3-di-*O*-methyl- α -L-rhamnopyranosyl-(1 \rightarrow 2)-3-*O*-methyl- α -L-rhamnopyranose¹². The principal antigenic determinant of PGL-I is the ultimate di- and trisaccharide part of the molecule^{2, 9}. Monoclonal antibodies were used to further analyze this antigenic determinant of PGL-I. It was shown that removal of the terminal sugar residue resulted in loss of binding of most antibodies, while removal of the long chain fatty acids of the molecule had no effect on antibody binding¹⁷. These and other results suggested that chemical synthesis of the ultimate disaccharide part would provide an antigenic epitope that would be specific enough for application in the serology of leprosy. The sugar part of PGL-I was synthesized and conjugated to bovine serum albumin (BSA) either directly or through different linkers, namely an octyl (O) or phenyl (P) linker arm. More recently it was conjugated to human serum albumin.

Since the identification of PGL-I, the following neoglycolipids have been produced: monosaccharide-octyl-BSA (M-O-BSA), disaccharide-BSA (D-BSA)¹⁰, natural disaccharide-octyl-BSA (ND-O-BSA)⁷, natural trisaccharide-phenyl-BSA (NT-P-BSA)⁸ and natural disaccharide-octyl-HSA (ND-O-HSA)⁷.

Based on native PGL-I and its semi-synthetic derivatives, numerous serological assays have been developed to detect the presence of antibodies of the immunoglobulin IgM, IgG and IgA classes. Since the semi-synthetic antigens can be produced in larger quantities and can be applied more easily than native PGL-I, a wide variety of applications ranging from clinical diagnosis to large epidemiological studies became feasible.

The techniques used to develop tests include the enzyme-linked immunosorbent assay (ELISA)³, the passive hemagglutination test (PHA)¹⁵, a gelatin particle agglutination test (MLPA)¹³, the dipstick⁵ and the lateral flow test⁶. An expressive part of the studies published so far have used the ELISA, dipstick and lateral flow techniques. However, the question remains as to which technique is the most suitable for a specific study. There are advantages and potential pitfalls that may occur when using each technique.

Advantages and pitfalls of techniques

ELISA

ELISA is a well established in-house technique³. Despite being laborious to standardize, its low cost and quantitative results make it unique. For large epidemiological studies and for patient follow-up, ELISA is preferable.

The advantages of the ELISA lie in (i) the wide availability of the necessary equipment, since the technique is almost universally applicable; (ii) the easy separation of solid and liquid phases, and (iii) the lack of a requirement for labeling the *Mycobacterium leprae*-specific components of the test (either antigen or antibody). Even so, ELISA is a laborious technique and pitfalls exist due to differences in antigen preparations, antigen concentrations, buffers, washing procedures, serum dilution and type of substrate used in the technique.

ELISA for PGL-I derivatives involve the immobilization of the protein part of the molecule (BSA or HSA) to a plastic surface via passive interactions. The ability of the plastic surface to interact with proteins is an essential feature. However, nonspecific binding of other proteins or biomolecules to unoccupied spaces on the surface during the performance of the assay can be detrimental to the specificity and sensitivity of the assay results. Blocking is therefore essential.

The use of non-sugar conjugated BSA or HSA-coated wells is crucial to enable the correction of any (nonspecific) binding that may occur. Similarly, correction for nonspecific binding to uncoated wells (but treated with the same buffers) should occur when using native PGL-I.

The ratio of sugar molecules to the protein (BSA or HSA) will vary in every antigen preparation. Thus, it is important to

report the sugar/protein ratio of the antigen, to allow comparison between studies.

In order to maximize assay precision and sensitivity, complete removal of loose or unbound fractions is required. In addition, daily variation in assay performance will also influence the results and is mainly related to the enzyme-substrate reaction. Several requirements, such as timing and development conditions, need to be optimized to result in a precise, accurate and reproducible assay. Since enzyme-substrate reactions are kinetic, the time elapsed from the start of the reaction to the end of the reaction, as well as the temperature, can and will affect the final concentration of product developed. In order to obtain comparable results, an internal standard serum must be included in the assay and to stop the reaction when the standard serum has achieved a certain optical density (OD) value. Positive and negative controls should also be used in order to validate the assay results.

Dipstick

The dipstick assay (ML Dipstick)⁴ is an immunochromatographic test composed of a PGL-I antigen line bound to a nitrocellulose strip adhered to a plastic support. To obtain an internal control, anti-human IgM antibodies are coated onto the nitrocellulose as a separate band. The detection reagent is a monoclonal anti-human IgM antibody conjugated to palanil red, a colloidal dye¹¹, and lyophilized for preservation. Serum dilutions (1:50) are made directly into the reconstituted detection reagent in reaction vials and the dipsticks are incubated for 3 hours at room temperature.

The advantages of the dipstick technique are: (i) there is no need for refrigeration or equipment; (ii) it has an easy protocol, and (iii) the lack of a requirement for labeling the *Mycobacterium leprae*-specific components of the test (either antigen or antibody). Still, the dipstick technique includes serum dilution, washing procedure and 3 hours incubation.

Since the test is not commercially available, it is unlikely that a researcher would consider using this technique. However, the dipstick might be the technique of choice when planning a large epidemiological study in which the performance of several new antigens would be tested and compared to PGL-I in the same study population (Figure 1). Using this technique would be fairly cost-effective even when considering the in-house development of such a test.

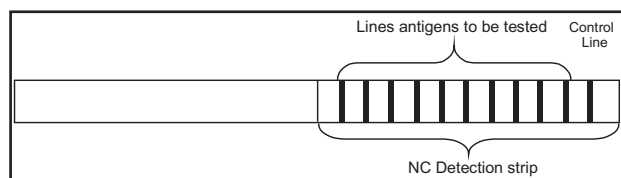


FIGURE 1

Outline of a dipstick test for several antigens.

Lateral Flow

The lateral flow assay (ML Flow)⁶ is an immunochromatographic test composed of a nitrocellulose detection strip that is flanked at one end by a reagent pad made from wool fiber containing the

dried colloidal gold-labeled antihuman IgM antibody, and at the other end by an absorption pad (Figure 2). In the test line, a semi-synthetic antigen linked to bovine serum albumin is used as the antigen. Human IgM is deposited as a second line parallel to the antigen line to function as a reagent control. The assay is performed by the addition of 5 µl undiluted serum or 10 µl whole blood to the sample receptacle followed by the addition of 130 µl running buffer. The test is read after 10 minutes for serum and after 5 minutes for blood.

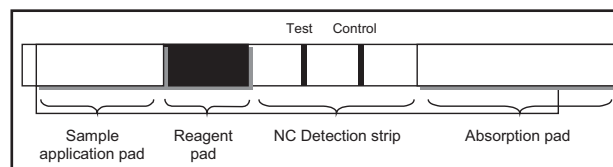


FIGURE 2

Outline of the lateral flow test.

Another immunochromatographic test (ICA) is available (Dr. Ray Cho personal communication), produced by Standard Diagnostics Inc, Suweon, Korea, using the two neoglycoconjugate antigens, ND-O-BSA and ND-O-HSA. One of the neoglycoconjugate antigens is conjugated with gold particles and the other is fixed onto the membrane. The manufacturer suggests a serum dilution of 1:5 and reading the results after no more than 10 minutes.

Serology applicability

Detection of IgM antibodies to PGL-I is not a diagnostic test, but can be used for the diagnosis of leprosy when the results are considered together with other diagnostic and clinical data. Antibody detection is especially useful for the diagnosis of MB leprosy; antibody levels may be low or undetectable in PB patients.

During treatment of MB patients, repeated testing in ELISA can be used as an additional tool to measure the effect of therapy. Whether serology could be used to diagnose or even predict reactions is still not clear¹⁴. Furthermore, increasing antibody levels may indicate a relapse. However, serological results must always be interpreted in combination with other diagnostic information.

Clearly, PGL-I antibodies are indicative of bacterial load in an individual, reflecting subclinical infection or disease. Serological screening and follow-up of contacts of leprosy patients is a useful tool for early detection of new cases. In addition, it may be useful to measure the extent of the leprosy problem in a population.

In this edition, the results of several studies conducted in Brazil are presented. The first, a systematic review conducted by Moura *et al* analyzes and contextualizes the different publications that present evidence on how serology can be used in leprosy work. Subsequently, Castorina-Silva *et al* confirm the specificity of the PGL-I antigen. The correlation between serology and bacilloscopy is further studied by Lyon *et al*.

Several studies discuss the use of serology as an auxiliary tool for the diagnosis and classification of leprosy patients and how this might benefit health units in leprosy control. Through

the presentation of a set of multibacillary cases that were particularly difficult to diagnose, Oliveira *et al* outline how PGL-I serology results may be used as a complementary tool for leprosy diagnosis, clarifying that serology should not be used as a diagnostic test *per se*. Grossi *et al* present evidence of the influence of the use of the ML Flow test in the reduction of the number of MB cases in the State of Minas Gerais. Another article sets out to prove that the number of nerves affected by the disease is not important in the classification of patients as MB or PB. Barreto *et al* discuss dimorphous cases that have as many as five skin lesions, thereby receiving a paucibacillary classification, but that have biopsy results with acid-fast bacilli and positive serology.

Teixeira *et al* provide a complete agreement study between clinical/laboratory examinations for leprosy diagnosis, suggesting that serology should be used in the classification of patients for treatment in order to minimize the risk of disabilities.

The application of serological tests in leprosy reactions is explored by Brito *et al*. The results of this paired study show the importance of post-MDT follow-up of patients that presented reactions during treatment and that maintained highly-positive serology results at the time of release from treatment.

Household contact studies show the need for continued research in this area. Andrade *et al* present seropositivity results in the contacts of leprosy patients and Alves Ferreira *et al* discuss the factors associated with the seropositivity in patients and contacts under the age of 18 years.

Deps *et al* studied seropositivity in wild armadillos in Brazil.

The two populational studies presented involved schoolchildren. Ferreira *et al* conducted an active case finding study for leprosy, demonstrating the epidemiological situation, including the seropositivity rate among 68 cases diagnosed in a total population of 16,623 children examined. Research conducted by Bührer *et al* included 7,073 children and showed that a significant difference occurred in the seropositivity between private and public schools, providing further evidence that socioeconomic conditions are a determinant factor for subclinical infection, a fact that has already been observed in leprosy transmission, where it was witnessed that the majority of cases occur in families from underprivileged social classes

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