


Sensitivity of a Rapid Mix Test with Combined Synthetic Antigens Derived from Mycobacterium Leprae PGL-1 for Diagnosis and Surveillance of Leprosy

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Abstract

Introduction: In the Americas, Brazil contributes 91.63% of the total cases and the state of Pará still has high endemia for leprosy. **Objective:** To analyze the performance of a rapid test for the diagnosis and epidemiological surveillance of leprosy in endemic areas. **Methods:** The sample consisted of 70 MB multibacillary leprosy (MB) patients, 63 paucibacillary (PB) patients, and 80 intradomiciliary consanguineous contacts (ICSCO) of patients. A rapid test with a 15-minute reading was applied using two prototypes: prototype 1, double test with trisaccharide antigen (NT-P-BSA) at 1a. line (83.2 ng/test) and disaccharide antigen (ND-O-BSA) at 2a. (83.2 ng/test), both with a flow of 0.08 μ L/mm with a 10 μ C membrane, anti-IgM conjugate with a flow of 0.040 μ L/mm and a Tris-Triton and prototype 2 runner buffer with MIX antigen (trisaccharide + disaccharide) in the same concentrations and conditions of prototype 1. **Results:** The comparison of the MIX test positivity rate and the disaccharide or trisaccharide doublet test across all samples was statistically significant, demonstrating that the MIX test had higher seropositivity rates compared to the ND-O-BSA or NT-P-BSA. It was demonstrated that the MIX test showed a good performance, with 25.39% of the PB patients negative for the disaccharide and trisaccharide duplet test, but positive for MIX. **Conclusions:** These data suggest the potential for further optimizing the performance by adding other synthetic antigens to the MIX antigens.

Keywords

Leprosy, Diagnosis, Rapid Test

1. Introduction

Leprosy is an endemic disease prevalent in developing countries [1]. It is still associated with precarious living conditions and occurs in socially vulnerable populations [2] [3], which often have restricted access to health services. Scientific studies have sought to establish immunological diagnostic methods applicable to, and suitable for endemic regions with few technical-diagnostic resources [3].

The latex agglutination technique in the 1950s represented the beginning of the studies towards the production of immunochromatographic rapid reading tests. Subsequently, rapid tests were widely studied in the 1980s and finally established in the 1990s and 2000s [4] [5] [6] [7] [8]. The most well-known formats of immunochromatographic tests are the lateral flow immunochromatography test (ML Flow), double migration immunochromatography, and immunoconcentration, and solid phase devices [8]. The lateral flow immunochromatographic test detects anti-PGL-1 (phenolic-glycolipid-1) IgM antibodies. The sensitivity of the ML Flow test is 97.4% in patients with multibacillary leprosy (ML) and the specificity is 90.2%; it has an excellent correlation with ELISA data (91%; $k = 0.77$) [6].

The ML Flow test uses the trisaccharide antigen (NT-P-BSA), colloidal gold and conjugate containing anti-human IgM, dispensed into the test control line [4] [5] [6]. Another rapid test developed by the Infectious Disease Research (IDRI), the Institute of Infectious Diseases Research in the United States, in cooperation with the Brazilian company Orange Life [7] was approved by ANVISA (National Health Surveillance Agency) in 2012. The NDO-LID-1 test, which merges the two recombinant proteins ML0405 and ML 2331 together with the NDO antigen showed 93.3% specificity for both MB and PB patients. The sensitivity was 95.7% for the MB and 73.7% for the PB patients [7].

Therefore, it is important that these studies are carried out in countries where leprosy still represents a public health problem and leads to social and economic difficulties for the affected population, making it more vulnerable and increasing the burden on the state through the need of treatment and disability pensions.

The objective of this study was to determine the sensitivity of a rapid test with synthetic antigens derived from *Mycobacterium leprae*. PGL-1 combined, disaccharide antigen (ND-O-BSA) [4] was produced in North America and trisaccharide antigen (NT-P-BSA) [4] in Japan for the diagnosis and epidemiological surveillance of leprosy, especially of patients with paucibacillary leprosy (PB). These patients have a genetic-immunological profile expressing a cellular-based immune response and have low bacillary loads and milder symptoms. It is more

challenging to define the clinical classification for specific treatment purposes in these cases [5]. Therefore, a rapid sensitivity test for such cases may be of great help to health professionals responsible for clinical diagnosis [4] [5].

2. Methods

This cross-sectional study involved municipalities of the State of Pará in the Brazilian Amazon: Curionópolis, Goianésia do Pará, and Canaã dos Carajás. All participants or legal guardians provided written informed consent, and the study was approved by the municipal health authorities and the Research Ethics Committee of the Evandro Chagas Institute's (Ministry of Health) (CAAE 48723115.1.0000.0019).

The study included individuals older than 18 years old who did not have immunosuppressible diseases and who agreed to participate in the study. Exclusion criteria were individuals under 18 years of age and/or with immunosuppressible diseases and pregnant women. Patients with multibacillary leprosy (MB), patients with paucibacillary leprosy (PB), and Intradomiciliary consanguineous contacts (ICSCO) of patients with leprosy were selected from the active registry of the health units through the SINAN Program (Notification Disease Information System). The ICSCO group included individuals without clinical symptoms of leprosy and who lived with the patient.

The health unit team was previously contacted through the community health agents. The sample consisted of 70 MB patients (Dimorfa and Virchowiana forms), 63 PB patients (indeterminate and Tuberculoid forms), and 80 ICSCO patients, in total, 213 individuals were recruited for the study.

An epidemiological record with personal, clinical, and epidemiological data was filled out for all individuals. After signing the Free and Informed Consent Term, the dermato-neurological clinical examination was performed and blood/serum was collected to perform the immunoserological tests. The rapid tests Duplete and Mix were developed in a Technological Development Program of Reagents for Diagnostic (Bio Mnaguinhos, Fio Cruz, Rio de Janeiro, Brasil).

The test was established using two prototypes: The Duplete test with trisaccharide antigen (NT-P-BSA) on the first nitrocellulose strip lane (83.2 ng/test) and disaccharide antigen (ND-O-BSA) on the second (83.2 ng/test). Both were run with a flow rate of 0.08 $\mu\text{L}/\text{mm}$ on a 10 μm membrane. The anti-IgM conjugate was run with a flow rate of 0.40 $\mu\text{L}/\text{mm}$ and Tris-Triton-X-100 running buffer. Prototype 2 with the MIX antigens (trisaccharide and disaccharide) was run in a single lane on the nitrocellulose strip and at the same concentrations and conditions of prototype 1. The test is read out within 15 minutes after the initial placement of the tested serum in the sample reservoir together with four drops of the running buffer.

Epi-Info 2000 and BioStat 5.0 software were used to calculate the Odds Ratio (OR) and determine the cut-off point, sensitivity and specificity of the screening test, the MIX rapid test and the Duplete test for the studied samples. In addition,

we applied descriptive statistics for the predictor variables of the analyzed data

3. Results

The seropositivity of the MIX rapid test was compared to the ND-O-BSA test and NT-P-BSA test across all groups, according to the established classification. The results showed that 84.28% (59/70) of the MB patients were positive for the MIX test, followed by 80.00% (56/70) NT-P-BSA positives, and 51.42% (36/70) ND-O-BSA positives. Among the PB patients, 46.03% (29/63) were positive for the MIX test, followed by 12.69% (8/63) positive for the NT-P-BSA positive test and 3.17% (2/63) positive for the ND-O-BSA test. Among the ICSCO, 32.5% (26/80) were positive for the rapid MIX test, followed by 16.25% (13/80) positive for the NT-P-BSA test and 6.25% (5/80) positive for the ND-O-BSA test (**Table 1**).

The comparison of the MIX test positivity rate and the disaccharide or trisaccharide doublet test across all samples was statistically significant for the hypothesis that the MIX test had higher seropositivity rates compared to the ND-O-BSA or NT-P-BSA test, $\chi^2 = 9.33$, $p = 0.002$. The odds ratio or probability that the MB group had a higher positivity rate for the MIX test compared to the ICSCO group was statistically significant OR = 80.45, $p < 0.0001$ (95% CI $13.81 \leq \mu \leq 98.45$) (**Table 2**). Among PB patients for the MIX test compared to the ICSCO group for the ND-O-BSA test the odds ratio was OR = 12.79 and $p < 0.0001$ (95% CI $5.84 \leq \mu \leq 115.98$) (**Table 3**).

Table 1. Performance of MIX and Duplete test according to the classification of the studied sample.

Classification/ Positivity	NT-P-BSA			ND-O-BSA			MIX		
	Positive	%	Negative	Positive	%	Negative	Positive	%	Negative
MB	56	73	15	36	83.72	34	59	52	11
PB	8	10	55	2	5	61	29	25.2	34
ICSCO	13	17	67	5	11.28	75	26	22.8	54
Total	77	100	136	43	100	170	114	100	99

NT-P-BSA trisaccharide; ND-O-BSA disaccharide; MB Multibacillary; PB Paucibacillary; ICSCO Intradomiciliary consanguineous contacts.

Table 2. MIX rapid test performance among patients with multibacillary leprosy (MB) compared to performance of the Duplete (disaccharide) test among the Intradomiciliary Consanguineous Contact Group (CCOSI) in the study sample.

Groups	Test MIX		Test disaccharide		OR	p-value
	Positive	Negative	Positive	Negative		
MB	59	11	56	14	*80.45	<0.0001
ICSCO	26	54	5	75		
Total	85	65	69	81		

*Odds ratio (OR) between MB (MIX) and ICSCO (Disaccharide) = 80.45, $p < 0.0001$, IC95% - $13.81 \leq \mu \leq 98.45$. MB Multibacillary; PB Paucibacillary; ICSCO Intradomiciliary consanguineous contacts.

The screening test for the MB group using the MIX test compared to the ICSCO group for the ND-O-BSA test showed a sensitivity of 84.29%, a specificity of 93.75%, false-positive = 6.25%, false-negative = 15.71%; Prevalence = 0.467 or 46.67%; Predictive value of positive test (PPV) = 92.19%, negative predictive value (NPV) = 87.21%, accuracy = 0.89 or 89.33%, +LR Likelihood Ratio positive = 13.49, -LR Likelihood Negative ratio = 0.17 (**Table 2**). For the group of PB patients using the rapid MIX test compared to the CCOSI group using the ND-O-BSA test, a sensitivity of 46.03%, specificity of 93.75%, false-positive = 6.25%, false-negative = 53.97%, prevalence = 0.441 or 44.06%, PPV = 85.29%, NPV = 68.81%, accuracy = 0.73% or 72.73%, +positive LR = 7.37, and -LR negative = 0.58. The comparison between PB patients and the ICSCO group using the ND-O-BSA test showed a sensitivity of 12.70%, specificity of 93.75%, false-positive = 6.25%, false-negative = 87.30%, prevalence = 0.441 or 44.06%, PPV = 61.54%, NPV = 57.69%, accuracy = 0.58 or 58.04%, +LR = 2.03%, -LR = 0.93 (**Table 3**).

The comparison between the Mix test and the ND-O-BSA test in the PB group showed a probability of seropositivity of 26.01, $p < 0.0001$ (95% CI-5.84 $\leq \mu \leq$ 115.78) for the MIX test, a sensitivity of 46.03% and a specificity of 93.75% (**Table 1** and **Table 4**). The comparison between the Mix test and the NT-P-BSA test for the probability of seropositivity for the MIX test was 5.86, $p < 0.0001$ (95% CI - 2.4 $\mu\text{M} \leq$ 14.30), a sensitivity of 46.03% and a specificity of 87.30% (**Table 1**).

Table 3. MIX rapid test performance among patients with paucibacillary leprosy (BP) compared to the performance of the Duplete (disaccharide) test among the Intradomiciliary Consanguineous Contact Group (CCOSI) in the study sample.

Groups	Test MIX		Test disaccharide		OR	p-value
	Positive	Negative	Positive	Negative		
MB	29	34	2	61	^a 12.79	<0.0001
ICSCO	26	54	5	75		
Total	55	88	7	136		

^aOdds ratio (OR) between PB (MIX) \times CCOSI (Disaccharide) = 12.79, $p < 0.0001$, IC95% - 4.55 $\leq \mu \leq$ 35.90. MB Multibacillary; ICSCO Intradomiciliary consanguineous contacts.

Table 4. MIX rapid test performance among patients with multibacillary leprosy (MB) compared to the performance of the Duplete (disaccharide) test among the group of patients with paucibacillary leprosy (PB) in the study sample.

Groups	Test MIX		Test disaccharide		OR	p-value
	Positive	Negative	Positive	Negative		
MB	59	11	36	34	^a 26.55	<0.0001
PB	29	34	2	61		
Total	88	45	38	95		

^aRelative Risk (RR) between MB (MIX) and PB (Disaccharide) = 26.55, $p < 0.0001$, IC95% - 6.76 $\leq \mu \leq$ 104.22. MB Multibacillary; PB Paucibacillary.

The MB group presented an excellent performance using the MIX rapid test compared to the ICSCO group using the ND-O-BSA test with probability of seropositivity of 80.45, $p < 0.0001$ (95% CI - 26.49 $\leq \mu \leq$ 244.32), and using NT-P-BSA test with a seropositivity probability of 27.64, $p < 0.0001$ (95% - 11:51 $\leq \mu \leq$ 66.37) (**Table 2** and **Table 5**). The analysis of the MIX test performance among MB patients compared to the ICSCO group for the ND-O-BSA test showed a sensitivity of 84.29%, specificity of 93.75% and accuracy of 90.00%. In the analysis of the MIX test among MB patients compared to the ICSCO group for the NT-P-BSA test, the probability of seropositivity was 27.64, $p < 0.0001$ (95% CI - 11.51 $\leq \mu \leq$ 66.37), a sensitivity of 84.29%, a specificity of 83.75% and accuracy of 84.00% (**Table 5**).

4. Discussion

Epidemiological studies using immunological and molecular methods have been carried out to improve the understanding of the mechanisms of pathogenicity of *M. leprae* in the host and to minimize the incapacitating effects of leprosy in a socially vulnerable population in countries endemic to this disease [4] [5] [6] [7] [8].

Leprosy is considered to be a multifactorial disease with a possible genetic component in the development of the disease in the host [9] [10] [11]. These candidate genes may modulate the immune response, which may be protective and involve T-helper-1 lymphocytes or ineffective with activation of B lymphocytes and expressive production of anti-PGL-1 antibodies [10] [11] [12]. The challenge to reach the diagnosis in PB patients with a cell-based immune response profile, with low bacillary loads and low production of anti-PGL-1 antibodies, is the development of an immunological test with an improved sensitivity.

Among the PB patients, the MIX rapid test reached a sensitivity of 46.03% and a specificity of 93.75%, while in the ND-O-BSA test the sensitivity was 12.70% and specificity was 93.75%, demonstrating a significantly better performance of the MIX test compared to the ND-O-BSA test ($p < 0.0001$) for the diagnosis of PB patients (**Table 1**). The sensitivity of the MIX test was similar to that of the ML Flow test (40%) [6], which uses the trisaccharide antigen. Moreover, in PB

Table 5. MIX rapid test performance among patients with multibacillary leprosy (MB) compared to the performance of the Duplete (trisaccharide) test among the group of Intradomiciliary Consanguineous Contacts (CCOSI) in the study sample.

Groups	Test MIX		Test trisaccharide		OR	p-value
	Positive	Negative	Positive	Negative		
MB	59	11	56	14	^a 27.64	<0.0001
ICSCO	26	54	13	67		
Total	85	65	69	81		

^aOdds ratio (OR) between MB (MIX) and; ICSCO (Dissaccharide) = 27.64, $p < 0.0001$, IC95% - 11.51 $\leq \mu \leq$ 6.37. MB Multibacillary; PB Paucibacillary; ICSCO Intradomiciliary consanguineous contacts.

patients negative for the ND-O-BSA or NT-P-BSA test alone, it was observed that 25.39% (16/63) were positive in the MIX test, suggesting that the combination of trisaccharide and disaccharide antigens potentiates the performance of the rapid MIX test.

In the analysis of the performance of the MIX test between PB patients compared to the performance of the ND-O-BSA rapid test between PB patients demonstrated OR = 26.01, $p < 0.0001$ (95% CI - $5.84 \leq \mu \leq 15.78$) and a sensitivity of 46.03% and specificity of 96.83%. The NT-P-BSA test resulted in, OR = 5.86, $p < 0.0001$ (95% CI - $2.4 \leq \mu \leq 14.30$), the sensitivity was 46.03% and the specificity was 87.30% (**Table 1**). These data demonstrate that the MIX test had the best performance, which was also better than the NDO-LID-1 rapid test with a specificity of 93.3% and a sensitivity of 73.7% [7]. It is important to emphasize that one of the objectives of this study was to evaluate the performance of the rapid MIX test for the diagnosis of PB patients and the biggest obstacle to this objective is the proportion of patients whose negative test result is 53.97% for the MIX test, 87.30% for the NT-P-BSA test and 53.97 for the ND-O-BSA test, observing that the NT-P-BSA test is the least detectable for patients with PB leprosy. It is possible, that we can reduce this rate using other synthetic antigens combined with trisaccharide and disaccharide for this purpose.

MB patients have a genetic profile for the development of a TH2-type immune response with B lymphocyte activation, large production of anti-PGL-1 antibodies, and invariably positive bacteriological indices, with diagnosis [4] [5] [6] [7] [8]. Thus, the ML Dipstick (disaccharide antigen), [12] [13] ML Flow (Trisaccharide antigen) [6] [8], NDO-LID-1 (recombinant antigen) [7] rapid test will have an excellent performance in this group with a seropositivity rate ranging from 80% - 100%, similar to the one found in this study for the MIX test among MB patients, which was 84.28%.

Among clinically healthy individuals classified as ICSCO group, 32.5% were positive for the MIX test, 16.25% for the trisaccharide double test, and 6.25% for the disaccharide doublet test. An improved performance for the detection of infected individuals in the endemic area was observed for the MIX test. The seropositivity rate detected with our Mix test was much higher than in some previous Brazilian studies [12]. The positivity ranged from 18.4% to 19.7% using the ML Flow test (trisaccharide antigen) to diagnose leprosy patients [12] [13] [14] [15], which is approximately the 16.25% rate found in this study for the double-trisaccharide test.

Accordingly, we demonstrated that the MIX test showed a good performance, with 25.39% of the PB patients negative for the disaccharide and trisaccharide duplet test, but positive for MIX. These data suggest the potential for further optimizing the performance by adding other synthetic antigens to the MIX antigens.

5. Conclusions

The rapid MIX test with combined *M. leprae* antigens proved to be an excellent

tool for the diagnosis and surveillance of leprosy in endemic areas, compared to the rapid double-disaccharide and/or trisaccharide test.

In this study, the need to improve the performance of the MIX test, adding other synthetic antigens was determined. This may reduce the false negative rate and improve the diagnosis of multibacillary leprosy, especially paucibacillary leprosy.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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