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Detection of *Mycobacterium leprae* infection employing a combinatorial approach of anti-45 kDa and modified anti-PGL-I antibody detection assays

Leprosy is a contagious infectious disease caused by *Mycobacterium leprae*, which is non-cultivable *in vitro*. The routine diagnosis of leprosy is largely based on clinical manifestations. However, even experienced clinicians face difficulties in the definitive diagnosis of leprosy, which could result in improper or unnecessary treatment. The integration of leprosy control programmes into general health services has posed a threat to the availability of sufficiently experienced health workers to diagnose leprosy. According to a recent report, leprosy is limited mainly to developing countries like: India, Brazil, Myanmar, Madagascar, Nepal, Mozambique, Democratic Republic of Congo, Tanzania, Angola and Central Africa (WHO, 2005). Improved laboratory tests that can assist in the diagnosis of leprosy or *M. leprae* infection will be of considerable value in leprosy control programmes. Since serological tests can easily be implemented under the conditions commonly encountered in developing countries, these are the preferred methods. Hence, developing serological tests for diagnosis of leprosy has long been the subject of investigation. Unfortunately, specific serological assays described thus far are applicable for multibacillary (MB) patients but have limited sensitivity for detection of paucibacillary (PB) forms of leprosy (Parkash, 2002; Oskam *et al.*, 2003; Parkash *et al.*, 2006a, b, 2007a). Furthermore, comparative study among PGL-I (phenolic glycolipid-I), serine-rich 45 kDa protein (45 kDa antigen), culture filtrate protein-10 (CFP-10), and early secreted antigenic target-6 (ESAT-6) have shown that the performance of the 45 kDa antigen was comparatively the best in terms of sensitivity and specificity in our setting (Parkash, *et al.*, 2006a, O. Parkash, A. Kumar, R. Pandey, B. K. Girdhar, K. L. M. C. Franken & T. H. M. Ottenhoff, unpublished data). In another study (Parkash *et al.*, 2007b), we recently reported a modified anti-PGL-I antibody detecting ELISA in which incubating the reactants at low temperature significantly improved the sensitivity of the conventional anti-PGL-I antibody detecting ELISA. On the other hand, in the case of 45 kDa antigen-based ELISA low temperature incubation did not show such improvement over the conventional assay (A. S. Purvia, A. Kumar & O. Parkash, unpublished data). The aim of the present study, therefore, was to assess the performance of the combined conventional anti-45 kDa and modified anti-PGL-I low temperature antibody detecting ELISAs.

The approval for the study was obtained from the National JALMA Institute for Leprosy and Other Mycobacterial Diseases ethics committee for scientific researches. In total, serum samples from 106 subjects were examined, including 18 smear-positive MB leprosy patients and 38 smear-negative PB leprosy patients classified as described elsewhere (WHO, 1988). The diagnosis of leprosy was based on clinical criteria as defined by World Health Organization (WHO, 1998). All leprosy patients either were as yet untreated or had started therapy less than one month before inclusion in the study. To evaluate specificity, serum samples from 16 clinically active pulmonary tuberculosis patients, 14 patients with other skin diseases and 20 healthy individuals were analysed.

Circulating serum antibodies against the 45 kDa antigen were detected by ELISA as previously reported (Parkash *et al.*, 2006a). For detection of anti-PGL-I antibodies the reactants (antigen and serum samples) were allowed to react for 2 h at 37 °C, followed by a 24 h extended incubation at 4 °C (Parkash *et al.*, 2007b). The chi-square test was used for statistical validation of the test performance between the independent groups. Table 1 shows the results of the two assays separately as well as in combination. Importantly, there were differences noted in the results of the two assays, particularly when considering the PB group. When analysing the results from the two assays in a joint fashion, the combined results for PB patients differed significantly ($P<0.05$) from that of 45 kDa antigen-based assay alone. In contrast, combining the results of the conventional PGL-I-based assay and the 45 kDa antigen-based assay did not differ significantly from the 45 kDa antigen-based assay alone, in line with our previous work (Parkash *et al.*, 2006a). When considering the combined groups of MB and PB leprosy patients together, the recognition frequencies with the PGL-I-based modified ELISA alone were found to be slightly but not significantly higher than that of the 45 kDa antigen-based ELISA (76.8 % vs 64.3 %, taking all MB and PB leprosy patients together). Interestingly, in both the cases, the specificity was found to be >98.0 %. When combining the results of the two assays together, the overall performance (considering MB + PB patients together) was found to be better (sensitivity about 84.0 %, specificity 98.0 %) than those of the individual antigen-based assays. It is important to note that with the combined approach more than 76.0 % of the samples from PB patients were found to be reactive. Thus, the combinatorial approach discussed here could help, particularly, in detecting more PB patients compared to the 45 kDa antigen-based and modified PGL-I-based ELISAs alone. Since PB patients are a group in which, generally, the sensitivity of detection of anti-*M. leprae*-specific antibodies remains low, we consider this combined approach of the anti-45 kDa and the modified anti-PGL-I antibody detecting assays to be a better alternative for serodiagnosis of leprosy.

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Table 1. Results of ELISA for detection of anti-45 kDa and anti-PGL-I antibodies

Group	No. of patients	No. positive (%) by 45 kDa	No. positive (%) by PGL-I	No. positive by 45 kDa but negative by PGL-I	No. positive by PGL-I but negative by 45 kDa	No. positive (%) by either 45 kDa or PGL-I
Leprosy						
MB	18	18 (100.0)	17 (94.1)	1	0	18 (100.0)
PB	38	18 (47.4)	26 (68.4)	3	11	29* (76.3)
Total	56	36 (64.3)	43 (76.8)	4	11	47 (83.9)
Control						
Tuberculosis	16	0 (0)	1 (6.3)	0	0	1 (6.3)
Other skin diseases	14	0 (0)	0 (0)	0	0	0 (0)
Healthy	20	0 (0)	0 (0)	0	0	0 (0)
Total	50	0 (0)	1 (2.0)	0	0	1 (2.0)

*Significantly ($P<0.01$) different when compared to 45 kDa antigen.

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