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METHODS AND PROTOCOLS

Comparative evaluation of antibody detection tests to facilitate the diagnosis of multibacillary leprosy

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Abstract Despite control efforts, leprosy persists as a significant health concern in many regions. Diagnosis is achieved by a combination of clinical, histopathological, and bacteriological examinations, each of which presents a barrier to expeditious diagnosis, particularly by non-experts. Immunological investigations in research laboratories have clearly indicated that antibody detection tests could aid the diagnosis of leprosy. In this study, we detected circulating antibodies with two rapid diagnostic tests (RDT) involving immunochromatographic lateral flow platforms and one rapid ELISA system. Leprosy patients were identified with a high degree of sensitivity in each assay (over 80 % in all; over 90 % among cases with bacterial indices >1+), although critical differences were observed in specificity. While the specificity of CTK OnSite Leprosy Ab Rapid Test and InBios Leprosy *Detect*™ fast ELISA were high (96.4 and 93.7 % in the general population, respectively), there was a marked reduction in OrangeLife NDO-LID® RDT (only 25.0 %). As anticipated, seropositivity rates were marginally higher in contacts of leprosy patients than in endemic controls. Although we observed a slight drop in test band intensity when blood, rather than serum, was used to develop OnSite Leprosy Ab Rapid Tests, the sensitivity and specificity of these tests was unaffected. When we contrasted test performance with clinical and bacteriological information, we found that RDT and ELISA results positively correlated with the bacteriological index. These data indicate that these assays could be a ready replacement of invasive, insensitive, and time consuming skin slit smear procedures that additionally require expert microscopic examinations. We propose that, due to their speed and point of care applicability, the RDT could be used as an initial entry point to the diagnostic protocols, with confirmation of results attained in a highly quantitative manner following serum transfer to a reference laboratory.

Keywords Leprosy · Diagnosis · Mycobacteria · Serology

Introduction

Leprosy, the clinical consequence of infection with *Mycobacterium leprae*, presents across an extremely diverse range of symptoms with varying severity (Scollard et al. 2006). Either directly through the unrestricted replication of *M. leprae* or indirectly via the granulomatous immune response to the infection, leprosy manifests as peripheral nerve damage presenting as autonomic, sensory, and motor dysfunction. Loss of sensation also means that leprosy patients can unwittingly suffer damage due to ordinary hazards (Cross 2006; Jacob and Franco-Paredes 2008; Visschedijk et al. 2000). Since 1995, based upon the annual reporting of new leprosy cases, the World Health Organization (WHO) has disseminated a cocktail of antibiotics for free of charge multidrug therapy (MDT). The widespread provision of MDT and revised treatment regimens have been major contributors to the massive reduction of registered leprosy cases, from approximately 12 million in 1985 to less than 250,000 in 2012 (WHO 2013). The new case detection rate has stabilized, however, indicating that vigilance is still required.

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When sufficient resources are available, leprosy patients can be stratified into five categories based on histological examination: leprotomatous leprosy (LL), borderline leprotomatous (BL), mid-borderline (BB), borderline tuberculoid (BT), and tuberculoid (TT) (Pardillo et al. 2007). Those who are seeing patients typically have more limited facilities, however, so to simplify diagnosis and the initiation of treatment, the World Health Organization (WHO) guidelines rely on a categorization based on number of skin lesions, bacterial count, and nerve involvement. Patients are categorized as either multibacillary (MB; including LL, BL, BB, and smear positive BT forms) or paucibacillary (PB; including TT and smear negative BT forms); MB patients have either more than five skin lesions or more than one nerve involvement or positive skin smears; while PB patients only have a maximum of five skin lesions, have no or only one nerve involvement and negative skin smears. Immunologically, MB patients skew toward antibody-mediated responses that do not control *M. leprae* replication and present with positive bacterial indices (BI; a measure of the number of acid-fast bacilli in the dermis expressed in a logarithmic scale) while PB patients have cellular responses that restrict bacterial dissemination and present with negative BI.

The current reliance on expert clinical recognition to achieve the correct diagnosis of leprosy presents a bottleneck that can negatively impact control programs. A reduced proportion of clinicians can now recognize leprosy relative to the years preceding widespread MDT provision. The consequence is that many leprosy patients are initially treated for other conditions, and appropriate treatment is delayed. Although difficult to accurately assess, in most regions it takes extended periods of time (often years) from the first recognition of signs or symptoms for a patient to obtain an accurate diagnosis. Indeed, late diagnosis is indicated by reports that approximately 10 % of new leprosy cases registered each year have signs of neuropathy (WHO 2013).

Simple serological tests could enable clinicians with a minor understanding of leprosy to enhance their diagnostic acumen, facilitating informed referral of suspected patients to experts for formal diagnosis and case management. Many studies have demonstrated that phenolic glycolipid (PGL)-I, a membrane component exclusive to *M. leprae*, is readily detected by antibodies in the majority of MB patients (Geluk et al. 2011; Spencer and Brennan 2011; Young and Buchanan 1983). A synthetic mimetic of PGL-I, natural disaccharide epitope (3,6-di-O-methyl-β-D-glucopyranosyl(1 → 4)2,3-di-O-methylrhamnopyranoside (NDO), behaves similarly when conjugated to inert carrier proteins such as albumin (Chatterjee et al. 1986; Spencer and Brennan 2011). Although of limited application for the serological diagnosis of all leprosy clinical forms (most PB patients lack detectable anti-PGL-I antibodies), the detection and measurement of anti-PGL-I antibodies has been suggested as an adjunct tool for a simpler categorization of MB and PB leprosy (Buhrer-Sekula et al. 2000; Buhrer-Sekula et al. 2007). Over the past few years, we and others have extensively examined immune responses against *M. leprae* protein antigens, demonstrating that the detection of antibodies against the LID-1 fusion protein (produced by fusing the *ml0405* and *ml2331* genes) is also an effective diagnostic and prognostic indicator (Duthie et al. 2007; Qiong-Hua et al. 2013; Rada et al. 2012; Spencer et al. 2012). Conjugation of NDO with LID-1 (NDO-LID) combines the diagnostic potential of each component and has recently been integrated into rapid diagnostic formats (Cardoso et al. 2013; de Freitas Mizoguti et al. 2015; Duthie et al. 2014a; Duthie et al. 2014b). In this report, our data indicate that OnSite Leprosy Ab Rapid Test and Leprosy *Detect*™ fast ELISA could be used as replacements of invasive techniques to assist in the confirmation and characterization of leprosy cases.

Materials and methods

Subjects

Volunteers of both sexes and a range of ages were recruited at the Cebu Skin Clinic, the leprosy treatment facility of the Leonard Wood Memorial Center for Leprosy Research (LWM), Mandaue City, Cebu, Philippines, under a study

Table 1 Characteristics of total study population

	Patients	Contacts	General population
Total	147	264	224
Age in years (mean; range)	34 (10–69)	31 (12–71)	36 (12–78)
Sex (M/F)	96/51	155/109	79/145
MB/PB	145/2	–	–
Ridley-Jopling classification	75 LL, 55 BL, 16 BT (1 undefined)	–	–
Lesions (total lesion count; number of patients)	>20 (94), >5 (25), 2–5 (18), <1 (10)	–	–
Bacterial indices	86 3+, 34 1–3, 26 < 1	–	–

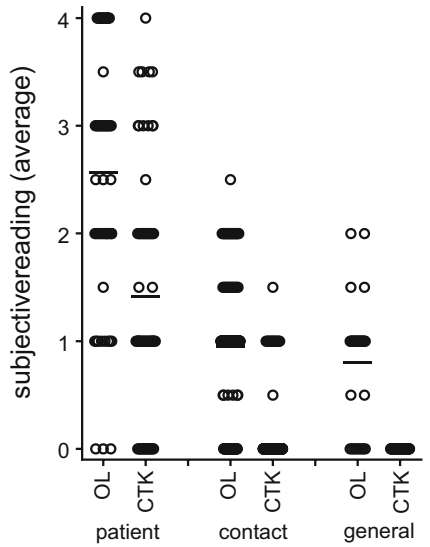

Fig. 1 Comparison of RDT performance. Serum samples from various groups were added to either OrangeLife NDO-LID ® (OL) or OnSite Leprosy Ab Rapid Test (CTK). Each test was scored by two independent readers and *each symbol* represents the highest score attributed to each individual sample. *Horizontal bars* mark the mean signal intensity determined for each group

protocol approved by the local Ethics Committee. Only subjects who signed an informed consent were included. For participants below 18 years of age, the informed consent form was signed by either a parent or legal guardian. Leprosy was diagnosed after a thorough clinical exam to provide preliminary diagnosis as MB or PB, after which each patient was fully characterized by examination of skin slit smear (SSS) and biopsy to permit placement into the more rigorous Ridley-Jopling scale (LL; BL; BB; BT; TT). Patients were recruited in consecutive order based on clinic attendance and with no bias toward clinical presentation. Two groups of control individuals were recruited: (a) healthy household contacts (HHC) of MB patients, enrolled as individuals at elevated risk of developing leprosy (Moet et al. 2006) and (b) endemic controls (EC), categorized as individuals presenting with other skin conditions/diseases. Individuals with active tuberculosis or history of close exposure to tuberculosis and/or HIV infection were excluded.

Samples Blood was collected by venipuncture, and serum prepared by centrifugation, at the time of clinical diagnosis. Sera were also stored at −20 °C until thawed for repeat evaluations or conductance of laboratory testing.

Rapid diagnostic tests Two rapid diagnostic tests (RDT) were evaluated; the NDO-LID® fabricated by OrangeLife (Rio de Janeiro, Brazil) and the OnSite Leprosy Ab Rapid Test fabricated by CTK Biotech (San Diego, CA, USA). Each RDT is a simple immunochromatographic lateral flow

Table 2 Comparative performance of RDT

RDT	Group	Total (*n*)	Subjective score (*n*)[a]					Discordance (*n*)[b]	Positive (*n*)	Positive (%)
			0	1	2	3	4			
OL NDO-LID	Patients	66	3	8	19	18	18	5	63	95.5
	Contacts	209	37	137	34	1	0	18	172	82.3
	General	36	9	23	4	0	0	4	27	75.0
CTK OnSite	Patients	66	15	22	17	7	5	7	51	77.3
	Contacts	209	194	14	1	0	0	2	15	7.2
	General	36	36	0	0	0	0	0	0	0.0

[a] Score assigned by two independent readers, with the highest value assigned in the event of discordance

[b] Number of tests that were scored differently by independent readers

Table 3 Comparative performance of blood and serum in OnSite Leprosy Ab Rapid Test

	Total (*n*)	Positive (*n*)[a]		Discordance[b]	% Positive	
		Blood	Serum		Blood	Serum
Patients	30	25	25	10	83.3	83.3
Contacts	49	3	4	1	6.1	8.2
General	28	0	0	0	0.0	0.0

[a] Score assigned by two independent readers, with the highest value assigned in the event of discordance

[b] Number of tests that were scored differently by independent readers

test with the purpose of detecting circulating IgM antibodies to PGL-I and IgG antibodies specific to LID-1 (the synthetic mimetic conjugated to the recombinant fusion protein product of the *M. leprae* genes *ml*0405 and *ml*2331) (Duthie et al. 2007). Evaluations involved the addition of undiluted serum (5–10 μl) and running buffer (2–3 drops; ~100 μl) to a sample well, followed by readings of line development in the detection window after 15–20 min. Validation of the results required the visualization of a colored control line. A positive result was defined by the staining of both the control band and the test band; development of the control band coincident with no coloration of the test band was considered as a valid negative result. Visual readings were performed by a minimum of two independent readers unaware of the categorization of the sample's source.

Leprosy *detect*™ fast ELISA Serum antibodies against NDO-LID were also assessed in a rapid ELISA system, according to the manufacturer's instructions (Leprosy *Detect*™ fast ELISA; InBios International, Inc., Seattle, WA). Briefly, each serum was diluted 1:100 in Tris-HCl-buffered solution with 0.05 % Tween 20 added to a single well of pre-coated 96-well polystyrene plate. Each plate was quality controlled by the inclusion of two positive controls and two negative controls, with two blank wells included on each plate to provide background readings. After 30 min incubation at 37 °C, each plate was washed with PBS. HRP-conjugated anti-human Ig diluted in Tris-buffered solution was then added, followed by 30 min incubation at 37 °C. After another wash, 3, 3′, 5, 5′-tetramethylbenzidine (TMB) and hydrogen peroxide in a citric-acid citrate buffer (peroxidase color substrate) was added. The colorimetric reaction was quenched after 10 min by the addition of 1N H_2SO_4. The optical density (OD) of each well was then read at 450 nm. To be deemed valid, each plate was required to provide an average OD of the positive controls above 0.5 and an average OD of the negative controls below 0.15. In addition, a discrimination capacity (defined as the mean positive control divided by the mean negative control) greater than 5 was required. To provide inter-assay comparability, values were standardized as test sample OD divided by mean EC OD and expressed as "immune status ratio."

Statistical analyses Graphs, mean values, and linear regression goodness of fit were generated using GraphPad Prism (version 5). Statistical significance was assessed by one-way analysis of variance and Sidak's or Tukey's multiple

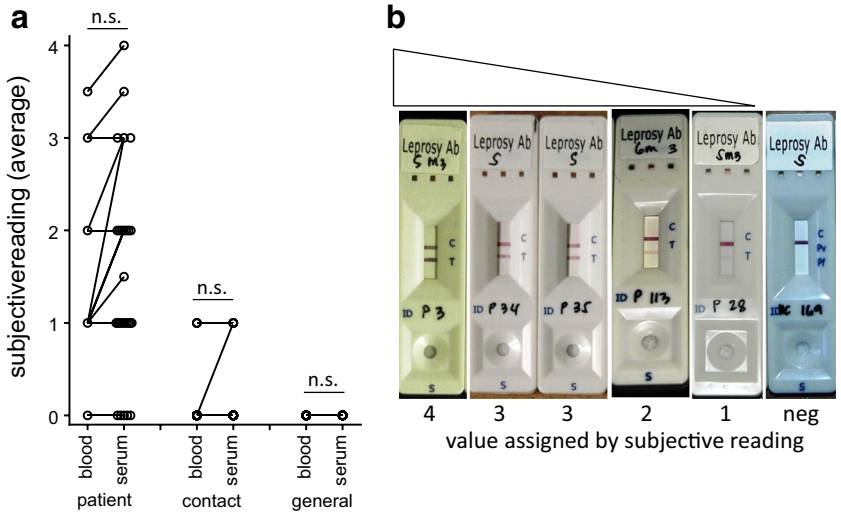

Fig. 2 Comparison of blood and serum reactivity in OnSite Leprosy Ab Rapid Test. Blood and matched serum samples from various groups were added to OnSite Leprosy Ab Rapid Test (CTK), then in **a** each test was scored by two independent readers. *Each symbol* represents the highest score attributed to each individual sample, and the *lines* join the paired blood and serum samples. Statistical significance was assessed by one-way analysis of variance and Sidak's multiple comparison test used to compare the groups indicated by the *bars*, *n.s.* not significant. In **b**, images of tests are shown as representative examples of each subjective scoring group

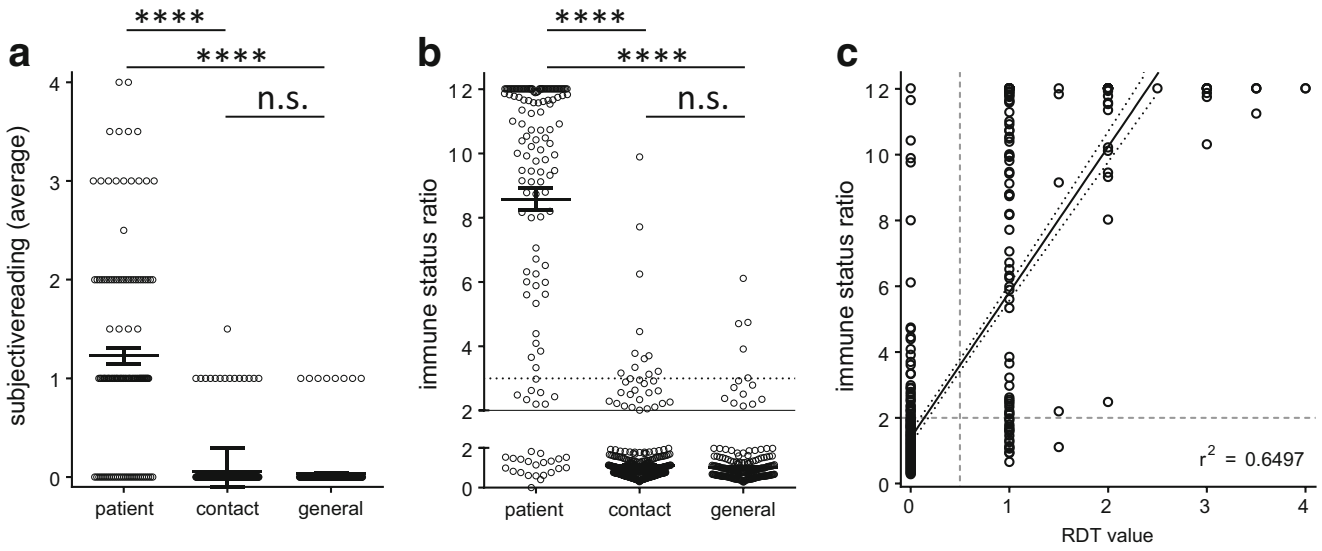

Fig. 3 Comparison of the performance of OnSite Leprosy Ab Rapid Test and Leprosy *Detect*™ fast ELISA. In **a**, an expanded evaluation of serum samples was conducted using OnSite Leprosy Ab Rapid Test. Each test was scored by two independent readers and *each symbol* represents the highest score attributed to each individual sample. In **b**, the same samples were subjected to Leprosy *Detect*™ fast ELISA and the immune status ratio of each is plotted. The *solid horizontal line* marks the immune status ratio threshold of 2 and the *dotted horizontal line* marks the immune status ratio threshold of 3. In each plot, the *horizontal bars* mark the mean of each group, with *error bars* representing standard error in the mean (*SEM*). Statistical significance was assessed by one-way analysis of variance and Tukey's multiple comparison test used to compare the groups indicated by the *bars*, *n.s.* not significant and **** = p value <0.0001. In **c**, results from the tests developed with patient sera are plotted against each other, with *each symbol* representing an individual serum. The *gray hatched lines* represent the threshold for positive reactions with each test, while the *solid line* represents the correlation of results and the *black dotted lines* represent the 95 % confidence intervals. The r^2 value is indicated in the text insert

comparison test used to compare two groups. Results were considered statistically significant when p values <0.05 were obtained.

Results

Patient population Leprosy patients were recruited in consecutive order based on clinic attendance, with the vast majority being clinically characterized as MB in accordance with WHO classification (145 of 147; Table 1). A majority (63.9 %; 94 of 147) presented with more than 20 lesions/patches. When assessed more rigorously by histological and bacteriological measures, 43.5 % (64 of 147) presented with the LL phenotype in accordance with the Ridley-Jopling classification and 58.9 % (64 of 147) had BI over 3. These data indicate that the patients attending the leprosy clinic are already in an advanced state of disease.

Comparison of RDT performance In an initial evaluation, we analyzed sera using a two RDT fabricated on the basis of detecting IgM antibodies against NDO and IgG antibodies against the LID-1 protein. Subjective interpretation indicated that when developed with sera from patients, NDO-LID® tests produced a stronger test band than the test bands observed in CTK Biotech tests

Table 4 Comparative performance of OnSite Leprosy Ab Rapid Test and Leprosy *Detect*™ fast ELISA

		Total (*n*)	Positive (*n*)	% Positive
CTK OnSite Leprosy Ab RDT	Patients	147	118	80.3
	Contacts	264	15	5.7
	General	224	8	3.6
InBios Leprosy *Detect*™ fast ELISA	Patients	147	125	85.0
	Contacts	264	21	8.0
	General	224	14	6.3

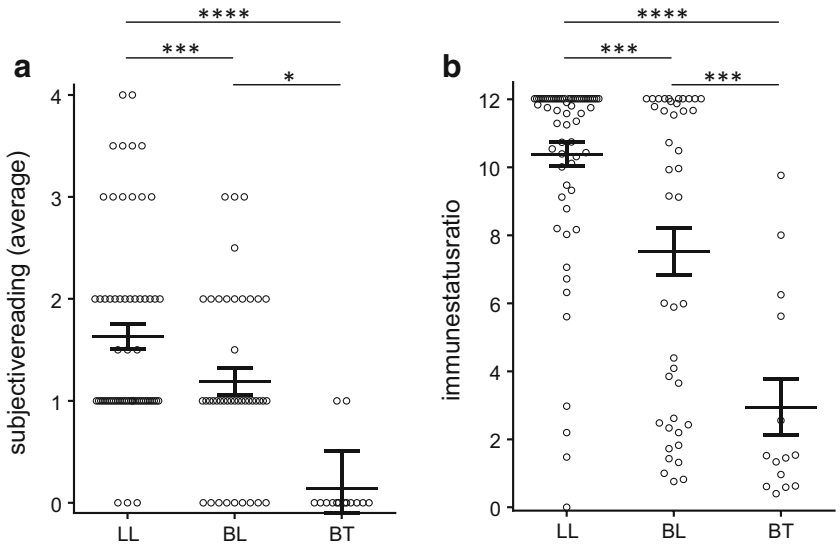

Fig. 4 Relationship of test results and clinical status. Results from patient serum samples evaluated in **a** OnSite Leprosy Ab Rapid Test and **b** Leprosy *Detect*™ fast ELISA are plotted versus clinical status. *Each symbol* represents, in **a**, the highest score attributed to each individual sample in RDT or, in **b**, the immune status ratio determined by ELISA. Statistical significance was assessed by one-way analysis of variance and Tukey's multiple comparison test used to compare the groups indicated by the *bars*, *n.s.* not significant and * = p value <0.05, *** = p value <0.001, and **** = p value <0.0001

developed with the same samples (Fig. 1). Sensitivity for patients in this comparison cohort was found to be 95.5 % (63 of 66) in NDO-LID® versus 77.3 % (51 of 66) in OnSite Leprosy Ab Rapid Test (Table 2). The stronger results with patient samples in the NDO-LID® tests arose, however, at the expense of specificity. When developed with EC sera, 75.0 % (27 of 36) of the NDO-LID returned a positive test band whereas 0.0 % (none) of the OnSite Leprosy Ab Rapid Tests were positive (Table 2). Together, these data indicate that the OnSite Leprosy Ab Rapid Tests are a highly specific tool that can aid the clinical diagnosis of leprosy patients.

Impact of sample type on OnSite Leprosy Ab Rapid Test performance A major advantage of RDT over other tests is the potential to use them, and obtain results, directly at the point-of-care. This asset is enhanced further if tests perform well with whole, unfractionated blood samples such that the need for processing to serum can be removed. We therefore evaluated the performance of OnSite Leprosy Ab Rapid Tests when developed with matched blood and serum samples. Of 30 blood samples that were collected from patients, 25 (83.3 %) tested positive when added to OnSite Leprosy Ab Rapid Tests (Table 3). While 10 matched serum samples returned stronger signals in these RDT than blood, no samples converted from negative to positive (Fig. 2). Most

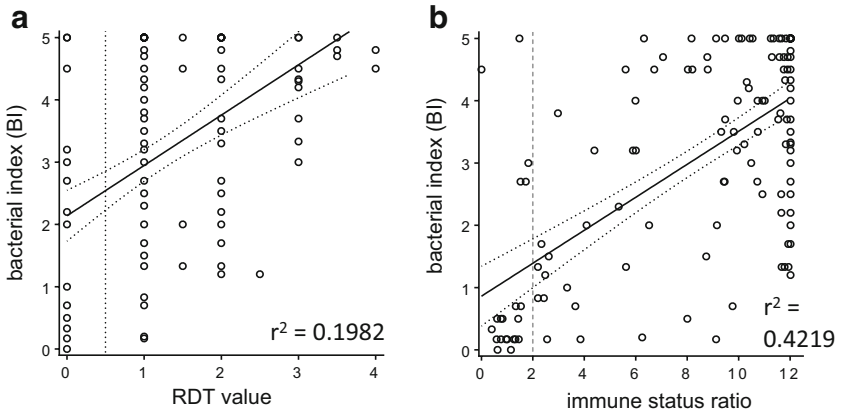

Fig. 5 Relationship of test results and bacterial burden. Results from patient serum samples evaluated in **a** OnSite Leprosy Ab Rapid Test and **b** Leprosy *Detect*™ fast ELISA are plotted versus BI. *Each symbol* represents, in **a**, the highest score attributed to each individual sample in RDT or, in **b**, the immune status ratio determined by ELISA. The *gray hatched lines* represent the threshold for positive reactions with each test, while the *solid line* represents the correlation of results and the *black dotted lines* represent the 95 % confidence intervals

importantly, regardless of test development with either blood or serum, all EC samples tested negative (Fig. 2). Thus, blood can be used in OnSite Leprosy Ab Rapid Test with similar sensitivity, and the same specificity, as sera.

Diagnostic performance of Leprosy *Detect*™ fast ELISA To refine the sensitivity and specificity calculations for OnSite Leprosy Ab Rapid Test, we expanded the analyses of serum to greater numbers of patients and controls (from both the contact and the general populations). Expansion of the evaluations yielded results consistent with the earlier, more limited analyses and indicated good sensitivity (80.3 %) and excellent specificity of the tests (5.7 and 3.6 % positive tests in contacts and the general population, respectively; Fig. 3a and Table 4). We also further evaluated the antibody responses of the same serum samples in Leprosy *Detect*™ fast ELISA. ELISA were marginally more sensitive (85.0 %) but slightly less specific (8.0 and 6.3 % positive tests in contacts and the general population, respectively; Fig. 3b and Table 4). The highly quantifiable nature of the Leprosy *Detect*™ fast ELISA, however, allows potential refinement of the analyses. Indeed, by moving the threshold to an immune status of 3, 79.6 % patient samples were positive versus 2.65 and 2.23 % in contacts and the general population, respectively.

Concordance of tests with infection status We then evaluated test performance across patients with highly defined clinical status and BI at the time of clinical diagnosis. As expected, RDT test bands and immune status ratio were most intense for LL patients and diminished across the Ridley-Jopling scale (Fig. 4). OnSite Leprosy Ab Rapid Tests were positive in 91.7 % (111 of 121), and Leprosy *Detect*™ fast ELISA were positive in 95.9 % (116 of 121) of patients with BI >1, with test results similarly diminished as BI decreased (Fig. 5). Results from both OnSite Leprosy Ab Rapid Test and Leprosy *Detect*™ fast ELISA had highly significant correlation with BI (both $p < 0.0001$; $r^2 = 0.1982$ and 0.4219, respectively). The theoretical intersect of a positive result in RDT occurred at a BI of 2.54, and this was reduced to 1.39 in Leprosy *Detect*™ fast ELISA. These data indicate that ELISA has a greater capacity to detect individuals with lower infection levels.

Discussion

At present, the diagnosis of leprosy is achieved almost entirely by clinical examination. This is not simple, however, and many patients undergo multiple clinical exams, laboratory investigations, and mistreatments before finally reaching experts and receipt of a proper diagnosis (Nicholls et al. 2003; Nicholls et al. 2006). This is because, due to the relative scarcity of the disease, most clinicians lack sufficient training or experience in recognizing and distinguishing the signs and symptoms of leprosy. The elongated period of time taken before receiving proper treatment has two clear and important negative effects: a personal impact because the infection has more chance of causing irreversible damage; and a community impact because it extends the window for potential transmission to others. Improvements to integrate and expedite the diagnostic processes appear necessary, and standardized and simplified tests could be a relatively easy addition that would benefit this. Our data indicate the highly specific nature of both OnSite Leprosy Ab Rapid Test and Leprosy *Detect*™ fast ELISA for the detection/confirmation of leprosy. These tests detected more than 80 % of the leprosy patients attending the leprosy clinic (increased to greater than 90 % sensitivity for patients with BI >1) with the important note that recruitment occurred using an unbiased, unfiltered strategy based simply upon order of appearance at the clinic. It is worth noting that in this particular setting, the vast majority of patients were classified as MB cases. We propose that, due to their speed and point of care applicability, OnSite Leprosy Ab Rapid Test be used as an initial entry point to the diagnostic protocols, with confirmation of results attained in highly quantitative manner by Leprosy *Detect*™ fast ELISA following serum transfer to a reference laboratory.

The lack of material resources and infrastructure in many of the areas in which leprosy is endemic is also an important factor that limits the ability to detect and characterize patients (Deps et al. 2006; Zhang et al. 2009). A major advantage of RDT over other tests is the potential to use them, and obtain results, directly at the point of contact. Although we observed a slight drop in test band intensity, the retention of overall performance characteristics when blood, rather than serum, was used to develop OnSite Leprosy Ab Rapid Test indicates testing could be conducted directly by finger prick, removing the need to collect into a vial for serum preparation. As such, these tests could be used in the office of general practitioners or even in field situations without the need for additional supplies. Considering its high level of sensitivity and specificity, OnSite Leprosy Ab Rapid Test could be especially useful in geographically challenged areas where most health workers are not trained to detect leprosy; detection of mid- and late-stage MB leprosy through this tool is therefore highly important, particularly in field settings hardly reached by clinical experts who can confirm the disease. We therefore propose that blood testing could be conducted for individuals with even the slightest suspicion of having leprosy to serve as a mechanism of triaging for subsequent clinical exams, diagnosis, and case management by leprosy experts.

Downstream of the clinical exam, confirmation of the diagnosis of leprosy is currently achieved by histological and bacteriological assays that require, at a minimum, moderately painful collection of biopsies or SSS, respectively. Both methods are heavily reliant on evaluation by an experienced microscopist and even then SSS are not particularly sensitive

(Banerjee et al. 2011; Bhushan et al. 2008; Contin et al. 2011; Kamal et al. 2010). Furthermore, neither of these support assays can be adapted to allow the high throughput analysis of multiple samples. Our findings support earlier recommendations that these antibody detection assays could be utilized as a ready replacement of SSS procedures, particularly among mid- to late-stage MB patients with BI >1 who were sensitively detected with these antibody detection tools. At 86.6 %, MB cases with more than five lesions (typically presenting as BI >1) were more readily detected than PB and early-stage MB patients who usually have fewer than five lesions (typically presented as BI ≤1) for whom sensitivity in OnSite Leprosy Ab Rapid Test was reduced to 53.6 %. Given the good correlation of BI with the tests evaluated, we propose that blood could be analyzed by RDT to provide immediate objective information and guidance to both the clinician and the patient. Subsequent analysis of serum by Leprosy *Detect*™ fast ELISA in a reference laboratory setting would verify the RDT findings and provide a more refined measurement. Given its high level of specificity (96.4 %), a positive RDT result is likely conclusive of a MB leprosy diagnosis. A negative RDT result does not, however, necessarily exclude PB or early MB leprosy. Diagnosis of these presentations continues to be more difficult and may require additional clinical signs and levels of suspicion alongside the need for further diagnostic work-up such as SSS and histopathology to be confirmed.

In summary, we believe that the antibody detection tests described here, principally the OnSite Leprosy Ab Rapid Test and Leprosy *Detect*™ fast ELISA, have significant potential to detect *M. leprae*-infected individuals, facilitate referral for MB leprosy diagnosis by experts who can then provide appropriate counsel and case management, and serve as replacements of painful and technically prohibitive histological and bacteriological examinations. In the continued effort to limit the spread of *M. leprae* infection and the burden of leprosy, each of these properties represents an improvement over current practice.

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Compliance with ethical standards This article does not contain any studies with animals. All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Conflict of interest MSD provided antigen to companies for fabrication of the tests assessed in this study. All other authors have declared that they have no conflict of interest. On behalf of all authors, I hereby certify that this article contains the original data from our research activities and is for the first time submitted for publication.

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