Leprosy remains an important health problem in a number of regions. Early detection of infection, followed by effective treatment, is critical to reduce disease progression. New sensitive and specific tools for early detection of infection will be a critical component of an effective leprosy elimination campaign. Diagnosis is made by recognizing clinical signs and symptoms, but few clinicians are able to confidently identify these. Simple tests to facilitate referral to leprosy experts are not widely available, and the correct diagnosis of leprosy is often delayed. In this report, we evaluate the performance of a new leprosy serological test (NDO-LID). As expected, the test readily detected clinically confirmed samples from patients with multibacillary (MB) leprosy, and the rate of positive results declined with bacterial burden. NDO-LID detected larger proportions of MB and paucibacillary (PB) leprosy than the alternative, the Standard Diagnostics leprosy test (87.0% versus 81.7% and 32.3% versus 6.5%, respectively), while also demonstrating improved specificity (97.4% versus 90.4%). Coupled with a new cell phone-based test reader platform (Smart Reader), the NDO-LID test provided consistent, objective test interpretation that could facilitate wider use in nonspecialized settings. In addition, results obtained from sera at the time of diagnosis, versus at the end of treatment, indicated that the quantifiable nature of this system can also be used to monitor treatment efficacy. Taken together, these data indicate that the NDO-LID/Smart Reader system can assist in the diagnosis and monitoring of MB leprosy and can detect a significant number of earlier-stage infections.
against PGL-I or the components of LID-1 could lead to improved sensitivity within tests.

In conjunction with OrangeLife, Rio de Janeiro, we have created simple immunochromatographic lateral flow tests with the capacity to detect PGL-I and LID-1-specific antibodies. In this report, we assessed the performance of this new rapid diagnostic test against that of a previously available test using newly acquired and archived serum samples from clinically confirmed leprosy patients in Cebu, Philippines.

**MATERIALS AND METHODS**

**Study site and participants.** Blood samples were collected following local ethics committee approval from Cebu Skin Clinic attendees after they signed informed consent forms. Patients were fully characterized on the Ridley-Jopling scale by slit skin smear and biopsies (4). Healthy household contacts (HHCs) of MB leprosy patients were enrolled as individuals at elevated risk of developing leprosy (29). Individuals presenting with other skin conditions/diseases were also enrolled as endemic controls (ECs). Serum samples from a total of 208 newly diagnosed MB leprosy patients, 62 newly diagnosed PB leprosy patients, 51 healthy household contacts of MB leprosy patients, and 63 endemic controls were evaluated in distinct panels (Tables 1, 2 and 3). Sera were prepared by centrifugation. Following diagnosis, each leprosy patient was provided with a standard multidrug therapy (MDT) regimen as recommended by WHO (for MB leprosy patients, rifampin [600 mg once a month], dapsone [100 mg daily], and ethambutol [600 mg daily] for 6 months).

**Rapid diagnostic tests.** Sera were tested either within 2 h of collection or after thawing following storage at −20°C for up to 6 years. Two rapid diagnostic tests were evaluated: the SD Leprosy test was purchased from Standard Diagnostics (Yongin, South Korea), and NDO-LID was fabricated by OrangeLife (Rio de Janeiro, Brazil). Each is a simple immunochromatographic (lateral flow) test with the purpose of detecting circulating antibodies. The SD Leprosy test detects IgM antibodies to *M. leprae*-specific PGL-I through the use of NDO-bovine serum albumin (NDO-BSA); a synthetic mimetic of PGL-I conjugated to BSA), while NDO-LID detects 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 ML0405 and ML2331 (18)). Evaluations with each rapid diagnostic test involved the addition of undiluted sera ([10 μl] and running buffer (2 to 3 drops; 100 μl) to a sample well, followed by readings of line development in the detection window after 10 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 line and the test line; faint or no staining was considered a negative result. Visual readings were performed by a minimum of two independent readers.

**Objective measurement of NDO-LID.** NDO-LID tests have been adapted such that they can be read electronically by a Smart Reader, an Android-based smartphone rapid test reader platform mechanically attached to the existing camera unit (Fig. 1). This reader collected test images and objectively quantified the signal intensities of the control and test lines in each NDO-LID test. The calculation of Smart Reader cutoff values was based on the receiver operating curve, taking into account the visual results of the tests obtained with a panel of Brazilian MB leprosy patient samples and control samples. Assuming a sensitivity of 87%, as determined by visual readings, the Smart Reader cutoff was calculated as 9.99. For data analysis, the cutoff for positive results by the Smart Reader was therefore considered 10.0. Assuming this cutoff, the sensitivity of the test among the registration cohort of Brazilian MB leprosy patients was 87% (95% confidence interval [CI], 79.2 to 92.7%) and the specificity was 96.1% (95% CI, 91.7 to 98.6%), with an area under the curve (AUC) of 0.96 (standard deviation, 0.01; P < 0.0001) (30).

**Statistical analysis.** Statistical significance was assessed using an unpaired t test for comparison between two groups. Results were considered statistically significant when *P* values of <0.05 were obtained.

## RESULTS

### Comparison of two rapid diagnostic tests for leprosy.

We analyzed sera using both the SD Leprosy test (based on the detection of IgM antibodies against the PGL-I mimetic NDO antigen) and the NDO-LID rapid diagnostic test (based on the detection of IgM antibodies against NDO and IgG antibodies against the LID-1 protein) to permit direct comparisons between these tests. In an initial study, subjective interpretation indicated that when developed with sera from MB leprosy patients, NDO-LID tests produced a significantly stronger band than that observed with SD Leprosy tests (Fig. 2A). This was true for both fresh and frozen samples (P values, 0.011 and 0.003, respectively). Sensitivity for
MB leprosy patient samples in this initial study was found to be 93.8% (45 of 48 samples) with NDO-LID versus 77.1% (37 of 48) for SD Leprosy (Table 1). While previous storage did not affect performance in the NDO-LID tests, the signal intensity of SD Leprosy tests was, surprisingly, lower when freshly prepared sera were added (Fig. 2A). These results were verified against another panel of sera (Fig. 2B, P value of 0.02).

PB leprosy patients have low or absent antibody responses and are not well recognized in rapid diagnostic tests containing only PGL-1 mimetics (14, 16). In agreement, when NDO-LID and SD Leprosy rapid diagnostic tests were developed with PB leprosy patient sera, only a subset of PB leprosy patient samples could be distinguished. A stronger signal was, however, observed with the NDO-LID tests than with the SD Leprosy tests, such that a greater proportion of PB leprosy patient samples were positive (Fig. 2A, P value of <0.0001) (Table 1, 52.6% positive by NDO-LID versus 0.0% by SD Leprosy). Despite returning stronger results with patient samples, the NDO-LID tests were less likely to be positive than SD Leprosy tests when developed with sera from control individuals (Fig. 2A and Table 1, 0.0% positive by NDO-LID versus 25.0% by SD Leprosy). An independent follow-up study using only fresh sera confirmed that the NDO-LID test had a greater band intensity when developed with leprosy patient sera (Fig. 2B) and that a greater proportion of patients could be discriminated (Table 2). Together, these data indicate that the NDO-LID test provides a greater differential of positive and negative results than the SD Leprosy test, with improved discrimination of leprosy patients from healthy individuals.

**Objective and quantitative evaluation by NDO-LID.** Visual interpretation of results is highly subjective and represents an important limitation when performed by personnel lacking expertise, limiting their scope of use. Results from any diagnostic test would ideally also be blinded from the clinical evaluation, but this is difficult to achieve in rural settings with limited resources. To address this deficit, a simple test reader (Smart Reader) can be used to permit objective scrutiny of data following the subjective evaluation of each NDO-LID. While readings on the control line were relatively consistent, a wide range of values were obtained when tests developed with sera from patients with MB leprosy were analyzed (Fig. 3A). The Smart Reader identified an additional 1 and 5 samples as positive, respectively, increasing sensitivity to 95.8% in study A (46 of 48 samples) and 97.5% in study B (39 of 40). When signal intensities were compared, there was a highly significant correlation of readings (Spearman r, 0.967) (Fig. 3A). Repetitive Smart Reader quantification of the same test, and repeat testing of the same sample, yielded highly consistent results (data not shown). In addition, although the manufacturer does not provide guidelines for reevaluation, only a minor but consistent drop in signal intensity was measured when values were reevaluated approximately 1 month later (Spearman r, 0.980) (Fig. 3B). Thus, when coupled with a Smart Reader, NDO-LID tests provide rapid, consistent, robust, and objective quantification of seroreactivity.

**Correlation of rapid diagnostic test results with BI.** We then evaluated rapid diagnostic test performance across sera from patients with MB leprosy identified to have either high (>4.0), medium (2.0 to 3.9), or low (<2.0) BI at the time of clinical diagnosis. As expected, test bands were most intense for the high-BI patients and diminished as BI decreased (Fig. 4A). While both rapid diagnostic tests performed well in detecting MB leprosy patients, the signal intensity was significantly greater in NDO-LID tests than in SD Leprosy tests. NDO-LID tests detected 20% of the PB leprosy serum samples in this testing round versus 10% detected by SD Leprosy tests. More importantly, while the SD Leprosy tests returned positive results for similar proportions of healthy household contacts and endemic controls (12.0 and 12.5%, respectively), the NDO-LID tests improved specificity (2.5% healthy household contacts were identified as being positive, and there were no positive results against endemic controls) (Table 2). The subjective NDO-LID results were corroborated by Smart Reader (Fig. 4B). Thus, the NDO-LID test can readily detect MB leprosy patients, and compared to the SD Leprosy test, it permits improved discrimination of PB leprosy patients from the general population.

**Monitoring treatment by NDO-LID and Smart Reader.** Smart Reader measurements could confer additional utility beyond initial detection and patient classification. To evaluate if the rapid diagnostic test/Smart Reader combination was sensitive enough to monitor treatment, we contrasted results generated using sera collected from patients at the time of diagnosis against sera collected at MDT completion. Overall, there was a reduced signal intensity in the after-treatment samples, with the decline most obvious in sera from patients that had the highest BI at intake (Fig. 4B, P value of 0.003). In these high-BI MB leprosy patients, the mean reading of 46.3 at diagnosis declined to 27.7 after treatment, while in medium-BI MB leprosy patients, the de-
cline was from 27.2 to 20.7, and in low-BI MB leprosy patients, it was from 10.2 to 6.1. These data suggest the utility of continued testing during, and even after, MDT.

DISCUSSION

Leprosy control programs are currently structured around the treatment of cases as they are reported. Case numbers are now relatively low in most regions, however, such that fewer clinicians have experience with the disease and only a limited number can confidently recognize the early signs of leprosy. Diagnosis is therefore commonly delayed, and the appearance of leprosy-associated disabilities may become more frequent (2, 3). By lessening the reliance on the clinical exam and the recognition of symptoms, simple tools like the rapid diagnostic tests evaluated here could address this shortcoming. Tests could greatly aid general practitioners in their evaluation of suspected cases. This would appear particularly prudent in regions where a large proportion of patients present as MB leprosy cases, such as the Philippines (31). Importantly, the ability to objectively read and quantify NDO-LID test results using a Smart Reader eliminates the need for prior training/experience in interpreting test results. This also provides an objective threshold and generates results that are consistent regardless of the many variables that could adversely affect test interpretation (different users, days, times of day, locations, etc.). This is of particular importance considering the possibility of individual bias in subjective reading of rapid tests in field conditions.

FIG 2  Improved performance of NDO-LID over SD Leprosy. In an initial study (A), stored (from MB leprosy patients only, n = 38) or fresh (MB leprosy patients, n = 10; PB leprosy patients, n = 19; and EC, n = 12) sera were tested by either the SD Leprosy or the NDO-LID rapid diagnostic test. The strength of the test band was then subjectively interpreted on a scale of 0 to 4 (negative to strong positive). In a follow-on study (B), fresh sera (MB leprosy patients, n = 40; PB leprosy patients, n = 13; HHC, n = 26; and EC, n = 11) were evaluated in the same manner, with the exception that the scoring scale had a maximum value of 3. Results from one interpreter are shown, and they were verified corroborated by the additional interpreter. Strength of the NDO-LID test and control bands was then objectively measured using the Smart Reader (panel C indicates the objective measurement of the tests that were subjectively assessed in panel A, and panel D depicts the objective measurement of the tests subjectively assessed in panel B). *, P < 0.05; **, P < 0.01; ***, P < 0.001; and ****, P < 0.0001 between the indicated groups. Results in panels A and B are shown as means and standard errors of the means (SEM) for each group; horizontal bars in panels C and D indicate means.
The quantitative information could also be used to monitor individuals suspected of being infected with *M. leprae* over time or to expedite an informed referral to a leprosy expert.

The uptake of previous rapid tests for leprosy was restricted due to the relatively high proportion of seropositive results in the general population in regions of endemicity, despite the fact that the individuals with these results did not display clinical symptoms (15). In any test, the threshold for a positive result is obviously critical to establish test performance, balancing sensitivity against specificity. Our previous results, obtained in the laboratory setting by enzyme-linked immunosorbent assays (ELISA), indicate that LID-1 provides an improved discrimination of leprosy patient samples from those of control subjects (18). This would appear to continue through to the NDO-LID that contains a combination of NDO and LID-1. Our data not only demonstrate the utility of the NDO-LID but also strongly indicate an improved performance, in terms of both sensitivity and specificity, over that of the SD Leprosy rapid diagnostic test.

Consistent with WHO recommendations, Cebu Skin Clinic staff clinically examine contacts of MB leprosy patients at 6-month intervals over the course of 2 years following index case reporting (6). While this system facilitates the earlier recognition of leprosy among these contacts, it is labor-intensive and time-consuming, especially given Cebu’s size. Additional practical and economic considerations (presence during visits, ensuring that work is not impacted, etc.) necessitate vigorous and sustained efforts to ensure that as many individuals can be observed as possible. Because the NDO-LID/Smart Reader is simple and rapid (10 to 20 tests can be conducted by one individual in 30 min), their integration could simplify and enhance this type of monitoring. The duration of any household visit could be markedly reduced, and evaluations could potentially be made at a much greater fre-
quency than that of clinical exams. Any marked increase in test values could trigger a full clinical exam along with regularly scheduled visits. In this regard, strong results in LID-1 laboratory-based ELISA have already been used to draw attention to individuals who have subsequently developed clinical symptoms (24, 27). The levels detected in ELISA that have triggered such attention are readily detected in the NDO-LID test (30). In addition, the robustness/stability of developed tests suggest that if tests perform equally well when using whole blood, they could be sent in advance to patients so that each of their household members could use them at a convenient time proximate to a surveillance visit. The long-term preservation of signal in the tests also suggests that they could even be returned to a central facility for quantitation. By simplifying the referral system, enhancing surveillance programs, or a combination of both, the use of an objective and quantifiable rapid diagnostic test could provide earlier detection and, through prompt treatment, a further reduction in leprosy-associated disabilities.

By lab-based ELISA, we previously identified reductions in patient antigen-specific antibody responses during treatment (24, 32). These subtle changes can be captured by the NDO-LID/Smart Reader combination. In another study, we identified patients who were mistakenly undertreated or who had poor compliance with treatment (28). We hypothesize that, in parallel with clinical examinations, thorough quantification of serological antibody responses by Smart Reader will allow us to capture nonresponse to treatment. Given that truncated treatment regimens are being proposed (33–36), projecting how a patient will respond to treatment without the need for invasive skin slits or biopsies would be an important and practical tool in trial design. Expanding evaluations into the treatment phase could ultimately provide objective guidelines for clinicians to identify high-risk groups requiring additional monitoring, permitting streamlining and prioritization within currently stretched control programs.

In summary, the highly quantifiable nature of the NDO-LID test/Smart Reader platform appears to have utility for detection and monitoring of MB leprosy. We believe it could enhance surveillance, facilitate referrals, and be an important tool in trials of new interventions and treatments.

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Marco Collovati is the owner of OrangeLife, the company producing and marketing the NDO-LID rapid test. Ronaldo Ferreira Dias is an employee of OrangeLife.

REFERENCES


