

Recent Advances in Leprosy

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Abstract

The present review briefly summarizes the highlights of the recent advances in Mycobacterium leprae specific tests for early diagnosis of leprosy. In addition to establishing the diagnosis of clinical cases of leprosy, these tests have also been used to detect subclinical infections in endemic population. Several attempts have been made from 1980 onward for standardization of specific diagnostic assays for early detection of leprosy. Brief account about the development and use of these assays has been described in this review article.

Keywords: Leprosy; Recent advances; Vaccines.

INTRODUCTION

Humans have been known to contract leprosy since ancient times. It is a chronic infectious illness of the skin and nerves caused by Mycobacterium leprae (ML) and Mycobacterium lepromatosis. It is still common in a lot of nations, like India. India continues to harbor 63% of the global leprosy population¹, and over 70% of newly diagnosed cases of leprosy worldwide are found there each year.² While India achieved the elimination threshold in December 2005, with a prevalence rate

(PR) of less than 1 case per 10,000 population size (<0.9/10,000), PR is still continuing at 0.74/10,000 (April 2017), suggesting no appreciable decrease in PR over the last ten years. Additionally, the annual new case detection rate, or ANCDR, has shown a growing trend recently, going from 9.71/100,000 in 2016 to 10.12/100,000 in 2017. This rate had nearly plateaued earlier.³ These patterns suggest that leprosy burden and transmission in India remain serious health concerns even with the introduction of multidrug therapy (MDT). Early detection and treatment of leprosy are crucial to reducing its impact and spreading throughout the population.

Use of M. Leprae-Specific Serology

Lepromin is an intradermal saline suspension of entire M. leprae that is used to assess an individual's CMI or delayed type hypersensitivity (DTH) reaction to the organism. It is applied to the volar surface of the forearm. Patients at the BL/LL end do not react skin wise to lepromin, but those at the TT/BT end elicit a robust DTH skin reaction. Subsequently, purified M. leprae derived from armadillos were disrupted or sonicated to prepare M. leprae soluble antigens. These soluble antigens,

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also referred to as "leprosin," demonstrated a background pattern of population sensitization to mycobacterial antigens with bimodal distribution, such as lepromin.⁷ These antigens have been more useful in the categorization of leprosy and in assessing the CMI of leprosy patients during therapy. Due to the fact that *M. leprae* shares antigens with other environmental mycobacteria that are found all over the natural world.⁸ A positive lepromin or leprosin test will only reveal an individual's CMI to *M. leprae* or cross-reactive mycobacterial antigens. Lepromin, sometimes

known as "leprosin," cannot be used to diagnose leprosy since the positive results of these tests are not specific for *M. leprae* infection. A person may benefit from immunomodulation to increase their CMI to *M. leprae*, though, if they have a negative reaction to lepromin, which indicates a lack of host CMI to *M. leprae*. Therefore, there is a scope for lepromin to be used for mass survey to identify the prospective lepromin negative candidates in a population who can benefit from vaccines to *M. leprae*.

Table 1: Comparative efficacies of immunological and molecular markers in diagnosis of leprosy

Name of the Test	Type of Leprosy		Sensitivity/Specificity (%)
	PB Patients Percent (%)	MB Patients Positivity	
Serological marker (PGL-1)	0-40 ^(10-12,27)	70-95% ^(10-12,27)	91% ⁽²⁷⁾
Evaluation of dipstick assay using ND-O-BSA-based ELISA			94.4% / 90.2% ^(27,28)
ML=flow test	40% ^(27,28)	97.4% ^(27,28)	97.4% / 90.2% ^(27,28)
35-kD-based serology	46.7% ⁽³⁹⁾	98.5% ⁽³⁹⁾	98.4% / 100% ⁽⁴⁰⁾
			90% / 97.5% ⁽⁴²⁾
NDO-LID rapid test	15.4-21.2% ⁽⁵⁸⁾	83.3-87% ⁽⁵⁸⁾	87% / 96.1% ⁽⁵⁸⁾
PCR using gene target RLEP	73% ⁽⁶⁷⁾	100% ^(67,68)	73.6% / 100% ⁽⁶⁷⁾
	83% ⁽⁷⁰⁾	96.6% ⁽⁷¹⁾	87.1% ⁽⁷²⁾
PCR using 16SrRNA gene target	50% ⁽⁷¹⁾	100% ⁽⁷¹⁾	51% / 100% ⁽⁷⁰⁾
PCR using Ag85B gene target	80% ⁹⁽⁷²⁾	100% ⁽⁷²⁾	56% / 100% ⁽⁷⁰⁾
PCR using 18kDa gene target	74% ⁽⁷³⁾	99% ⁽⁷³⁾	100% / 83% ⁽⁷³⁾
Proline-rich antigen (pra-36 kDa)	36-60% ⁽⁷⁵⁻⁷⁷⁾	87-100% ⁽⁷⁵⁻⁷⁷⁾	-
Multiplex PCR	83% ⁽⁶⁸⁾	100% ⁽⁶⁸⁾	-

Use of *M. leprae*-Specific Serology

Leprosy-specific serological tests emerged only after identification of *M. leprae*-specific antigens. Specificity and sensitivity of the serological assays have been summarized in Table 1.

Serological test using phenolic glycolipid-1

One of the earliest mycobacterial antigens to be recognized and separated from the primary glycolipid cell wall antigen of the bacteria is phenolic glycolipid-1 (PGL-1).⁹ An enzyme linked immunosorbent test (ELISA) was first created to diagnose leprosy using this as an antigen.¹⁰⁻¹² Approximately 26% of household contacts tested positive for PGL-1 antibodies in this assay, despite the endemic controls' sensitivity testing being primarily negative. Subsequently, trisaccharide [3,6-di-O-methyl-β-d-glucopyranosyl-(1→4)-2,3-di-O-methyl-α-l-rhamnopyranosyl-(1→2)-3-O-meth

yl-α-l-rhamnopyranose]¹³⁻¹⁵ and it was discovered that PGL-1's disaccharide components are the ones that specifically react with IgM antibodies in patient samples. In order to standardize ELISA for the diagnosis of leprosy, these synthetic sugars natural disaccharide (ND) and natural trisaccharide (NT) were separately synthesized and conjugated with either bovine serum albumin (BSA) or human serum albumin (HSA) using either octyl (O) or phenyl (P) linker arms (ND-O-BSA/HSA or NT-O-BSA/NT-P-BSA).^{16,17} It was shown that these glycoconjugates exhibited a growing trend in antibody levels from tuberculoid to lepromatous spectrum correlated with rise in bacterial load, and that they had stronger affinity for IgM antibody than PGL-1.^{18,19-22} Nevertheless, there was not always a favorable association between PGL-1 antibody levels and bacterial load²³. Newer assays like the particle agglutination assay and the *M. leprae* dipstick assay^{24,25} were created using this

neoglycoconjugate.²⁶ There are two antigen bands in the dipstick format; one indicates reactivity to ND-O-BSA, while the other serves as an internal control for human IgM. A 94.9% agreement was obtained between the dipstick assay and the ND-O-BSA based assay.²⁴ The development of the gelatin particle agglutination test, an alternative particle agglutination assay, involved first activating colored gelatin particles with tannic acid and then combining them with NT-P-BSA.²⁶ With an average cutoff value for positive spanning between serum dilutions of 1:64 and 1:128, these NT-P-BSA-labeled gelatin particles agglutinated with serial two-fold dilutions of patients' blood.

Status of PGL-1 antibody level with treatment

Given that the bacterial load and antibody levels have been observed to correlate¹⁹⁻²², it is reasonable to assume that following appropriate treatment, PGL-1 antibody levels will decrease. Therefore, PGL-1 antibody based serology may offer a way to keep track of leprosy patients receiving treatment.²⁶ A new cohort research with 105 leprosy patients using MDT regimen and monitored BI and PGL-1 antibody levels every 6 months to 2 years revealed that measuring antibody levels during chemotherapy helps assess the effectiveness of MDT for leprosy patients.²⁸ The levels of PGL-1 antibody in leprosy patients significantly decreased during chemotherapy, according to a number of other earlier investigations.²⁸⁻³³ based on 35 kDa serology.

The existence of 35kD protein in the membrane of *M. leprae* was confirmed by immunobiochemical method, and the monoclonal antibody MLO3-A1 responded exclusively with the ML 35kD antigen epitope. Following the discovery of the gene encoding 35kD of ML, *Mycobacterium smegmatis* allowed for its cloning, and adequate amounts of pure recombinant 35kD (r35kD) were accessible. Subsequently, it was discovered that ML 35kD shares 82% of its DNA and 90% of its amino acids with *Mycobacterium avium*, another mycobacterial species. For serological investigations, a different set of monoclonal antibodies (MLO4) with specificity for the same 35 kD was also used. The technique was first created as a radioimmunoassay based on competitive inhibition between the patient's serum and MLO4 that was labeled with I125. Later, it was standardized as an ELISA using MLO4 that was labeled with horse radish peroxidase. A large number of blood samples from patients with MB and PB were screened using 35kD ELISA, and the results showed a sensitivity of 46.7% and 98.5%,

respectively. To conduct field based research, a filter paper based blood sample collecting procedure from a remote field region was standardized. Even though *M. avium*, *Mycobacterium kansasii*, and *Mycobacterium paratuberculosis* share certain genes with the 35kD antigen, the standardized serodiagnostic assay was found to be 90% sensitive and 97.5% specific in the diagnosis of leprosy. Another study which compared PGL-1-based ELISA with 35kDa based serology, found both the assays to be reproducible and comparable. Roche *et al.* compared PGL-1-based ELISA and 35-kD inhibition based ELISA for their accuracy in diagnosis of leprosy with different levels of antibodies. It was noted that while PGL-1-based ELISA was suitable for diagnosis of cases with all the levels of antibodies, 35-kD inhibition-based ELISA did not perform well for diagnosis of patients having antibody levels near the cutoff value.⁴⁴

Afterwards, the specificity and sensitivity of r35kD were evaluated directly. The assay's sensitivity for diagnosing MB and PB cases was found to be 83.0% and 17.0%, respectively, despite its 94.3% specificity. The cloned pure recombinant protein may contain cross-reactive *M. smegmatis* mycobacterial proteins, which could explain the assay's limited sensitivity. Alternatively, the presence of subclinical infection in the exposed contacts could be the cause. Additionally, a dipstick ELISA was created and compared to the traditional ELISA utilizing both PGL-1 and r35kD. It was shown that there was a good concordance between the two methods.

A 35kD test card identified 59% of untreated PB cases compared to that of 27% detection by PGL-1; however, the sensitivity was found to be 90% by the r35kD test card and 100% by PGL-1 dipstick.

Status of 35kD antibody level with treatment

It has been demonstrated that the quantity of antibodies strongly correlates with the number of anesthetic patches used on patients. [40,45] Eventually, it was discovered that there was a positive correlation between the antibody levels and the number of nerves affected by primary neuritic leprosy. Additionally, efforts were undertaken to determine whether patient skin scraping samples, urine, or cerebrospinal fluid contained antibodies. These samples, however, did not prove to be more accurate in detecting leprosy cases than blood samples. It was discovered that after patients received efficient chemotherapy, their antibody levels against 35kD decreased.

Search for new antibody reactive *M. leprae* recombinant proteins and development of LI-1 and NDO-LID rapid test.

A vast panel of produced recombinant proteins was examined in a protein array format for their reactivity with classified leprosy sera due to the low level of false positivity with PGL-1 antigen. For additional examination, antigens that reacted significantly with patient sera and barely at all with control sera were chosen. The proteins that were chosen for the diagnosis of MB leprosy were ML0405 and ML2331. The fusion construct of these two proteins is known as LID-1 (Leprosy Infectious Disease Research Institute Diagnostic-1). It has been demonstrated that LID-1 can identify MB patients in Brazil, China, Japan, and the Philippines in particular. As PGL-1 or ND-O-BSA/HAS conjugate assay demonstrated positive results sometimes in uninfected controls as well,²⁷ LID-1 assay has been preferred for diagnosis of MB leprosy. Both of these antigens LID-1 and ND-O-BSA have been synthetically conjugated to work in one platform and a rapid test based on NDO-LID has been developed and has been named as NDO-LID rapid test (Orange Life, Rio de Janeiro, Brazil). NDO-LID kit is a ready-to-use kit for testing in field. Serum sample (10 µl) and running buffer (100 µl) are charged in the sample well causing the migration of sample and colloidal gold beads loaded with anti-IgG and anti-IgM through the membrane across the detection window. The reaction of the test and control yields a red color. Within 20 minutes of the samples being charged, readings are taken. The test is validated by a distinct development of the control line. When the test and control lines are developed, a favorable outcome is established. A visual reading score of 1+, 1.5+, or 2+ is assigned, and the development of a weak or absent color is seen as a negative. A "point-of-care" test was created for field use that uses a smartphone reader to capture the color development density. When compared to PGL-ELISA tests conducted in laboratories, the proportion of leprosy cases diagnosed by these quick tests was greater. The positive of PGL-ELISA was increased from 83.3% to 87% for MB cases and from 15.4% to 21.2% for PB cases using this NDO-LID fast test. The percentage of serological positivity with NDO-HSA, LID-1, and NDO-LID has not changed recently when screening a Venezuelan MB population; nevertheless, the study's limited sample size may have contributed to this finding. The incidence of anti-NDO-LID and anti-NDO-HSA positive was much higher in the general population than in home contacts,

indicating subclinical infection or community exposure to the virus, according to screening of endemic normal population household contacts.

Status of LID-1/NDO-LID antibody levels with treatment

It was observed that the antibody level to LID-1 decreased more quickly following the MDT regimen than the antibody level to PGL-1.⁶¹ A recent study that measured antibody levels utilizing PGL-1, LID-1, and NDO-LID discovered that after 6 months of uniform MDT (UMDT) or 12 months of the entire course of MDT, the antibody levels dramatically decreased. There was a correlation between the decrease in bacillary load and the drop in antibody levels. Additionally, this group proposed that in terms of lowering antibody levels and bacillary burden, UMDT was shown to be comparable to full course MDT.

Use of *M. leprae*-specific molecule employing polymerized chain reaction

Numerous biological specimens, including skin biopsies, skin sections, skin smears, nerve sections, and biological fluids including blood, pleural effusions, ascetic fluid, cerebrospinal fluid, saliva, nasal swabs, etc., can be routinely subjected to *M. leprae*-specific polymerase chain reaction (PCR). Even 10-30 fg of *M. leprae* component, or 2.8-8.3 bacilli, can be found by PCR. Using genes like RLEP, hsp65, 18kDa, 36kDa, 16SrRNA, and sodA, ML-specific PCRs were created because of the several regions of the *M. leprae* genome that are specific for ML. Leprosy diagnosis has been made using the majority of these genes alone. A quantitative PCR (qPCR) was used in clinical samples using RLEP, 16SrRNA, Ag85B, 18kDa, 36kDa, gene targets and it was noted that RLEP-PCR was most sensitive out of all these specified gene based PCRs. RLEP-PCR was also used by several other workers and was found to be most sensitive and specific of all the other gene targets.

Post chemotherapy related status of the *M. leprae*-specific PCR

The result of treatment can also be ascertained by ML-specific PCR. A technique using ML PCR was developed as early as 1993. It was observed that, despite no discernible change in BI, the number of genomes detected by PCR sharply decreased after 3, 6, 12, and 24 months of chemotherapy, and this reduction was correlated with a decrease in the morphological index of the bacilli.⁸⁰ A

recent quantitative real time (RT)-PCR based on hsp18mRNA showed that no viable ML could be detected in 47 leprosy cases after two years of MDT treatment; however, a significant amount of DNA was detected in many of these samples, indicating that RT-PCR could be used to monitor patients receiving chemotherapy. The method using RT-PCR was not further developed because of its complexity to perform under field conditions. However, with the emergence of drug resistant ML, this technique is presently being employed in reference laboratories in samples collected from the remote areas.

Use of molecular based technology for drug resistance in leprosy

Leprosy drug resistance has lately been documented in a number of nations, including India. Patients who are not responding to MDT are being treated with molecular based approaches to uncover mutations in the drug-resistant determining region (DRDR) of ML, as the mouse foot pad methodology for drug resistance identification takes at least six months. It has been demonstrated that several mutations in the folP1 region for DDS, GyrA region for Ofloxacin, and rpoB region for Rifampicin cause drug resistance in multiple lateral ligaments. In order to identify mutations by gene sequencing in the corresponding DRDR regions of medications responsible for drug resistance, reference laboratories may receive slit skin smears or biopsies preserved in 70% ethanol from patients who are not responding to treatment.

M. leprae-specific Antibody or PCR Positivity in the Context of Normal Household Contacts and Endemic Population.

From the discussion above, it may be concluded that the above mentioned ML-specific antibody and PCR tests are valuable tools in the diagnosis of a doubtful or a definite case of leprosy. However, clear guidelines in case of positive results of any of these assays in household contacts or an individual from endemic population are lacking. It is known that many normal household contacts of cases turn out to be leprosy cases in future, and household contacts of MB cases have been shown to have 3.8-10 fold more chance of getting leprosy than the general population. Several studies in Indonesia, India, and Brazil have indicated that in an endemic community as population are exposed to infection, the biological samples such as blood, nasal swabs, saliva, and slit skin smears of contacts of patients remain positive either for ML specific antibody or for specific component of ML. How many of these

biomarker positive contacts of the population will transform into cases is generally uncertain and depends on the immune status of the individual having subclinical infection. These diagnostic tests are performed only once in individuals who pass through a dynamic state of the immune system, and therefore every individual who test positive to these assays do not develop leprosy in future. Rather, it has been noted in a 2-year follow-up study that large number of cases appear from the ML-specific test negative group from the community which outnumber the cases that appear from the small cohort population of household contact group. Therefore, these tests performed only at a single point of time may not be useful for prediction of a future case. However, these tests could be applied in a cohort population at risk under surveillance but will not prove to be a cost-effective proposition for the leprosy control program.

VACCINE

In 2013, the WHO published new recommendations for manufacturing and evaluating BCG vaccine (for tuberculosis). In 2018, the WHO officially included leprosy in the single dose BCG vaccination recommendation.

The ICRC vaccine consists of a collection of slow growing, cultivable mycobacteria from the M. avium complex that were identified in 1958 from a leprosy patient and inactivated by gamma radiation. In an extensive comparative study conducted in India, Gupte *et al.* found that after a 4-6 year follow-up, 66% of participants were protected by ICRC while 34% were protected by BCG. It's interesting to note that in the same comparison trial, BCG and killed M. leprae provided 64% protection, which is comparable to ICRC. Human immunoglobulin G coating ICRC candidate strain C-44 was discovered during a recent review of the ICRC formula; this coating may have an effect on immunological responses.

The National Institute of Immunology in India produced the MIP vaccine, which had encouraging early results. Patients responded less well to the MIP vaccination: at 3, 6, and 9 year follow-ups, the protective effectiveness was 43%, 31%, and 3%, respectively. Smaller trials have discovered, however, that MDT and MIP used as immunotherapy for patients with multibacillary leprosy could speed up recovery, lower the bacterial load, remove granuloma, and lessen neuritis.

The most recent vaccination candidate via the

clinical trial pipeline is LepVax (Fig. 4.3). LepVax is a specified subunit vaccine that contains a chimeric recombinant protein (LEP-F1) made up of a synthetic glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE) and a tandem linkage of *M. leprae* antigens ML2531, ML2380, ML2055, and ML2028. 24 healthy adult volunteers in the United States participated in the phase 1a clinical trial to assess the dose, safety, and immunogenicity aspects of LepVax. The 2020 trial findings indicated that LepVax was immunogenic and safe.

Vaccine and Drug Combinatory Therapy

Chemotherapy and immunotherapy together can reduce the length of leprosy treatment and possibly enhance its effectiveness. Patients have to follow the regimen for at least two years after the WHO authorized MDT for leprosy in 1981. In a preliminary assessment of the MIP vaccine candidate, Talwar *et al.* discovered that patients who received MDT concurrently with vaccination saw faster bacterial clearance. Zaheer *et al.* looked at whether inducing cell mediated immune responses using chemotherapy in conjunction with immunotherapy, such as MDT + MIP, could shorten the duration of treatment. They came to the conclusion that MIP added to MDT could shorten treatment duration from 4-5 years to 2-3 years.

Immunotherapy added to patients receiving MDT typically has favorable clinical results. What about the transmission of leprosy in close proximity to patients? Research demonstrates that giving a single dose of rifampicin, one of the medications in the leprosy multidisciplinary team, to a patient's close contacts can prevent leprosy in 57% of cases within two years, but has little effect beyond that time. Richardus *et al.* looked into the possibility of reducing transmission among contacts of leprosy patients by administering rifampicin based chemoprophylaxis and BCG based immuno prophylaxis.

Conclusion and Vaccine Outlook

There is now only limited protection against leprosy from the BCG vaccine. Despite the World Health Organization's initial "elimination" announcement, which was defined as "the reduction of prevalence to a level below one case per 10,000 population," leprosy remains a problem. Due to the fact that it altered public opinion and diverted funds and resources required to conduct important, lengthy epidemiological investigations, this has garnered harsh criticism. *M. leprae* is still a bacterium that needs to be grown in animals. The

precise process of *M. leprae* transmission, the way it triggers immunological responses, and the cause of nerve injury remain unknown. The WHO's acknowledgement that BCG is a leprosy vaccine is a crucial statement that can encourage societal changes and further current vaccine research.

The above discussion has briefly described the recent progresses that has been made in the area of specific diagnostic tests for leprosy. Despite the attempts to develop a definitive early diagnostic test for leprosy especially for patients in whom cardinal signs of leprosy are not fulfilled, the objective of an ideal diagnostic test is still to be attained. Rather, these assays fail to detect almost 60% cases of PB leprosy patients demonstrating one of the cardinal signs. Another major concern with these tests is the positive results in significant number of contacts not showing any clinical signs of leprosy. These contacts have been found to have the same level of antibodies or markers of ML in their biological samples like early cases of PB leprosy. However, these antibody based assays using any of the antigens like PGL-1 or LID-1 or NDO-LID may prove to be useful in cases of early diffuse lepromatous or MB leprosy having no major nerve deficit or thickening which may be missed by leprosy experts. In spite of the above advancement in technology, there is still a need for development in early diagnosis of leprosy. Future efforts could be directed to search for new and novel antigens or host biomarkers which will be mainly expressed only in subclinical, preclinical, and in early leprosy cases and at the same time will also be able to discriminate these cases from uninfected endemic contacts.

REFERENCES

1. WHO. Weekly epidemiological record, September 1,2017;92:501-20. <http://www.who.int/wer>.
2. NLEP Annual Report, 2015-2016; WHO. Available from: <http://www.who.int/lep/en/>. [Last accessed on 2017 Sep 12].
3. NLEP Annual Report, 2016-2017; Available from: <http://www.nlep.nic.in/>. [Last accessed on 2018 Feb 22].
4. Ridley DS, Jopling WH. A classification of leprosy for research purposes. *Lepr Rev*1962;33:119-28.
5. Ridley DS, Ridley MJ. Classification of nerves is modified by the delayed recognition of *M. leprae*. *Int J Lepr Oth Myc Dis* 1986;54:596-600.
6. Forno C, Hausermann P, Hatz C, Itin P, Blum J. The difficulty in diagnosis and treatment of leprosy. *J*

- Travel Med 2010;17:281-3.
7. Gupte MD, Anantharaman DS, Nagaraju B, Kannan S, Vallishayee RS. Experiences with *Mycobacterium leprae* soluble antigens in a leprosy endemic population. *Lepr Rev* 1990;61:132-44.
 8. Sengupta U. Experience and lessons from the use of lepromin and *Mycobacterium leprae*-specific serology. Workshop Proceedings. *Lepr Rev* 2000; 71:S63-6.
 9. Brennan PJ, Barrow WW. Evidence for species-specific lipid antigens in *Mycobacterium leprae*. *Int J Lepr Other Mycobact Dis* 1980;48:382-7.
 10. Cho SN, Yanagihara DL, Hunter SW, Gelber RH, Brennan PJ. Serological specificity of phenolic glycolipid I from *Mycobacterium leprae* and use in serodiagnosis of leprosy. *Infect Immun* 1983;41:1077-83.
 11. Young DB, Buchanan TM. A serological test for leprosy with a glycolipid specific for *Mycobacterium leprae*. *Science* 1983;221:1057-9.
 12. Brett SJ, Draper P, Payne SN, Rees RJ. Serological activity of a characteristic phenolic glycolipid from *Mycobacterium leprae* in sera from patients with leprosy and tuberculosis. *Clin Exp Immunol* 1983;52:271-9.
 13. Hunter SW, Fujiwara T, Brennan PJ. Structure and antigenicity of the major specific glycolipid antigen of *Mycobacterium leprae*. *J Biol Chem* 1982;257:15072-8.
 14. Brett SJ, Payne SN, Draper P, Gigg R. Analysis of the major antigenic determinants of the characteristic phenolic glycolipid from *Mycobacterium leprae*. *Clin Exp Immunol* 1984;56:89-96.
 15. Fujiwara T, Hunter SW, Cho S-N, Aspinall GO, Brennan PJ. Chemical synthesis and serology of disaccharides and trisaccharides of phenolic glycolipid antigens from the leprosy bacillus and preparation of a disaccharide protein conjugate for serodiagnosis of leprosy. *Infect Immun* 1984; 43:245-52.
 16. Chatterjee D, Cho SN, Brennan PJ, Aspinall GO. Chemical synthesis and seroreactivity of O-(3,6-di-O-methyl-beta-D-glucopyranosyl)-(1----4)-O-(2,3-di-O-methyl-alpha-L-rhamnopyranosyl)-(1----9)-oxynonanoyl-bovine serum albumin-- the leprosy-specific, natural disaccharide-octylneoglycoprotein. *Carbohydr Res* 1986;156:39-56.
 17. Fujiwara T, Aspinall GO, Hunter SW, Brennan PJ. Chemical synthesis of the trisaccharide unit of the species-specific phenolic glycolipid from *Mycobacterium leprae*. *Carbohydr Res* 1987;163:41-52.
 18. Gigg J, Gigg R, Payne S, Conant R. The allyl group for protection in carbohydrate chemistry Synthesis of propyl O-(3,6-di-O-methyl-beta-D-glucopyranosyl)-(1-4)-O-(2,3-di-O-methyl-alpha-L-rhamnopyranosyl)-(1-2)-3-O-methyl-alpha-L-rhamnopyranoside: The oligosaccharide portion of the major serologically active glycolipid from *Mycobacterium leprae*. *Chem Phys Lipids* 1985;38:299-307.
 19. Schwerer B, Meeker HC, Sersen G, Levis WR. IgM antibodies against phenolic glycolipid I from *Mycobacterium leprae* in leprosy sera: Relationship to bacterial index and erythema nodosum leprosum. *Acta Leprologica* 1984;2:394-402.
 20. Bach MA, Wallach D, Flageul B, Hoffenbach A, Cottenot F. Antibodies to phenolic glycolipid-1 and to whole *Mycobacterium leprae* in leprosy patients: Evolution during therapy. *Int J Lepr Other Mycobact Dis* 1986;54:256-67.
 21. Sinha S, McEntegart A, Girdhar BK, Bhatia AS, Sengupta U. Appraisal of two *Mycobacterium leprae*-specific serological assays for monitoring chemotherapy in lepromatous (LL/BL) leprosy patients. *Int J Lepr Other Mycobact Dis* 1989;57:24-32.
 22. Roche PW, Britton WJ, Failbus SS, Williams D, Pradhan HM, Theuvenet WJ. Operational value of serological measurements in multibacillary leprosy patients: Clinical and bacteriological correlates of antibody responses. *Int J Lepr Other Mycobact Dis* 1990;58:480-90.
 23. Lyons NF, Shannon EJ, Ellis BP, Naafs B. Association of IgG and IgM antibodies to phenolic glycolipid-1 antigen of *Mycobacterium leprae* with disease parameters in multibacillary leprosy patients. *Lepr Rev* 1988;59:45-52.
 24. Bühner-Sékula S, Cunha MGS, Ferreira WA, Klatser PR. The use of whole blood in a dipstick assay for detection of antibodies to *Mycobacterium leprae*: A field evaluation. *FEMS Immunol Med Microbiol* 1998;21:197-201.
 25. Bühner-Sékula S, Cunha MGS, Foss NT, Oskam L, Faber WR, Klatser PR. Dipstick assay to identify leprosy patients who have an increased risk of relapse. *Trop Med Int Health* 2001;6:317-23.
 26. Izumi S, Fujiwara T, Ikeda M, Nishimura Y, Sugiyama K, Kawatsu K. Novel gelatin particle agglutination test for serodiagnosis of leprosy in the field. *J Clin Microbiol* 1990;28:525-9.
 27. Bühner-Sékula S, Smits HL, Gussenhoven GC, van Leeuwen J, Amador S, Fujiwara T, et al. Simple and fast lateral flow test for classification of leprosy patients and identification of contacts with high risk of developing leprosy. *J Clin Microbiol* 2003;41:1991-5.
 28. Zenha EMR, Ferreira, MAN, Foss NT. Use of anti PGL-1 antibodies to monitor therapy regimens in leprosy patients. *Braz J Med Biol Res* 2009; 42:968-72.

29. Bach MA, Wallach D, Flageul B, Hoffenbach A, Cottenot F. Antibodies to phenolic glycolipid-1 and to whole *Mycobacterium leprae* in leprosy patients: Evolution during therapy. *Int J Lepr Other Mycobact Dis* 1986;54:256-67.
30. Roche PW, Britton WJ, Failbus SS, Neupane KD, Theuvenet WJ. Serological monitoring of the response to chemotherapy in leprosy patients. *Int J Lepr Other Mycobact Dis* 1993;61:35-43.

