Meta-analysis of the performance of a combined treponemal and non-treponemal rapid diagnostic test for syphilis and yaws.

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**BRIEF SUMMARY:**

A combined treponemal and non-treponemal rapid diagnostic test was found to have good sensitivity and specificity for both test syphilis and yaws. The performance of both the treponemal and non-treponemal test components was strongly associated with the RPR titre.

**ABSTRACT:**

Background:

The human treponematoses are important causes of disease. Mother-to-child transmission of syphilis remains a major cause of stillbirth and neonatal death. There are also almost 100,000 cases of endemic treponemal disease reported annually, predominantly yaws. Rapid diagnostic tests would improve access to screening for these diseases. Most RDTs cannot distinguish current and previous infection. The Dual Path Platform Syphilis Screen and Confirm test includes both a treponemal (T1) and non-treponemal (T2) component and may improve the accuracy of diagnosis.

Methods:

We conducted a meta-analysis of published and unpublished evaluations of the DPP Rapid Diagnostic Test (RDT) for the diagnosis of syphilis and yaws. We calculated the sensitivity, specificity and overall agreement of the test compared to reference laboratory tests.

Results:

Nine evaluations including a total of 7,267 tests were included. Sensitivity was higher in patients with higher titre RPR (≥1:16) for both the T1 (98.2% vs 90.1%, p<0.0001) and the T2 component (98.2% vs 80.6%, p<0.0001). Overall agreement between the DPP test and reference serology was 85.2% (84.4-86.1%). Agreement was highest for high titre active infection and lowest for past infection

Conclusions:

The RDT has good sensitivity and specificity of the treponemal and non-treponemal components both in cases of suspected syphilis and yaws, although the sensitivity is decreased at lower antibody titres.

**WORD COUNT: 2,999**

**KEY WORDS:**

Syphilis

Yaws

Sexually transmitted infections

Point of care test

Meta-analysis

**Introduction**

The human treponematoses comprise venereal syphilis and the endemic treponematoses yaws, bejel, and pinta. Syphilis, caused by *Treponema pallidum* subsp. *pallidum,* remains an important cause of both morbidity and mortality. The prevalence of syphilis is known to be particularly high among women attending ante-natal clinics in sub-Saharan Africa[1] and mother-to-child transmission of syphilis remains a major cause of stillbirth and neonatal death worldwide. It has been estimated that mother-to-child transmission of syphilis results in as many as 300,000 stillbirths and neonatal deaths a year in Africa alone[2]. These adverse pregnancy outcomes are entirely preventable through syphilis screening and appropriate treatment.

Yaws is an endemic treponemal infection caused by *Treponema pallidum* subsp. *pertenue*[3]. Although closely related to *T.p.* subsp. *pallidum*, yaws is not sexually transmitted and predominantly affects children living in poor, rural humid communities in the tropics. Untreated yaws progresses to destructive lesions of the bones and soft tissues. Between 2008 and 2012 there were 300,000 cases of yaws reported to the World Health Organization (WHO). In 2012 the WHO launched a global effort to eradicate the disease by 2020[4] and the development of a rapid diagnostic test for yaws has been identified as priority for the eradication programme. As yaws is serologically indistinguishable from syphilis [5], tests developed for syphilis may also be of value in the diagnosis of yaws.

Diagnosis of treponemal infections is based on serological tests that are classified as treponemal specific, such as the *Treponema pallidum* particle agglutination assay (TPPA) *Treponema pallidum* haemagglutination assay (TPHA) , enzyme-linked immmunosorbent assay (ELISA), enzyme immunoassay (EIA) or the fluorescent treponemal antibody test, or non-treponemal, such as the Venereal Disease Research Laboratory (VDRL) or the Rapid Plasma Reagin (RPR) assay. Treponemal tests are highly specific but frequently remain positive for life following infection, regardless of treatment or natural clearance. Non-treponemal tests are less-specific but reflect active disease more accurately, although positive non-treponemal results may also be seen in serofast patients Diagnosis of treponemal infections is generally based on a combination of both types of test as well as clinical findings and history.

Rapid diagnostic tests (RDT) for treponemal infections are a relatively recent development, which allow wider access to diagnostic testing, particularly for communities where routine laboratory facilities are not available. RDTs facilitate improved screening, diagnosis and treatment of syphilis in women presenting to antenatal clinics in low resource settings[6]and reduce the morbidity and mortality associated with mother-to-child transmission of syphilis. For yaws, a rapid diagnostic test would be of value due to the low positive predictive value of clinical diagnosis alone[7,8]. Validation and roll out of a rapid diagnostic test would lead to improved epidemiological data on yaws worldwide, which is a priority in facilitating eradication [4,9].

 A major limitation to most treponemal RDTs is that they are based on detection of treponemal specific antibodies, and therefore cannot distinguish between current and past infection. Resulting false positives lead to over-treatment of syphilis, as well as problems in interpreting epidemiological data for both syphilis and yaws. The Dual Path Platform (DPP-RDT) Syphilis Screen and Confirm test kit (Chembio, Medford, NY, USA) is the first commercial RDT to give both a “treponemal” result and a “non-treponemal” result[10] and can therefore assist in distinguishing between current and past infection, which may make it a more useful test in clinical practice. The kit is a lateral flow assay detecting both IgM and IgG antibodies against a recombinant *T.pallidum* antigen and a non-treponemal antigen. Several recent publications have reported on the performance of this assay and have reported good test performance, although variations have been noted in both the sensitivity and the specificity of the non-treponemal component of the test in particular. To provide more accurate estimates of performance we conducted an individual patient-level meta-analysis on the performance of the DPP-RDT for the diagnosis of both syphilis and yaws.

**Methods**

This review and meta-analysis utilized the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines[11].

Search strategy

We searched Pubmed to 1 August 2015 using the terms “Rapid Diagnostic Test” OR “Point of Care Test” AND “Syphilis” OR “Yaws” (and variations). We searched reference lists of identified articles and contacted individuals and research groups known to have undertaken unpublished evaluation studies to identify other relevant datasets.

Inclusion criteria

A paper was included if it evaluated the sensitivity and specificity of the Chembio Syphilis Screen & Confirm Rapid Diagnostic Test to detect syphilis or yaws. Laboratory, clinic and field based studies sampling a consecutive series of patients, or randomly selected series of patients were eligible. Non-English language publications were eligible for inclusion.

Exclusion criteria

Papers were excluded if they did not contain primary data (e.g. editorials, reviews or commentaries); or referred to conference proceedings and were not accompanied by a full description of the research.

Data extraction and management

The first author screened all titles and abstracts and the full text was obtained for any potentially relevant articles. Full-text articles were reviewed to determine whether they met the inclusion criteria and where this was uncertain, articles were reviewed by a co-author and disagreements were resolved by discussion. Data were initially extracted by the first author and double-checked by the co-authors.

For each study we extracted data on the sample type used for the DPP-RDT and the reference treponemal and non-treponemal assay used. For each individual we recorded data on the presence and stage of clinical disease, the result of the reference treponemal and non-treponemal assays and the result of the DPP-RDT.

Sample types were classified as serum, plasma, whole-blood or finger-prick. RDT results are dichotomous; reference RPR results were deemed positive at a titre ≥ 1:1; an RPR titre ≥ 1:16 was considered a high-titre RPR.

Statistical Analysis

We estimated the sensitivity and specificity of the DPP-RDT, by comparing the performance of the DPP treponemal result to reference treponemal serology, and comparing the performance of the DPP non-treponemal result to reference non-treponemal serology. Exact confidence intervals were calculated for each of these estimates using the binomial distribution. The inverse variance was used to weight each study in the meta-analysis. We calculated the I2 statistic to quantify study variability, and sensitivities and specificities were compared between pre-specified subgroups including the RPR titre, disease, sample type and clinical disease stage. We conducted multivariable logistic regression to assess test variables including disease, RPR titre, sample type and reference treponemal test that were significantly associated with test performance.

We assessed the overall performance of the DPP-RDT in detecting categories of infection. We classified the outcome of reference serology as active infection (treponemal and non-treponemal test positive), past infection (treponemal test positive and non-treponemal test negative) and no history of infection (both tests negative). We classified the outcome of the DPP-RDT correspondingly, as defining active infection (both elements positive), past infection (only the treponemal element positive) and no previous infection (both negative). We calculated the overall agreement between the outcome of the DPP –RDT and the reference serology. All analyses were conducted in STATA 13.1 (Statacorp, Texas).

Ethics

The study was approved by the ethics committee of the London School of Hygiene & Tropical Medicine, UK (LSHTM Ref. 8908).

**Results**

One hundred and eighty five articles were identified as meeting the search criteria and ten studies met the inclusion criteria[10,12–18]. Of these, two studies reported data on the same set of patients; one study evaluated the treponemal component of four RDTs including the DPP-RDT[18], the second study evaluated both the treponemal and non-treponemal component of the DPP-RDT and was included in this analysis[13]. Two further unpublished studies were identified by contacting groups known to have undertaken evaluations of the DPP-RDT (Figure 1).

Overall, individual-level data from a total of nine studies involving 7267 test results were therefore included in the meta-analysis. In all included studies the individual conducting the RDT was reported to be blinded to the results of the reference serological tests. The countries and characteristics of the included studies are shown in Table 1. In one study[10] individual level data were only available for 61.8% of the full dataset. The performance of the DPP test did not differ between the full dataset and the individual level data that were available (data not shown).

Of patients included in the study 5,656 (77.8%) underwent treponemal testing for suspected syphilis and 1,611 for suspected yaws. Individual level clinical data on the presence of absence of disease status were available for 2,636 patients (36.3%). Of patients for whom clinical data were available 1,417 (53.8%) had clinical evidence of either active syphilis or active yaws at the time of testing and 1,219 (46.2%) had no clinical evidence of active disease. The reference treponemal test was positive in 4,075 individuals (56.1%) and the reference non-treponemal test was positive in 3,112 (42.8%).

There was significant heterogeneity across the studies in the sensitivity of both the treponemal (I2 95.70%, p<0.01) and non-treponemal components (I2 96.70%, p<0.01) (Figure 2). When the results were restricted to high-titre samples the heterogeneity was no longer statistically significant for either treponemal (I2 26.13%, p=0.21) or non-treponemal (I2 26.13%, p=0.79) components (Supplementary Figure 1). There was also heterogeneity in the specificity of the treponemal component(I2 78.98%, p <0.01,) and the non-treponemal component (I2 97.59%, p<0.01) (Figure 3). As there was significant heterogeneity between studies an overall pooled summary estimate of the sensitivity and specificity of the RDT across the full dataset is not reported. Sensitivity was higher for specimens from patients with a high titre RPR (≥1:16) (n= 1,351) compared to specimens with a lower RPR titre (<1:16) for both the treponemal component (98.2% vs 90.1%, p<0.0001) and the non-treponemal component (98.2% vs 80.6%, p<0.0001) (Table 2). If all RPR positive samples are considered positive, the specificity of the treponemal component was 98.0% and the specificity of the non-treponemal component was 89.4%. If only samples with a high-titre RPR (≥1:16) are considered positive, the specificity of the treponemal component was 91.2% (Table 2).

The sensitivities of both test components were higher in patients with syphilis than in patients with yaws at low titres but not at high-titres. For high-titre specimens the sensitivity of the treponemal component was 98.4% and 97.6% for syphilis and yaws respectively and the sensitivity of the non-treponemal components were 98.7% and 96.6% respectively. For low titre specimens the sensitivity of the treponemal component was (93.1% for syphilis compared to 73.5% for yaws and the sensitivity of the non-treponemal component was 85.0% for syphilis compared to 59.1% yaws (p<0.0001 in both cases). The specificity of the treponemal component was slightly higher in patients tested for syphilis compared to yaws for low-titre specimens whilst the converse was true for the non-treponemal component(Table 3).

There were only minor differences in the performance of the test based on the specimen type used (Supplementary Table 1). The sensitivity and specificity of the treponemal component varied depending on the reference treponemal assay used (Supplementary Table 2). As compared to when TPPA was used as the reference treponemal test, the sensitivity of the treponemal component was lower when the reference test was an ELISA (p<0.001) and the specificity was lower when TPHA was taken as the reference standard. The sensitivity of both the treponemal and non-treponemal components was higher in individuals with evidence of clinical disease than in asymptomatic cases (Supplementary Table 3).

In multivariable logistic regression a higher RPR titre was significantly associated with an increased sensitivity of both the treponemal and the non-treponemal components (P <0.001) after controlling for other variables. After controlling for other variables the sensitivity was lower for both test components in individuals being tested for yaws than in those tested for syphilis, and when the test was performed on serum (p<0.001 in both cases). After controlling for other variables the specificity of the treponemal component was higher when the reference standard was either TPPA or TPHA as compared to EIA or ELISA (p <0.0001 in both cases). The specificity of the non-treponemal component was significantly associated with the RPR titre after controlling for other variables (p<0.023).

Overall agreement between the DPP test and reference serology was 85.2% (84.4-86.1%). Agreement was highest for high titre active infection and lowest for past infection (Table 4). The lack of agreement in this group was due to misclassification of both treponemal components and non-treponemal components (data not shown).

**Discussion**

This meta-analysis has combined data collected from more than 7200 patients to evaluate the performance of the combined treponemal/non-treponemal RDT for the diagnosis of syphilis and yaws. The use of individual patient data from a large number of samples has allowed us to explore which factors are independently associated with test performance. We have shown that the DPP Syphilis Screen & Confirm RDT has good sensitivity and specificity compared to reference serology in cases of both suspected syphilis and yaws. As previously reported in one study, the sensitivity of the non-treponemal component of the RDT is related to the patient’s RPR titre[15], with significantly higher sensitivity of the DPP kit for high-titre RPRs. We have demonstrated that the sensitivity of the treponemal component is also related to the RPR titre. This finding was true even after controlling for other relevant variables such as the sample type, disease and reference test used. As a result the DPP-RDT showed excellent overall sensitivity for high-titre infections (RPR ≥1:16) (97.5%) but lower sensitivity for low-titre active infections (81.0%). Although treponemal tests are commonly reported as either positive or negative, quantitative testing is possible and titres to certain treponemal antigens decline following treatment[19] and it is likely that these findings reflect an overall reduced sensitivity of the RDT at lower antibody titres. As a result some low titre positive patients may be missed when using the DPP-RDT. As the DPP-RDT is designed to be used as a point-of-care test the lower sensitivity noted when testing serum samples may be of less clinical relevance, but clinicians should be aware of this when considering how to roll-out rapid diagnostic testing.

Improved screening of women attending antenatal care for syphilis is a priority intervention to reduce the mortality and morbidity associated with mother-to-child transmission of syphilis. Adverse pregnancy outcomes due to syphilis are almost all seen in mothers with an RPR titre ≥RPR in the mother[20], and our findings confirm that the DPP RDT has a high sensitivity in this group (97.6%). Adoption of the DPP assay as the basis for treatment decisions would therefore be likely to detect a high proportion of active infections. Conversely, it would reduce the number of women having unnecessary treatment, as around half of those with past, treated infection would show up as negative on the test, compared to positive on a standard treponemal only RDT. This study was not designed to assess the cost-effectiveness of a combined treponemal and non-treponemal RDT, but other studies have shown that this is highly dependent on both the prevalence of disease and the cost of the RDT[21]. A number of RDTs used for antental settings also now combine a treponemal test and an HIV test[22] and the decision about the correct RDT selection and testing strategy is likely to vary between countries, depending on the prevalence of syphilis, yaws and HIV and the cost and availability of the RDTs.

Clinical diagnosis alone of yaws does not have a high predictive value and the addition of a rapid diagnostic test would be a significant advantage. In this meta-analysis the DPP-RDT performed better in patients with suspected syphilis than in those with suspected yaws. This finding was explained predominantly by the lower sensitivity in yaws patients with low titres. Although lower titres are often found in patients with yaws compared to those with syphilis[23], it is unclear why the test performance should be worse in patients with yaws when controlling for antibody titre. These findings suggest that the DPP-RDT may be adequate in pre-mass treatment campaigns when there are many active cases with high titre disease but that, as the number of active cases declines, it may be necessary to utilise a different testing strategy, either using repeated testing or adoption of an alternative test with a higher sensitivity for low-titre disease.

A limitation of our study was that the full individual clinical dataset was not available for one study[10]. The missing data represents less than 10% of the total set included in this study and the reported results from the full dataset do not differ significantly from the subset used in this meta-analysis, so it seems unlikely that the missing data would substantially alter our findings. All the data analysed here were collected as part of research studies and it is recognised that test performance may not be as good in a real world setting in the hands of the end users (healthcare workers), and that the utilisation of test results may be influenced by factors other than simply the result[24]. Training and support to healthcare workers has been shown to significantly improve utilisation of RDTs in other areas[25] and should be a key component of the roll out of RDTs for syphilis and yaws

This meta-analysis demonstrates that the DPP-RDT has both a high sensitivity and specificity for both treponemal and non-treponemal antibodies. Our study includes a large number of patients enrolled in studies from many countries around the world being tested for syphilis or yaws. Our large sample size allows us to provide the most accurate estimates published to date of the test performance across a range of sub-groups. The major limitation of the DPP-RDT is the reduced sensitivity of the test for low titre disease. As RPR titres tend to be higher in patients with syphilis than in patients with yaws, this reduced sensitivity is likely to be a greater problem when using the test as part of yaws eradication efforts, especially as a high sensitivity assay will be needed to ensure all cases are detected to confirm the final eradication status of the infection. Combined treponemal / non-treponemal assays offer a number of advantages over treponemal only RDTs. Our data provide evidence to support the decision to use the DPP-RDT as one such assay.

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**Conflict of Interest**

The authors confirm that they have no relevant conflict of interest to declare.

**Author Contributions**

MM, OM, CB, RB, DM designed the study, conducted the analysis and drafted the manuscript. LC, RG, JG, LB, RC, FP, RW, AC, YT, AA, FT, YPY and XSC contributed to the analysis and revised the manuscript.

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| **Table 1** | **Study Characteristics** |  |  |  |  |  |
| **Study** | **Study Site** | **Disease** | **Reference Treponemal Test** | **Reference Non-Treponemal Test** | **Sample Type** | **Sample Size** | **Year of Publication** |
| Ayove [16] | Papua New Guinea | Yaws | TPHA | RPR | Finger-Prick | 199 | 2014 |
| Plasma | 504 |
| Aziz | Ghana | Yaws | TPPA | RPR | Finger-Prick | 255 | Unpublished Data |
| Castro [10] | United States | Syphilis | TPPA | RPR | Serum | 1168\* | 2010 |
| Castro [12] | Portugal | Syphilis | TPHA | RPR | Serum | 248 | 2014 |
| Causer [13] | Australia | Syphilis | ELISA/IA | RPR | Serum | 1,005 | 2015 |
| Guinard [17] | France | Syphilis | ELISA/IA | RPR | Serum | 100 | 2013 |
| Marks [15] | Solomon Islands | Yaws | TPPA | RPR | Serum | 415 | 2014 |
| Taleo | Vanuatu | Yaws | TPPA | RPR | Finger-Prick | 238 | Unpublished Data |
| Yin [14] | China | Syphilis | TPPA | TRUST | Plasma | 1,323 | 2013 |
| Whole-Blood | 1,324 |
| Finger-Prick | 488 |

\*The published study size was 1,889 but individual level data was only available for 1,168 tests

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| --- | --- | --- | --- | --- |
| **Table 2 :** | **Overall Sensitivity and Specificity of the DPP RDT by RPR Titre**  |  |  |  |
|  | **n** | **Reference Test Positive** | **DPP Positive** | **Sensitivity (95% CI)** | **Reference Test Negative** | **DPP Negative** | **Specificity (95% CI)** |
| RPR <1:16 |   |   |   |   |   |   |   |
| Treponemal Test | 5916 | 2736 | 2464 | 90.1% (88.9-91.2%) | 3180 | 3115 | 98.0% (97.4-98.4%) |
| Non-Treponemal Test | 5916 | 1761 | 1419 | 80.6% (78.7-82.4%) | 4155 | 3714 | 89.4% (88.4-90.3% |
|   |   |   |   |   |   |   |   |
| RPR ≥1:16 |   |   |   |   |   |   |   |
| Treponemal Test | 1351 | 1339 | 1315 | 98.2% (97.3-98.8%) | 12 | 11 | 91.2% (61.5-99.8%) |
| Non-Treponemal Test | 1351 | 1351 | 1327 | 98.2% (97.4-98.9%) |   |  |   |

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| **Table 3: Sensitivity and Specificity of the DPP-RDT Stratified by Disease and RPR Titre** |
| **Disease** | **n** | **Reference Test Positive** | **DPP Positive** | **Sensitivity (95% CI)** | **Reference Test Negative** | **DPP Negative** | **­­­Specificity (95% CI)** |
| *Syphilis* |   |   |   |   |   |   |   |
| RPR <1:16 |   |   |   |   |   |   |   |
| Treponemal Test | 4600 | 2310 | 2151 | 93.1% (92.0-94.1%) | 2290 | 2256 | 98.5% (97.9-99.0%) |
| Non-Treponemal Test | 4600 | 1460 | 1241 | 85.0% (83.1-86.8%) | 3140 | 2750 | 87.6% (86.4-88.7%) |
|   |   |   |   |   |   |   |   |
| RPR ≥1:16 |   |   |   |   |   |   |   |
| Treponemal Test | 1056 | 1049 | 1032 | 98.4% (97.4-99.1%) | 7 | 7 | 100% (59-100%) |
| Non-Treponemal Test | 1056 | 1056 | 1042 | 98.7% (97.8-99.3%) |   |   |   |
|   |   |   |   |   |   |   |   |
| *Yaws* |   |   |   |   |   |   |   |
| RPR <1:16 |   |   |   |   |   |   |   |
| Treponemal Test | 1316 | 426 | 313 | 73.5% (69.0-77.6%) | 890 | 859 | 96.5% (95.1-97.6%) |
| Non-Treponemal Test | 1316 | 301 | 178 | 59.1% (53.3-64.7%) | 1015 | 964 | 95.0% (93.4-96.2%) |
|   |   |   |   |   |   |   |   |
| RPR ≥1:16 |   |   |   |   |   |   |   |
| Treponemal Test | 295 | 290 | 283 | 97.6% (95.1-99.0%) | 5 | 4 | 80.0% (28.4-99.5%) |
| Non-Treponemal Test | 295 | 295 | 285 | 96.6% (93.8-98.4%) |   |   |   |

|  |  |
| --- | --- |
| **Table 4** | **Agreement of the DPP Test Kit classification relative to Reference Serology** |
| **Serological Classification** | **Reference Test Classification** | **DPP Test Classification** | **Agreement (95% CI)** |
| Active | 3021 | 2668 | 88.3% (87.1-89.4%) |
| Active (high titre - ≥ 1:16) | 1339 | 1306 | 97.5% (96.6-98.3%) |
| Active (low titre <1:16) | 1682 | 1362 | 81.0% (79.1-82.8%) |
| Past Infection | 1054 | 570 | 54.1% (51.1-57.1%) |
| No Infection | 3101 | 2938 | 94.7% (93.9-95.6%) |
| False Positive RPR\* | 91 | 19 | 20.9% (13.1-30.7%) |

\* An RPR was considered a false positive if the treponemal test was negative. This may be overly conservative as the RPR may become positive before the treponemal test. This definition would not effect our estimate of the agreement between the DPP test and the reference test in this scenario.

Figure Legends

Figure 1: Search Results

Figure 2: Forest Plot of Sensitivity of the T1 and T2 components in comparison to reference treponemal and non-treponemal assays.

Figure 3: Forest Plot of Specificity of the T1 and T2 components in comparison to reference treponemal and non-treponemal assays