Evaluation of a Rapid Diagnostic Test for Yaws Infection in a Community Surveillance Setting

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Abstract

Yaws is a non-venereal treponemal infection caused by Treponema pallidum ssp. pertenue. The WHO has launched a worldwide control programme, which aims to eradicate yaws by 2020. The development of a rapid diagnostic test (RDT) for serological diagnosis in the isolated communities affected by yaws is a key requirement for the successful implementation of the WHO strategy. We conducted a study to evaluate the utility of the DPP test in screening for yaws, utilizing samples collected as part of a community prevalence survey conducted in the Solomon Islands. 415 serum samples were tested using both traditional syphilis serology (TPPA and quantitative RPR) and the Chembio DPP Syphilis Screen and Confirm RDT. We calculated the sensitivity and specificity of the RDT as compared to gold standard serology. The sensitivity of the RDT against TPPA was 58.5% and the specificity was 97.6%. The sensitivity of the RDT against RPR was 41.7% and the specificity was 95.2%. The sensitivity of the DPP was strongly related to the RPR titre with a sensitivity of 92.0% for an RPR titre of >1/16. Wider access to DPP testing would improve our understanding of worldwide yaws case reporting and the test may play a key role in assessing patients presenting with yaws like lesions in a post-mass drug administration (MDA) setting.


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Introduction

Yaws is a non-venereal treponemal infection caused by Treponema pallidum ssp. pertenue (T. pertenue) [1] which is currently thought to be endemic in fourteen countries [2]. The emergence of azithromycin as an effective oral agent in the treatment of yaws [3] has prompted renewed calls to a coordinated worldwide programme to eradicate the disease by 2020 [4].

Despite this optimism there are significant barriers still to be overcome. The differential diagnosis of yaws can be broad [5] and serological testing is necessary to help establish a diagnosis. As with syphilis, latent infection occurs, and it is recognized that for every clinical case there may be 5–6 individuals with serological evidence of infection but no clinical manifestations [6,7]. Failure to adequately treat latent cases was one of the reasons for the failure of previous attempts to eliminate yaws [8]. Detection and treatment of these latent cases will be extremely important if the WHO eradication programme is to achieve its target.

Traditional syphilis serology includes a treponemal specific test, such as the Treponema pallidum particle agglutination assay (TPPA) or the fluorescent treponemal antibody test, and a non-treponemal test, such as the Rapid Plasma Reagin (RPR) or Venereal Disease Research Laboratory (VDRL) assays. The former tests are highly specific but normally remain positive for life following infection. The latter tests are non-specific but the RPR titre more accurately reflects disease activity and falls following successful treatment. Low-titre false-positive RPRs may occur in a number of conditions including acute viral infections, malaria and connective tissue diseases. Testing therefore requires combined treponemal and non-treponemal assays to give a more accurate diagnostic result.

The development of a rapid diagnostic test (RDT) that can be used to improve access to serological diagnosis in the isolated communities affected by yaws has been highlighted as a major research question [9] to be addressed. RDTs allow wider access to diagnostic testing in remote communities where laboratory
Author Summary

Yaws is a bacterial infection closely related to syphilis. The WHO has launched a worldwide campaign to eradicate yaws by 2020. If this goal is to be achieved, programme managers and clinical staff will need access to a rapid diagnostic test (RDT) for yaws that can be used in the remote communities where the disease is found. In this study, we present data evaluating one possible RDT for yaws as part of a community survey in the Solomon Islands. The test performed reasonably well—there were some false negatives but few false positives. The performance of the test was best in individuals with more active disease suggesting the test may be most appropriately used for confirming clinically diagnosed cases. These findings should prompt consideration of the use of this RDT as part of worldwide yaws control efforts.

Methods

Participant Recruitment

This study was embedded in a larger study investigating the epidemiology of yaws in the Solomon Islands. Briefly, we undertook a survey in Western and Choiseul provinces of the Solomon Islands in September and October 2013. Twenty-five clusters were randomly selected in each province. In each cluster, thirty households were visited, and children aged five to fourteen were enrolled in the study. The study team collected information on yaws symptoms, signs and treatment history. Venepuncture was performed and a serum sample was collected from all participants. Sera were kept on wet ice (4°C) in the field and transferred within 5 days of collection to the National Referral Hospital, Honiara, where they were frozen. Samples were shipped on dry-ice to the London School of Hygiene & Tropical Medicine (LSHTM).

Sample Size

We assumed that the prevalence of yaws sero-positivity by the gold standard assay would be 30%. We therefore calculated that a sample size of 415 was required to be 80% confident that the true sensitivity of the DPP, compared to the gold standard, was 85% or greater [13]. For the purposes of this study, simple random sampling was used to select samples to undergo parallel testing with both the DPP kit and traditional serology.

Laboratory Testing

Sera were tested using both the TPPA (Mast Diagnostics, Merseyside UK) and a quantitative RPR (Deben Diagnostics, Sheffield UK) at LSHTM by an operator masked to clinical findings. A second operator, masked to clinical findings and gold standard serology, tested samples using the DPP test kit. Samples for which the control line did not appear were repeated. The manufacturer’s instructions were followed for all test kits.

Statistical Analysis

The sensitivity, specificity, positive and negative predictive values of the DPP test kit were calculated using traditional serology as the gold standard. The DPP-T1 line was assessed against TPPA and the DPP-T2 line was assessed against RPR. Secondary analyses estimated these performance characteristics by RPR titre and presence or absence of clinical signs of yaws. Confidence intervals were calculated using robust standard errors to allow for clustering. Findings are reported in line with the STARD checklist for studies of diagnostic accuracy [14].

Ethics

Written, informed consent was obtained from the head of each household, who was the parent or guardian of children enrolled in

Table 1. Participant characteristics.

<table>
<thead>
<tr>
<th>Number of children</th>
<th>415</th>
</tr>
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<tbody>
<tr>
<td>Gender: Male(%)</td>
<td>216 (52.1%)</td>
</tr>
<tr>
<td>Age (yrs): Median (IQR)</td>
<td>9 (7–11)</td>
</tr>
<tr>
<td>Clinical signs of primary yaws</td>
<td>12 (2.9%)</td>
</tr>
<tr>
<td>Clinical signs of secondary yaws</td>
<td>7 (1.7%)</td>
</tr>
<tr>
<td>Clinical signs of healed yaws</td>
<td>34 (8.2%)</td>
</tr>
<tr>
<td>Recent treatment for yaws</td>
<td>54 (13.0%)</td>
</tr>
</tbody>
</table>

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Figure 1. Clinical lesions of yaws. a) Primary yaws ulcer. b) Healed yaws lesion. Images credit: Michael Marks. doi:10.1371/journal.pntd.0003156.g001
the study, and assent was obtained from all children. Ethical approval for the study was granted by the ethics committees of the Ministry of Health and Medical Services in the Solomon Islands, and LSHTM in the UK.

## Results

### Participant Characteristics

Four hundred and fifteen samples were randomly selected. The median age was 9, and 52.1% of participants were male. Individuals selected for this study did not differ significantly from the larger prevalence survey population with regards to demographic or clinical features (data not shown). Clinical findings consistent with active and healed yaws were found in 19 (4.7%) and 34 (8.2%) respectively of the 415 participants (Table 1 and Figure 1).

### Laboratory Testing

123 (29.6%) individuals had a reactive TPPA. 120 (28.9%) individuals had a reactive RPR at any titre. By gold standard serology there were 18 individuals with a false positive RPR (defined as a positive RPR and negative TPPA). All false positive RPRs in our study had an RPR titre of 1:2. The overall prevalence of true RPR reactivity was therefore 102/415 (24.6%). The distribution of RPR titres in the study population is given in Figure 2.

79 individuals (19.0%) had a positive T1 (treponemal) line on the DPP kit. 64 (15.4%) individuals had a positive T2 (non-treponemal) line on the DPP kit. There were ten individuals with a positive T2 line but a negative T1 line. The overall prevalence of dual-positivity was therefore 54/415 (13.0%).

The sensitivity of the T1 line against TPPA was 58.5% and the specificity was 97.6%. The positive predictive value was 91.1% and the negative predictive value was 84.5%. The sensitivity of the T2 line against RPR was 41.7% and the specificity was 95.2%. The positive predictive value was 78.1% and the negative predictive value was 79.9%. The sensitivity of combined T1 and T2 against combined TPPA and RPR was 47.1% and the specificity was 98.1%. The sensitivity of the DPP was strongly related to the RPR titre (Tables 2 and 3).

In individuals with clinical signs of active yaws the sensitivity of the T1 line, compared to TPPA, was 81.8% and the specificity was 100%. The sensitivity of the T2, compared to RPR, was 55.6% and the specificity was 83.3%. A similar association was seen between T2 sensitivity and RPR titre as in the overall population (data not shown).

### Discussion

In this study, we found that in a community surveillance setting, the sensitivity and specificity of the DPP rapid diagnostic test were markedly lower than in the only previous evaluation of the assay for use in clinically active yaws [15]. There was a strong association between the sensitivity of both the T2 line (against gold standard RPR) and RPR titre and also between the T1 line (against gold standard TPPA) and RPR titre. This finding might suggest that the apparent reduced sensitivity of the test reflects, at least in part, lower antibody titres in this population (where positive serology predominantly reflected asymptomatic latent cases) compared to populations in which the assay has previously been evaluated (where there were greater numbers of patients with active clinical disease), rather than a difference in test characteristics per se. Despite the reduced sensitivity and specificity the positive predictive values of the test remained relatively high, reflecting the high prevalence of treponemal infection in this endemic setting.

An association between the sensitivity of the T2 line and the RPR titre has previously been noted [12], but an association between RPR titre and T1 sensitivity has not been described before. It is possible that, in keeping with its lower pathogenicity compared to syphilis, yaws elicits less vigorous antibody production than does its venereal cousin, affecting both the non-specific (non-treponemal) and specific (treponemal) components of that response. ‘Attenuated yaws’ has previously been described in the Solomon Islands [16] with less florid clinical manifestations than noted elsewhere. Widespread use of antibiotics with treponemocidal activity has been postulated as one possible explanation for

### Table 2. Test characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Positive by Gold Standard</th>
<th>DPP Sensitivity</th>
<th>DPP Positive Predictive Value</th>
<th>DPP Specificity</th>
<th>DPP Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (Treponemal Line) vs TPPA</td>
<td>123</td>
<td>58.5% (43.4–72.2%)</td>
<td>91.1%</td>
<td>97.6% (95.2–98.8%)</td>
<td>84.5%</td>
</tr>
<tr>
<td>T2 (Non-Treponemal Line) vs RPR</td>
<td>120</td>
<td>41.7% (28.0–56.8%)</td>
<td>95.2%</td>
<td>95.2% (92–95%)</td>
<td>79.9%</td>
</tr>
<tr>
<td>Dual Positive DPP (T1+T2) vs nDual Positive Gold-Standard Serology (TPPA+RPR)</td>
<td>102</td>
<td>47.1% (31.1–63.6%)</td>
<td>88.9%</td>
<td>98.1% (95.2–99.2%)</td>
<td>85.0%</td>
</tr>
</tbody>
</table>

![Distribution of RPR Titres](image-url)
Identification of communities in which such individuals live is vital to allow adequate community targeted treatment to be undertaken [4]. The performance of the test in individuals with clinical evidence of yaws was better than in those without clinical evidence of disease, an association that is likely to be explained by the higher RPR titres found in individuals with clinically active disease. Because of inadequate access to point-of-care diagnostics, many national yaws surveillance systems only report clinically suspected cases. Wider access to DPP testing could allow a larger proportion of these cases to be evaluated serologically, which would both critically refine understanding of global yaws epidemiology as it evolves towards the eradication endpoint, but also provide a vital clinical aid in the post-MDA setting, where many conditions mimicking yaws will continue to present to health-care facilities. Further evaluation of the DPP in other yaws surveillance settings would be welcomed to provide the basis for guideline development.

Supporting Information

Checklist S1  STARD checklist. (DOC)
Dataset S1  Serology and DPP results. (XLS)
Figure S1  STARD flowchart. (TIFF)

Author Contributions
Conceived and designed the experiments: MM AG ZZ DM AWS. Performed the experiments: MM AG. Analyzed the data: OS EP ZZ TD CB DM AWS. Contributed reagents/materials/analysis tools: OS EP. Performed the experiments: MM AG. Analyzed the data: MM AG VV ZZ TD CB DM AWS. Designed and lead the fieldwork: MM VV OS EP ZZ TD CB DM AWS.

References


