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A Latex Agglutination Test for Rapid Serodiagnosis of Leprosy in the Field

TO THE EDITOR:

At present leprosy is diagnosed and classified according to clinical, histopathological, and bacteriological criteria ([1]). Nevertheless, in many developing countries where leprosy is endemic resources for these purposes are often very limited. Therefore, it is necessary to have simple, cost-effective, and rapid methodologies which can be complementary to, or even substitute for, conventional diagnostic procedures. The *Mycobacterium leprae*-specific phenolic glycolipid (PGL-I) in the semisynthetic disaccharide octyl bovine serum albumin (ND-O-BSA) form ([5]) has been widely used in indirect enzyme-linked immunosorbent assay (ELISA) techniques to detect anti-PGL-I antibodies in a number of diagnostic and epidemiologic studies (e.g., [2, 7, 11]). Even though the ELISA has many advantages, being fast, reproducible and quantitative, it does need the framework of an established laboratory and trained personnel.

The objective of this work was to develop a simple latex particle agglutination assay (LPAA) that can be performed under subtropical and tropical field conditions by untrained personnel which would give an easy, visual read-out within 5 minutes and which is cost-effective.

Latex particles (Estapor, KI-080, azure; Rhone-Poulenc, Aubervilliers, France) were covalently coupled with 400 μg/ml ND-O-BSA, obtained through WHO IMMLEP, according to the manufacturer's instructions (Rhone-Poulenc). Finally, precipitated antigen coupled latex particles were resuspended in 0.5 ml of the binding buffer (50 mM Na_2PO_4, 17 mM NaCl, 0.025% v/v Nonidet P-40, 0.1% w/v gelatin, 2% w/v Kollidon VA64, pH 6.6). A 5-μl suspension was spotted onto Perlalux Duplex rigid plastic films (Papierfabrik Perlen, Perlen, Switzerland) as a dot. The dots were dried at 40°C for 1 hr and stored at room temperature until used. Stability tests for binding to the plastic film, solubility, agglutination, and sensitivity were performed after 1 week, 2 weeks, 1 month, 2, 3, 6, 9, and 12 months using a standard pool of leprosy sera with a shelf-life of at least 12 months when stored at 15°C–35°C and 40%–80% humidity (data not shown). Hereby the criteria for use under subtropic and tropic conditions without refrigerating were obtained.

For the LPAA, 10 μl undiluted serum or twofold dilutions made in phosphate buffered saline, pH 7.2, of negative control serum, positive control serum and the unknown serum sample were added to the dried latex dots. After 60 sec, the dots were resuspended with a spatula and mixed gently. Four min later the results were read by visual inspection. The antibody titer was expressed as the highest dilution giving agglutination. A sample was considered positive when the titer value was 2 or more, determined on the basis of three times the standard deviation of the Ethiopian control group. The ELISA was used to test the reliability of the LPAA. The assay method was similar in principle to the one described by Cho, *et al.* ([5]). However, 2.5% w/v casein and 0.05% v/v Tween 20 were used as the blocking agent instead of 5% w/v BSA. Serum samples were tested as twofold dilu-

TABLE 1. *Results of latex particle agglutination assay (LPAA) and indirect ELISA for detecting anti-PGL-I-antibodies in leprosy patients, multidrug treated leprosy patients, tuberculosis patients and healthy control patients.*

Group	No. serum samples	No. specimens with test results[a]				% Agreement rate	% Positive	
		L^+E^+	U^+E^-	L^-E^+	L^-E^-		L	E
Active multibacillary (MB) leprosy	20	18	0	1	1	95.0	90.0	95.0
Multidrug-treated MB leprosy	7	2	1	1	3	71.4	42.9	42.9
Active paucibacillary (PB) leprosy	23	7	1	8	7	60.9	34.8	65.2
Multidrug-treated PB leprosy	7	2	0	0	5	100.0	28.6	28.6
Tuberculosis	12	1	1	0	10	91.2	16.7	8.3
Ethiopian controls	26	1	0	3	22	84.6	3.8	15.4
Danish controls	23	0	0	0	23	100.0	0.0	0.0
Total	118	31	3	13	71	85.6		

[a] L = Latex particle agglutination assay, + or − indicates positive or negative result. A sample was considered positive when the titer value was 2 or more determined on basis of three times the standard deviation of the Ethiopian control group. E = Indirect ELISA, + or − indicates positive or negative result. A sample was considered positive when the MONA value was 4 or more determined on basis of three times the standard deviation of the Ethiopian control group.

tions starting at the 1:200 dilution. The ELISA plates were Maxisorp Immunoplate (Nunc A/S, Roskilde, Denmark). Peroxidase-conjugated anti-human immunoglobulin M (P217; Dako A/S, Glostrup, Denmark) was used as the detecting secondary antibody. O-Phenylenediamine (OPD) and H_2O_2 in citrate phosphate buffer, pH 5.0, were used as the enzyme substrate. The value of the ELISA results are given as multiples of negative activity (MONA) values. A sample was considered positive when the MONA value was 4 or more, determined on the basis of three times the standard deviation of the Ethiopian control group.

The serum samples for this study were collected from 57 leprosy patients (43 Ethiopians and 14 Danes), 26 healthy Ethiopians, 12 Danish tuberculosis patients and 23 healthy Danes. The leprosy sera were classified according to the Ridley-Jopling scale ([12]) as 27 multibacillary patients (1 BB, 13 BL, 13 LL) and 30 paucibacillary patients (26 BT, 4 TT). Because of the subjective estimate of analysis on the degree of agglutination, all sera were tested under code at least twice before the results were sent to the Armauer Hansen Research Institute (AHRI) where the code was opened. At AHRI the results of both the LPAA and the ELISA were compared to previously obtained results.

Spearman's correlation coefficient was used for comparison between the groups ([14]). In ELISA we measured the IgM antibody responses (data not shown) because previously obtained data (e.g., [15]) showed only IgM responses to be of significance in the serodiagnosis of leprosy. The Spearman's correlation coefficients between the LPAA and the mean absorbance of IgM and IgG antibodies were 0.9215 and −0.3214 for the multibacillary cases, respectively.

Table 1 summarizes the results of the LPAA and ELISA. The agreement between the results obtained for the LPAA in Copenhagen and the ELISA performed previously in Addis Ababa was determined by Spearman's correlation coefficient. Values obtained included: 0.9992 for multibacillary leprosy patients, 0.9643 for multibacillary multidrug-treated patients, 0.9960 for paucibacillary patients, 1.0 for paucibacillary multidrug-treated patients, and 0.9985 for the Ethiopian control group, respectively, all showing good correlation between the data obtained by the two methods.

Because the aim of this study, among other things, was to develop a serodiagnostic leprosy assay to be used in the field under difficult conditions, we also tested undiluted sera in the LPAA (Table 2). Generally the sensitivity of the LPAA was higher than the results shown in Table 1, but when we consider the healthy Ethiopians as the negative control group, the specificity of the LPAA fell from 96.2% to 76.9%, compared to the result obtained in Table 1, as a result of an increased number of patients' sera having low antibody titers against PGL-I.

Recently, papers have been published describing the rapid serodiagnosis of leprosy using latex agglutination assays ([6, 8, 14]) and using a gelatin particle agglutination assay ([9]). Clearly, these efforts represent improvements, and may assist the clinician in the diagnosis of leprosy. However, the assay system described in the present work is even simpler to perform and is not dependent on access to any laboratory facilities. The reagents needed for the LPAA described here can be stored for 1 year at ambient temperature, and the assay may be performed using undiluted serum. The test may also prove useful in low-endemic areas where the number of patients suspected of having leprosy is so low that even an ELISA would be uneconomical to set up. We have compared the LPAA using both an undiluted and a diluted series of sera. The use of undiluted sera has a practical advantage and, in fact, increases the sensitivity of the system. When we prepared a twofold dilution series of the sera, the LPAA was found to be more specific than the indirect ELISA using the Ethiopian control group as a reference. The false-positive reactions (4 out of 26) observed in the Ethiopian control population may be caused by the relative hyper-gammaglobulinemia which is more common among Ethiopians compared to Scandinavians ([10]). It should also be kept in mind that the Ethiopian population was unselected and might have included healthy contacts or subclinical cases who had developed anti-PGL-I antibodies without any sign of the disease.

Using the dilution series of the sera we found that sensitivity was 90.0% for the LPAA when considering multibacillary patients, which is comparable with the other described assays 95.2% ([3]), 62.7% ([8]), 79.7% ([9]), and 88.4% ([14]). The paucibacillary patients generally displayed a very low level of anti-PGL-I antibodies; we detected 34.8% by the LPAA. Considering this, our test is as effective for the paucibacillary sera as the gelatin particle agglutination test described previously ([3, 9]). They obtained sensitivities of 21% and 33.3%, respectively, with specificities comparable to results obtained in this study. Wu, *et al.* ([14]) described a sensitivity at 62.5% for paucibacillary patients.

TABLE 2. *Results of latex particle agglutination assay (LPAA) showing the sensitivities and specificities for detecting anti-PGL-I antibodies in undiluted serum samples from leprosy patients, tuberculosis patients, and healthy control patients.*

Group	No. serum samples	No. positive LPAA samples	% Positive
Activity multibacillary (MB) leprosy	20	19	95.0
Multidrug-treated MB leprosy	7	4	57.1
Active paucibacillary (PB) leprosy	23	10	43.5
Multidrug-treated PB leprosy	7	2	28.6
Tuberculosis	12	2	16.7
Ethiopian controls	26	6	23.1
Danish controls	23	0	0.0

There have been many reports of decreases in antibody titer following effective chemotherapy (e.g., [4, 5]). The results of the LPAA presented here agree with those of earlier reports, indicating that the LPAA may be used to monitor anti-PGL-I titers in multibacillary patients under multidrug treatment. The LPAA results can (because the latex agglutination dot dries up on the plastic sheet) be stored in the patient's file for later evaluation of the treatment.

In conclusion, the evidence presented in this communication shows that the LPAA: a) has sensitivity and specificity that can be compared with ELISA, b) may be a useful tool in the diagnosis and monitoring of multibacillary leprosy, c) can be performed with undiluted sera, d) is very easy to apply by untrained personnel under difficult field conditions, f) the LPAA plates can be stored under subtropical and tropical conditions

without refrigeration for at least 1 year without losing sensitivity, g) is rapid and gives a clear, visual read-out within 5 min because of the use of azure latex particles on a white background, h) the LPAA plates can, after assay, be stored in the patient's file because the latex agglutinates dry up, and i) is cost-effective compared to other serodiagnostic methods.

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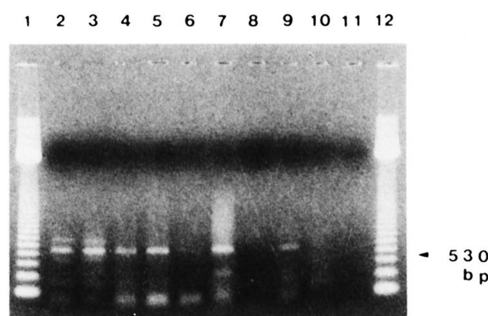
Detection of *Mycobacterium leprae* in Tissue and Blood by Polymerase Chain Reaction

TO THE EDITOR:

Leprosy is a chronic, systemic, infectious disease caused by *Mycobacterium leprae*. A new diagnostic method which is more sensitive and specific for the detection of *M. leprae* is required which might allow diagnosis of leprosy at a very early stage. Recently, specific DNA probes have been developed, and several investigators have studied the use of the polymerase chain reaction (PCR) to detect *M. leprae*([1-4]). Our present study applied PCR on sections of paraffin-embedded, frozen biopsy samples and peripheral blood from leprosy patients. Specimens were taken from 35 leprosy patients (19 males and 16 females between 14 and 73 years of age). Genomic DNA was isolated from paraffin-embedded or frozen tissues and peripheral blood, as described elsewhere. A set of primers, S13 (5'-CTCCACCTGGACCGGCGAT-3') and S62 (5'-GACTAGCCTGCCAAGTCG-3'), was selected on the basis of the nucleotide sequence positioned 530 bp of a gene encoding 36-kDa antigen of *M. leprae*([2]). DNA samples were first heated at 94°C for 10 min to denature the DNA and then subjected to 32 amplification cycles. For direct gel analysis 10 μl of the reaction mixture was subjected to electrophoresis on an 1.2% agarose gel for 45 min at 50 volts, and DNA was visualized as UV fluorescence (320 nm) after staining with ethidium bromide. For Southern blot analysis, DNA was separated by electrophoresis and transferred to nitrocellulose (NC) paper after treatment with 1.5 M NaCl, 0.5 N NaOH (denaturation solution) for 20 min then the filter was neutralized with 1.5 M NaCl, 1 M Tris-HCl (neutralizing solution) for 20 min at room temperature. Thereafter the DNA on the gel was transferred to NC paper for 20 hr at room temperature, and then baked for 60 min at 80°C. DNA samples were hybridized with digoxigenin-labeled *M. leprae* cDNA probe in hybridization solution. The filters were washed twice with 2×SSC (1×SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate, incubated in a blocking buffer for 30 min, and treated with alkaline phosphate for 1 hr for the color reaction.

In frozen and paraffin-embedded tissues of leprosy patients, relatively high detection rates of amplified PCR products were achieved by using direct gel analysis as well as Southern blot hybridization (The Figure). In the peripheral blood of five untreated



THE FIGURE. PCR detection of *M. leprae* DNA in biopsy samples from leprosy patients. DNA from: purified *M. leprae* (lane 2), frozen sections from LL (lanes 3–5) and BL (lane 6), paraffin-fixed sections from LL (lanes 7, 8), BT (lane 9) and TT (lane 10), and no DNA (negative control, lane 11). Lanes 1 and 12 contained molecular size markers.