A Rapid Quantitative Serological Test To Detect Infection with Mycobacterium leprae, the Causative Agent of Leprosy.

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Abbreviations used in this paper:
EC, endemic control
HHC, healthy household contact
LID-1, Leprosy IDRI Diagnostic-1
MB, multibacillary
MDT, multi-drug therapy
NEC, non-endemic control
PB, paucibacillary
PGL-I, phenolic glycolipid-I
TB, tuberculosis

Running title: Rapid serology test for leprosy.

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Abstract

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. Within this report we evaluated the performance of a new leprosy serological test (NDO-LID®). As expected, the test readily detected clinically-confirmed MB patients and the rate of positive results declined with bacterial burden. The NDO-LID® detected larger proportions of MB and PB patients than the alternative SD 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® tests provided consistent, objective test interpretation that could facilitate wider use in non-specialized settings. In addition, results obtained from sera at the time of diagnosis versus 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, as well as detect a significant number of earlier stage infections.
Introduction

Despite advances toward the elimination of leprosy over the last two decades, new case detection rates have stabilized over the last decade and leprosy remains an important health problem in many regions (36). Like the number of registered cases, however, the number of clinicians who can reliably diagnose leprosy has waned, and delays in correct diagnosis are common (6, 30). Efforts to develop and improve surveillance and referral systems appear necessary to achieve the early detection required to ensure that prompt and appropriate treatment can be provided to limit disabilities. The development of simple and practical tools to facilitate diagnosis would appear prudent.

Currently the diagnosis of leprosy is dependent on the appearance and recognition of clinical signs and symptoms. When skin-smear or pathology services are available, leprologists utilize the Ridley-Jopling scale to characterize five forms (27, 28). In practice, however, most field programs lack such services and the clinical system suggested by WHO to classify individual patients and to select their treatment regimen is used (35). The WHO system uses skin lesions, bacterial positivity by skin smear and the number of involved nerves to group leprosy patients into one of two simplified categories; multibacillary (MB) leprosy (typically smear positive or more than one nerve involvement or more than 5 lesions) and paucibacillary (PB) leprosy (smear negative, none to one nerve involvement, maximum of 5 lesions). In many settings, particularly the poorest rural settings where access to experienced leprologists is limited or entirely absent, primary care providers cannot identify the signs of leprosy and patients are commonly misdiagnosed and mistreated (5, 8, 9). With the delays in diagnosis of MB leprosy, transmission of *M. leprae* from infected individuals to their contacts continues and, in many cases, irreversible nerve damage has occurred before they are registered as patients (7, 18, 34). Simple tools that can facilitate leprosy diagnosis could help to address this deficit.

While PB leprosy patients have absent bacterial indices (BI; a measure of the number of acid-fast bacilli in the dermis expressed in a logarithmic scale) and generally have low or absent anti-*M. leprae* antibody responses, MB patients do not control bacterial replication and demonstrate titers of anti-*M. leprae* antibodies that correlate with their burdens (27). Phenolic glycolipid (PGL)-I is well recognized as a target of the antibody response of leprosy patients, with the magnitude of the response strongly correlating with bacterial index (BI) (14). To date, leprosy rapid diagnostic tests have comprised of only PGL-I mimetics as the antigenic target (ML Flow...
and Standard Diagnostic, containing tri- or disaccharides NTP and NDO, respectively)(1-3, 33).

Using laboratory-based assays our group and others have identified numerous native and recombinant proteins recognized by antibodies in MB patient serum (10, 11, 13, 20-22, 31, 32).

One example is the chimeric fusion protein Leprosy IDRI Diagnostic (LID)-1, an antigen specifically recognized by sera from leprosy patients from geographically and ethnically diverse populations with a direct correlation between seroreactivity and BI (10, 15, 24, 25).

Complementary detection of antibodies 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. Within 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 from Cebu Skin Clinic attendees after signing informed consent forms. Patients were fully characterized within the Ridley-Jopling scale by slit skin smear and biopsy (27). Healthy household contacts of MB patients were enrolled as individuals at elevated risk of developing leprosy (17). Individuals presenting with other skin conditions/diseases were also enrolled as endemic controls. Serum samples from a total of 208 newly diagnosed MB patients, 62 newly diagnosed PB patients, 51 healthy household contacts of MB 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 standard MDT regimen as recommended by WHO (MB: Rifampicin: 600 mg once a month; Dapsone: 100 mg daily; Clofazimine: 300 mg once a month and 50 mg daily; for 12 months. PB: Rifampicin: 600 mg once a month; Dapsone: 100 mg daily; for six months).

Rapid diagnostic tests. Sera were either tested within 2 hours of collection or after thawing following storage at -20°C for up to 6 years. Two rapid diagnostic tests were evaluated; SD Leprosy tests were purchased from Standard Diagnostics (Yongin, South Korea) and the NDO-LID® was fabricated by OrangeLife (Rio de Janeiro, Brazil). Each are simple immunochromatographic (lateral flow) tests with the purpose of detecting circulating antibodies. The SD test detects IgM antibodies to *M. leprae*-specific PGL-I, through the use of 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)(10). Evaluations with each rapid diagnostic test involved the addition of undiluted serum (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 10 minutes. 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 as a negative result. Visual readings were performed by a minimum of two independent readers.
Objective measurement of NDO-LID®. NDO-LID® 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 (Figure 1). This reader collected test images and objectively quantified the signal intensity of control and test lines in each NDO-LID® test. The calculation of Smart Reader® cut-off 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® cut-off was calculated as 9.99. For data analysis the cut-off for positive results by Smart Reader® was therefore considered as 10.0. Assuming this cut-off, 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% (CI 95%; 91.7 to 98.6%), with an area under the curve (AUC) of 0.96 (standard deviation, sd 0.01; $p < 0.0001$) (4).

Statistical analysis. Statistical significance was assessed using unpaired t-test for comparison between two groups. Results were considered statistically significant when $p$ values < 0.05 were obtained.
Results

Comparison of two rapid diagnostic tests for leprosy. We analyzed sera using both SD leprosy (based on the detection of IgM antibodies against the PGL-1 mimetic NDO antigen) and NDO-LID® rapid diagnostic tests (based on the detection of IgM antibodies against NDO and IgG antibodies against the LID-1 protein) to permit direct comparison between these tests. In an initial study, subjective interpretation indicated that when developed with sera from MB patients NDO-LID® tests produced a significantly stronger band than that observed in SD Leprosy tests (Figure 2A). This was true for both fresh and frozen samples ($p$-values = 0.011 and 0.003, respectively). Sensitivity for MB patients in this initial study was found to be 93.8% (45 of 48) in NDO-LID® versus 77.1% (37 of 48) in 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 (Figure 2A). These results were verified against another panel of sera (Figure 2B, $p$-value = 0.02).

PB patients have low or absent antibody responses and are not well recognized in rapid diagnostic test containing only PGL-I mimetics (1, 3). In agreement, when NDO-LID® and SD Leprosy rapid diagnostic tests were developed with PB patient sera, only a subset of PB patients could be distinguished. A stronger signal was, however, observed in the NDO-LID® tests than in the SD Leprosy tests such that a greater proportion of PB patients were positive (Figure 2A, $p$-value < 0.0001 and Table 1; 52.6% positive in NDO-LID® versus 0.0% in 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 (Figure 2A and Table 1; 0.0% positive in NDO-LID® versus 25.0% in SD Leprosy). An independent follow-up study using only fresh sera confirmed that NDO-LID® tests had a greater band intensity when developed with leprosy patient sera (Figure 2B) and that a greater proportion of patients could be discriminated (Table 2). Together, these data indicate that the NDO-LID® provide a greater differential of positive and negative results than SD Leprosy tests, improving upon the discrimination of 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 MB sera were analyzed (Figure 3A). The Smart Reader® identified an additional 1 and 5 samples, respectively, increasing sensitivity to 95.8% as positive in study A (46 of 48) 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; Figure 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 out with the test recommendations, only a minor but consistent drop in signal intensity was measured when values were re-evaluated approximately one month later (Spearman r = 0.980; Figure 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 MB patients identified to have either high (> 4.0), medium (2.0-3.9) 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 (Figure 4A). While both rapid diagnostic tests performed well in detecting MB patients, the signal intensity was significantly greater in NDO-LID® tests versus SD Leprosy tests. NDO-LID® tests detected 20% PB patients 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, no positive results against endemic controls) (Table 2). The subjective NDO-LID® results were corroborated by Smart Reader® (Figure 4B). Thus, the
NDO-LID® test can readily detect MB patients and, when compared against the SD Leprosy test, permits improved discrimination of PB 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 (Figure 4B; *p*-value = 0.003). In these high BI MB patients the mean reading of 46.3 at diagnosis declined to 27.7 versus after treatment, while in medium BI MB patients the decline was from 27.2 to 20.7 and in low BI MB 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 can become more frequent (6, 30). By alleviating the pressure on clinical exam and 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 cases, such as the Philippines (19). 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/ day/ time of day/ location etc.). This is of particular importance considering the possibility of individual bias in subjective reading of rapid tests in field conditions. 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 of endemic regions, despite these individuals not displaying clinical symptoms (2). 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 laboratory setting by ELISA, indicate LID-1 provides an improved discrimination of patients from controls (10). 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 the SD Leprosy rapid diagnostic test.

Consistent with WHO recommendations, Cebu Skin Clinic staff clinically examine contacts of MB patients at 6 month intervals over the course of 2 years following index case reporting (35). While this system facilitates the earlier recognition of leprosy within 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.,) necessitates 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 (10-20 tests can be conducted by one individual in 30 minutes), 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 frequency than clinical exams. Any marked increase in test values could trigger full clinical exam out with regularly scheduled visits. In this regard, strong results in LID-1 laboratory-based ELISA have already been used to draw the attention to individuals who have subsequently developed clinical symptoms (24, 31). The levels detected in ELISA that have triggered such attention are readily detected in the NDO-LID® test (4). In addition, the robustness/ stability of developed tests suggest that if tests perform equally well when using whole blood, they could either be sent in advance to patients so that each of their household members could use it at a convenient time proximate to a surveillance visit. The long-term preservation of signal in the tests also suggest that they could even be returned to a central facility for quantitation. Together, through either simplifying the referral system, enhancing surveillance programs or a combination of both, the use of an objective and quantifiable rapid diagnostic tests 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 (12, 31). These subtle changes can be captured by the NDO-LID®/ Smart Reader® combination. In another study we identified patients that were mistakenly under-treated or who had poor compliance with treatment (25). We hypothesize that, in parallel with clinical examinations, thorough quantification of serological antibody responses by Smart Reader® will allow us to capture non-response to treatment. Given that truncated treatment regimen are being proposed (16, 23, 26, 29), 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 within trials of new interventions and treatments.
Acknowledgements

The authors are extremely grateful to the patients and their contacts for generous participation, and would like to thank the field and laboratory staff of Cebu Skin Clinic and Leonard Wood Memorial for their excellent clinical and technical assistance.

Potential Conflict of Interest

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.
Table 1. Initial evaluation panel to determine specificity and sensitivity of rapid diagnostic tests by subjective interpretation.

<table>
<thead>
<tr>
<th></th>
<th>Study A</th>
<th></th>
<th>Study B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>NDO-LID®</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td>MB</td>
<td>48</td>
<td>93.8</td>
<td>77.1</td>
<td>40</td>
</tr>
<tr>
<td>PB</td>
<td>19</td>
<td>52.6</td>
<td>0.0</td>
<td>13</td>
</tr>
<tr>
<td>HHC</td>
<td></td>
<td>0.0</td>
<td>25.0</td>
<td>11</td>
</tr>
<tr>
<td>EC</td>
<td>12</td>
<td>0.0</td>
<td>25.0</td>
<td>26</td>
</tr>
</tbody>
</table>

a Per cent positive tests are shown. Tests were determined as rendering a positive result when a distinct band was observed (scored as 1 or greater).

b The total of 48 samples comprised 38 stored and 10 freshly collected sera. All other samples were freshly collected sera.
Table 2. Specificity and sensitivity of rapid diagnostic tests following development with a secondary serum panel.

<table>
<thead>
<tr>
<th>BI</th>
<th>n</th>
<th>NDO-LID®</th>
<th>SD Leprosy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high</td>
<td>40</td>
<td>100.0</td>
<td>97.5</td>
</tr>
<tr>
<td>medium</td>
<td>40</td>
<td>85.0</td>
<td>90.0</td>
</tr>
<tr>
<td>low</td>
<td>40</td>
<td>65.0</td>
<td>65.0</td>
</tr>
<tr>
<td>PB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>30</td>
<td>20.0</td>
<td>10.0</td>
</tr>
<tr>
<td>HHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>40</td>
<td>2.5</td>
<td>12.0</td>
</tr>
<tr>
<td>EC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>25</td>
<td>0.0</td>
<td>12.5</td>
</tr>
</tbody>
</table>

a Per cent positive tests are shown. Tests were determined as rendering a positive result when a distinct and was observed (scored as 1 or greater).

b low BI = < 2.0 ; medium = 2.0-3.9 ; high = >4.0.
<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>NDO-LID® (n, %)</th>
<th>SD Leprosy (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td>208</td>
<td>181 (87.0)</td>
<td>170 (81.7)</td>
</tr>
<tr>
<td>PB</td>
<td>62</td>
<td>20 (32.3)</td>
<td>4 (6.5)</td>
</tr>
<tr>
<td>HHC</td>
<td>51</td>
<td>2 (3.9)</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td>EC</td>
<td>63</td>
<td>1 (1.6)</td>
<td>6 (9.5)</td>
</tr>
<tr>
<td>(leprosy)</td>
<td>270</td>
<td>201 (74.4)</td>
<td>174 (64.4)</td>
</tr>
<tr>
<td>(not leprosy)</td>
<td>114</td>
<td>3 (2.6)</td>
<td>11 (9.6)</td>
</tr>
</tbody>
</table>

Table 3. Cumulative performance of rapid diagnostic tests following subjective interpretation. Tests were determined as rendering a positive result when a distinct band was observed (scored as 1 or greater).
Figure Legends

Figure 1. Representative images and subjective scoring system for NDO-LID®. Tests were developed and examples of each scoring group that was subjectively assigned are shown. The NDO-LID® have been adapted such that they can be read electronically by a Smart Reader® application, an Android-based cell phone rapid test reader platform mechanically attached to the existing camera unit.

Figure 2. Improved performance of NDO-LID® over SD Leprosy. In an initial study (A), stored (MB only, n = 38) or fresh (MB, n = 10; PB, n = 19; and EC, n = 12) sera were added to either SD Leprosy and NDO-LID® rapid diagnostic tests. The strength of the test band was then subjectively interpreted on a scale of 0-4 (negative to strong positive). In a follow-on study (B), fresh sera (MB, n = 40; PB, 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 were verified/corroborated by the additional interpreter. Strength of the NDO-LID® test and control bands was then objectively measured using the Smart Reader® (C indicates the objective measurement of the tests that were subjectively assessed in A, and D depicts the objective measurement of the tests subjectively assessed in B). \( * = p\)-value < 0.05, \( ** = p\)-value < 0.01, \( *** = p\)-value < 0.001, and \( **** = p\)-value < 0.0001 between the indicated groups.

Figure 3. NDO-LID®/Smart Reader provide a robust system for evaluation. In A, NDO-LID® were developed and the subjectively assigned values plotted versus objective Smart Reader® measurements to determine correlation. In B, Smart Reader® values obtained 10 minutes after test development (initial) are plotted versus values obtained by reading the same tests one month later. The solid line represents the best fit linear regression and the Spearman \( r \) is shown.

Figure 4. Measurement of patient response to treatment by NDO-LID®. Archived sera were selected based upon patient BI at time of collection then evaluated in NDO-LID(R) and SD
Leprosy (MB; high BI, n = 40; medium BI, n = 40 and low BI, n= 40; PB, n = 30; HHC, n = 30 and; EC, n = 25). In A, the strength of the test band in each rapid diagnostic test was subjectively interpreted on a scale of 0-3 (negative to strong positive). Results are shown as mean and SEM for each group. In B, the objective NDO-LID®/ Smart Reader® of sera collected from patients at either time of diagnosis or end of MDT are shown. The values for ‘at diagnosis’ samples were generated from the tests depicted in A, while the same number of ‘after treatment’ samples. * = $p$-value < 0.05 and ** = $p$-value < 0.01 between indicated groups.


Duthie et al., Figure 1

Subjective scores:
- 0
- 0.5
- 1
- 2
- 3

Objective reading:
Duthie et al., Figure 2

B Study B
A Study A

*2
* ** ****

2
3
effective value
0.17
1
-LID
SD
-LID
SD
-LID
SD
-LID
SD

0
1
subj
C ****
 D ****

MB PB HHC EC
NDO-
NDO-
NDO-
NDO-

stored fresh
MB MB PB EC
****
 ***
***
 *
Duthie et al., Figure 3

A

B

objective value

subjective assignment

initial value

re-read value

r = 0.967

r = 0.980