OPEN ACCESS

Improved rapid diagnostic tests to detect syphilis and yaws: a systematic review and meta-analysis

Ying Zhang ,[1] Su Mei Goh,[2] Maeve B Mello,[3] Rachel C Baggaley,[3] Teodora Wi,[3] Cheryl C Johnson,[3] Kingsley B Asiedu,[3] Michael Marks ,[4,5,6] Minh D Pham ,[7,8] Christopher K Fairley ,[2,9] Eric P F Chow ,[2,9,10] Oriol Mitjà,[11] Igor Toskin,[12] Ronald C Ballard,[12] Jason J Ong [2,4,9]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/sextrans-2022-055546).

For numbered affiliations see end of article.

Correspondence to
Dr Jason J Ong, Melbourne Sexual Health Centre, Carlton 3053, Victoria, Australia; jason.ong@monash.edu

YZ and SMG are joint first authors.

Received 9 June 2022
Accepted 16 August 2022

ABSTRACT

Background Current rapid tests for syphilis and yaws can detect treponemal and non-treponemal antibodies. We aimed to critically appraise the literature for rapid diagnostic tests (RDTs) which can better distinguish an active infection of syphilis or yaws.

Methods We conducted a systematic review and meta-analysis, searching five databases between January 2010 and October 2021 (with an update in July 2022). A generalised linear mixed model was used to conduct a bivariate meta-analysis for the pooled sensitivity and specificity. Heterogeneity was assessed using the I^2 statistic. We used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) to assess the risk of bias and Grading of Recommendations, Assessment, Development and Evaluations (GRADE) to evaluate the certainty of evidence.

Results We included 17 studies for meta-analyses. For syphilis, the pooled sensitivity and specificity of the treponemal component were 0.93 (95% CI: 0.86 to 0.97) and 0.98 (95% CI: 0.96 to 0.99), respectively. For the non-treponemal component, the pooled sensitivity and specificity were 0.90 (95% CI: 0.82 to 0.95) and 0.97 (95% CI: 0.92 to 0.99), respectively. For yaws, the pooled sensitivity and specificity of the treponemal component were 0.86 (95% CI: 0.66 to 0.95) and 0.97 (95% CI: 0.94 to 0.99), respectively. For the non-treponemal component, the pooled sensitivity and specificity were 0.80 (95% CI: 0.55 to 0.93) and 0.96 (95% CI: 0.92 to 0.98), respectively.

Conclusions RDTs that can differentiate between active and previously treated infections could optimise management by providing same-day treatment and reducing unnecessary treatment.

PROSPERO registration number CRD42021279587.

KEY MESSAGE

We systematically reviewed the performance characteristics and clinical utility of rapid diagnostic tests (RDT) for syphilis and yaws. We report a slightly lower sensitivity, but very high specificity compared with laboratory reference tests. RDTs could reduce time-to-treatment, over-treatment and lost-to-follow-up.

BACKGROUND

Syphilis and yaws are human treponematoses that remain significant causes of morbidity and mortality globally. Syphilis is caused by *Treponema pallidum* subspecies *pallidum* and is primarily transmitted through sex by skin-to-skin contact or through mother-to-child during pregnancy, causing congenital syphilis. Yaws is an endemic and neglected tropical disease caused by *Treponema pallidum* subspecies *pertenue* and is characterised by soft tissue and bone lesions.[1] Both infections are curable and preventable.

Globally, there are an estimated 6 million new cases of syphilis each year.[2] The burden of congenital syphilis is high, with an estimated 661 000 cases.[3] Further, syphilis disproportionally affects key populations such as sex workers, transgender women (TGW) and men who have sex with men (MSM). Recently, a 2021 study estimated a pooled prevalence of 7.5% among MSM worldwide.[4] Social and structural challenges often make it difficult for these populations to access healthcare services, resulting in delayed detection and lost to follow-up (LTFU) (from diagnosis to getting results or treatment).

For yaws, a systematic review in 2015 estimated the prevalence of active disease ranged from 0.3% to 14.5% in endemic areas, and of latent yaws from 2.5% to 31.1%.[1] Considering its severe morbidity, the WHO launched a strategy to eradicate yaws by 2020, later revised to 2030.[5] The revised strategy included using rapid diagnostic tests (RDTs) for *T. pallidum* as a priority for yaws eradication.[5]

Diagnostic methods for active syphilis and yaws include direct detection of treponemes or treponemal DNA sequences (ie, darkfield microscopy, direct immunofluorescence test or nucleic acid amplification tests performed on material obtained from primary or secondary lesions). In the absence of primary or secondary lesions, such as in latent syphilis or tertiary syphilis, serological tests for treponemal and non-treponemal antibodies using whole blood, serum/plasma or cerebrospinal fluid are required.[6] Over the past decade, several treponemal rapid screening tests have been developed with pooled sensitivity ranging from 85% to 98%, and specificity from 93% to 98%.[7] In 2015, syphilis RDTs were adopted into the WHO prequalification system.[8] However, these single-treponemal RDTs cannot differentiate between active and previously treated infections.

More recently, some novel RDTs have included both treponemal and non-treponemal test

Check for updates

To cite: Zhang Y, Goh SM, Mello MB, *et al*. *Sex Transm Infect* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/sextrans-2022-055546

components in the same device, such as the Dual Path Platform (DPP) Syphilis Screen and Confirm Assay (Chembio Diagnostic Systems, New York, USA), which will be referred to as the DPP-RDT.[9] The Burnet Institute (Melbourne, Australia) also developed an RDT for syphilis using a treponemal IgA-specific assay.[10 11] Furthermore, a new smartphone dongle triplex test targeting HIV, treponemal antibodies and anti-cardiolipin antibodies as the non-treponemal marker has been developed.[12] Of these novel RDTs, the only commercially available test currently is the DPP Screen and Confirm Assay which is accessible in Europe and the USA. The smartphone dongle and the Burnet tests are prototypes only at this stage and not yet commercially manufactured.[13]

In 2016, a meta-analysis on DPP-RDT to detect syphilis and yaws found an 85.2% concordance when comparing the DPP-RDT with reference serology.[9] Since that publication, there have been further studies evaluating DPP-RDT in various settings, including the use of digital readers[14] as well as newer RDTs.[10 12] Thus, we conducted a systematic review on the performance characteristics and clinical utility of RDTs for syphilis and yaws to inform forthcoming WHO guidance on testing for these diseases.

METHODS

This review follows the recommendations in the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy[15] and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) extension for Diagnostic Test Accuracy guidelines.[16]

Search strategy and selection criteria

Five databases (Medline, Embase, Global Health, CINAHL and Web of Science) were searched on 11 October 2021. The search strategy was adapted from a previous meta-analysis paper on DPP-RDT,[9] built around overarching terms, including 'syphilis', 'yaws', 'rapid diagnostic test', 'treponemal', 'nontreponemal' and their Medical Subject Headings (MeSH) terms (eg, syphilis congenital, syphilis latent, neurosyphilis), and was modified for each database (see online supplemental appendix 1). The search was limited from 2010 to October 2021, the period since the DPP-RDT assay became available. No language restrictions were set. Reference lists were checked to locate any other relevant papers.

Studies were included for meta-analysis if they contained primary quantitative data on the clinical performance of an RDT that detects treponemal and non-treponemal antibodies with no restrictions on populations, countries or study designs. Studies that evaluated secondary outcomes such as feasibility, usability and acceptability of the RDTs, testing uptake and cost-effectiveness were included for narrative synthesis.

Search results from each database were downloaded into the Covidence systematic review tool. After removing duplicates, two reviewers (YZ, SMG) independently screened the titles and abstracts of all articles potentially eligible for full-text retrieval, with a third reviewer (JJO) resolving any discrepancies. Non-English language articles were excluded at this stage. An updated literature search was undertaken in July 2022, and after screening, none of the articles met the inclusion criteria.

Data extraction

Two independent reviewers (YZ, SMG) extracted data from full-text articles that satisfied the inclusion criteria using a data extraction spreadsheet and checked by a third reviewer (JJO). We extracted data on the specimen type, disease (syphilis or yaws), RDT reading method (visual vs digital reader), type of laboratory-based reference test, sensitivity and specificity, country (classified by country income level as per the World Bank Group), study design, study setting and secondary outcomes (if available).[17]

Data analysis

The Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool was chosen to assess the risk of bias in diagnostic test accuracy.[18] Two reviewers (YZ, SMG) examined the risk of bias, and a third reviewer (JJO) resolved any discrepancies. Risk of bias was assessed using QUADAS[18] and the certainty of the evidence was evaluated using Grading of Recommendations, Assessment, Development and Evaluations (GRADE).[19]

Statistical analyses were conducted in Stata V.17 (StataCorp, College Station, Texas, USA) using the *midas* and *metandi* Stata modules for meta-analysis of diagnostic test accuracy studies.[20 21] We used a generalised linear mixed-model approach to conduct a bivariate meta-analysis of the sensitivity and specificity of the dual treponemal–non-treponemal RDTs. As the bivariate model assumes independent binomial distributions for the true positives and true negatives conditional on the sensitivity and specificity in each study,[21] we calculated their associated 95% CI. Forest plots and hierarchical summary receiver operating characteristic (HSROC) plots were created separately for syphilis and yaws. Statistical heterogeneity was assessed using the I^2 statistic, and Deeks' funnel asymmetry test was used to evaluate publication bias.[22] We calculated the positive and negative likelihood ratios. We used random-effects meta-regression to determine if any study-level covariates could explain the between-study heterogeneity. Meta-regression was not performed for yaws due to insufficient observations. We used narrative synthesis to describe the data for the secondary outcomes. This study is registered with PROSPERO (CRD42021279587).

RESULTS

The quality assessment results are summarised in online supplemental table 1 and online supplemental figure 1. There was potential for bias, particularly for client selection (33.3%, n=5). Most studies adequately described the index test 73.3%, n=11) and reference tests (73.3%, n=11). Results around the certainty of the evidence are shown in online supplemental table 2.

The PRISMA flow chart is presented in figure 1. In total, 750 records were retrieved and screened. We included 25 studies for data synthesis, 2 of which were unpublished data extracted from a previous publication following consultation with one of the coauthors (MM).[9] Characteristics of the 25 studies are outlined in table 1 . The majority of the studies were cross-sectional studies from high-income countries and conducted within clinical settings. In total, 13 articles on syphilis[10 11 14 23–32] and 4 articles on yaws[33 34] (including 2 unpublished studies) were included in the meta-analysis (see table 2). Fifteen articles contained enough information for the narrative synthesis of secondary outcomes.

Syphilis

For syphilis, the pooled sensitivity and specificity of the treponemal component were 0.93 (95% CI: 0.86 to 0.97) and 0.98 (95% CI: 0.96 to 0.99), respectively (figure 2). For the non-treponemal component, the pooled sensitivity and specificity for syphilis were 0.90 (95% CI: 0.82 to 0.95) and 0.97 (95% CI: 0.92 to 0.99), respectively (figure 3). High heterogeneity was observed for both the treponemal (sensitivity: I^2=96.9%;

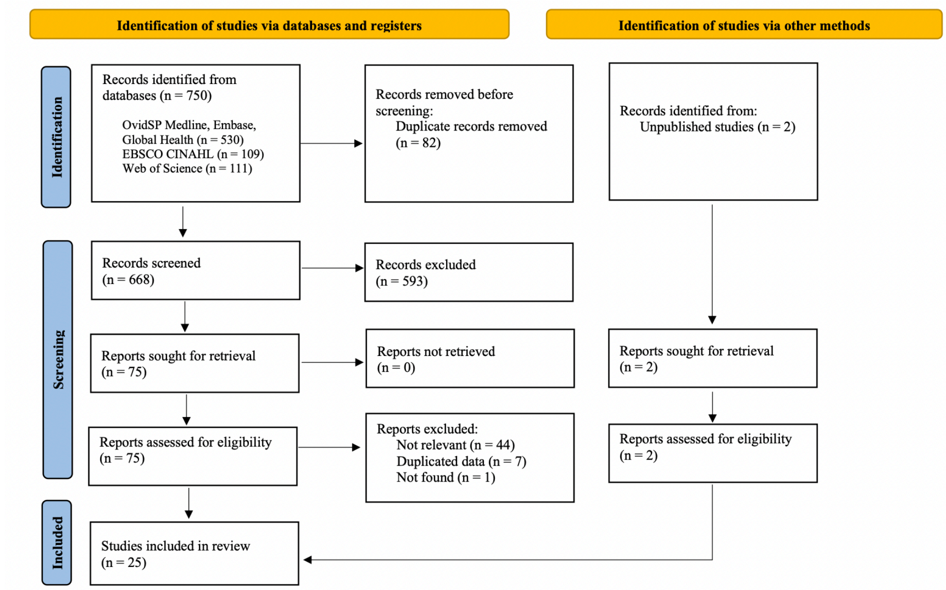


Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram.

specificity: I^2=94.7%) and non-treponemal (sensitivity: I^2=98.3%; specificity: I^2=99.3%) components. From the bivariate analysis, the positive and negative likelihood ratios were 55.1 (95% CI: 26.6 to 113.9) and 0.07 (95% CI: 0.04 to 0.14), respectively, for the treponemal component and 34.7 (95% CI: 11.4 to 106.1) and 0.10 (95% CI: 0.06 to 0.18) for the non-treponemal component. The diagnostic ORs were 777 (95% CI: 340 to 1776) and 339 (95% CI: 131 to 880), respectively.

Meta-regression was conducted using the study setting, sample type and RDT reading method (see online supplemental table 3). Serum samples performed better than whole blood samples in both treponemal (0.96 (95% CI: 0.93 to 1.00) vs 0.88 (95% CI: 0.79 to 0.97)) and non-treponemal sensitivity (0.95 (95% CI: 0.92 to 0.99) vs 0.83 (95% CI: 0.70 to 0.91)), but not for specificity. Studies conducted in laboratories had better sensitivity for both treponemal (0.95 (95% CI: 0.83 to 1.00)) and non-treponemal (0.93 (95% CI: 0.86 to 0.99)) test components compared with studies from clinical facilities (0.91 (95% CI: 0.82 to 1.00); 0.85 (95% CI: 0.72 to 0.98)). Although the use of digital readers to analyse RDT results resulted in greater specificity than the human eye (treponemal: 0.99 (95% CI: 0.99 to 1.00) vs 0.98 (95% CI: 0.96 to 0.99); non-treponemal: 0.99 (95% CI: 0.92 to 1.00) vs 0.97 (95% CI: 0.93 to 1.00), respectively), it only had slightly better sensitivity for the treponemal component (0.95 (95% CI: 0.86 to 1.00) vs 0.92 (95% CI: 0.87 to 0.98)) and added to the cost of the test.

Among all the studies, there were two outlier studies that were performed in clinical settings. A study in the USA reported the lowest sensitivity for both components due to participant selection as the sample included women who inject drugs and also reported higher-risk sexual behaviours.[28] In another outlier study exploring point-of-care tests for syphilis among MSM in Italy, Zorzi *et al* reported logistical problems with expired test assays that resulted in a subsample of recruited MSM's results being unavailable.[32] These might have contributed to the heterogeneity in the pooled studies. Further sensitivity analysis by removing these two studies increased the pooled sensitivity from 0.93 (95% CI: 0.86 to 0.97) to 0.95 (95% CI: 0.93 to 0.97) in the treponemal component, and 0.90 (95% CI: 0.82 to 0.95) to 0.98 (95% CI: 0.96 to 0.99) for the non-treponemal part. The I^2 statistic was reduced from 96.9% to 87.5%, and 98.3% to 98.2% for the sensitivity of the treponemal and non-treponemal components, respectively. The exclusion of these two studies increased the test performance of the non-treponemal component in clinical settings to 0.92 (95% CI: 0.85 to 1.00).

Yaws

For yaws, we found that for the treponemal component, the pooled sensitivity and specificity were 0.86 (95% CI: 0.66 to 0.95) and 0.97 (95% CI: 0.94 to 0.99), respectively, and for the non-treponemal component, 0.80 (95% CI: 0.55 to 0.93) and 0.96 (95% CI: 0.92 to 0.98), respectively (figures 4 and 5). The I^2 for sensitivity was 96.4% and 97.8%, and that for specificity was 84.2% and 88.5% for treponemal and non-treponemal components, respectively. The HSROCs for syphilis and yaws are depicted in online supplemental figure 2.

The positive and negative likelihood ratios were 27.8 (95% CI: 12.3 to 63.0) and 0.15 (95% CI: 0.06 to 0.39), respectively, for the treponemal component and 21.8 (95% CI: 8.9 to 53.5) and 0.21 (95% CI: 0.08 to 0.54) for the non-treponemal component. The diagnostic ORs were 187 (95% CI: 39 to 901) and 105 (95% CI: 20 to 553), respectively. Using Deeks' test, we did not detect any publication bias in the studies on syphilis (treponemal component: p=0.08; non-treponemal component: p=0.53) and yaws (treponemal component: p=0.74; non-treponemal component: p=0.70) (see online supplemental figure 3). The positive predictive values and negative predictive values for tests undertaken for syphilis and yaws are presented in online supplemental table 4.

Secondary outcomes

The narrative synthesis of the secondary outcomes is provided in online supplemental appendix 2. Briefly, RDTs were considered acceptable and feasible by healthcare workers and clients, and could reduce time to treatment, LTFU, overtreatment and improve cost-effectiveness. The usability of DPP-RDT was variable, with some studies advocating for digital readers to improve test accuracy.

Table 1 Characteristics of included studies

	Syphilis* (n=19) n (%)	Yaws* (n=7) n (%)
POCTs		
Chembio DPP-RDT	15 (78.9)	7 (100)
Smartphone dongle triplex test†	1 (5.3)	0
SpanDiagnostics‡	1 (5.3)	0
Burnet TP-IgA	2 (10.5)	0
Country income level§		
High	9 (47.4)	0
Middle	6 (31.6)	6 (85.7)
Low	2 (10.5)	1 (14.3)
Mixed	2 (10.5)	0
Study setting		
General practice/clinic	11 (57.9)	1 (14.3)
Laboratory	8 (42.1)	0
Field/non-clinical facility	0	3 (42.9)
Unclear (includes unpublished data)	0	3 (42.9)
Population		
General population	11 (57.9)	3 (42.9)
Pregnant women	4 (21.1)	0
MSM	2 (10.5)	0
People living with HIV	1 (5.3)	0
Children	0	2 (28.6)
Other¶	1 (5.3)	0
Unclear (from unpublished data)	0	2 (28.6)
Study design		
Experimental/randomised controlled trial	0	1 (14.3)
Observational/cross-sectional	16 (84.2)	2 (28.6)
Modelling	2 (10.5)	1 (14.3)
Qualitative	1 (5.3)	1 (14.3)
Unclear (from unpublished data)	0	2 (28.9)
RDT reading method		
Visual	16 (84.2)	6 (85.7)
Digital reader	3 (15.8)	1 (14.3)
Secondary outcomes		Total (n=15)
Acceptability		2 (13)
Feasibility		2 (13)
Usability		5 (33)
Appropriate treatment following testing		4 (27)
Cost/resources		2 (13)

*The total of studies for each category does not add up to 25 as one paper contained data for both syphilis and yaws.
†HIV, treponemal and non-treponemal RDT, not commercially available.
‡Similar to DPP-RDT, manufactured by Span Diagnostics.
§Country income level is classified as per the World Bank Group.
¶(>15 years old+behavioural risk group): (1) injection drug users (IDUs) with verified track marks (eg, visible signs of injection); (2) women who reported at least two male partners in the last 2 years or engaging in anal intercourse, sex trading, or sex with an MSM, an IDU, or an HIV-positive man; (3) MSM and men who have sex with men and women; and (4) transgender individuals.
DPP, Dual Path Platform; MSM, men who have sex with men; POCTs, point-of-care tests; RDT, rapid diagnostic test; TP-IgA, treponemal IgA-specific assay.

DISCUSSION

This systematic review synthesised current evidence regarding RDTs for detecting both treponemal and non-treponemal antibodies for syphilis and yaws. Since the last review by Marks *et al*,[9] new studies have evaluated DPP-RDT in various settings, and two new studies have data on the Burnet assay. We consolidated evidence regarding the acceptability, feasibility, usability, cost-effectiveness and uptake of treatment post-diagnosis, providing helpful information for policy and planning (see online supplemental appendix 2).

Syphilis

While we observed high pooled sensitivity and specificity in our results, we acknowledge that it is challenging to define active syphilis using diagnostics without further medical history (including past syphilis results) and clinical examination (for signs of syphilis). In addition, no test will be 100% accurate and have limitations. According to Shields's study, routine PCR has a sensitivity of 84–89% and a specificity of 93–100% for primary syphilis, but sensitivity dropped to 50% for secondary syphilis, rendering it unsuitable as a screening tool for secondary syphilis.[35] Other studies report that although venereal disease research laboratory (VDRL) is specific for syphilis, it is more prone to human error and lacks the sensitivity to be used as a first-line screening test for primary syphilis. [36] Serum RPR and VDRL have 62–100% sensitivity, depending on the disease stage.[37] Although we could not stratify our results by different syphilis stages, our results demonstrated strong test performance even with a mix of disease stages.

Notably, we found that serum samples performed better than whole blood samples in test sensitivity but not for specificity. This finding is concordant with Jafari *et al*, where diagnostic performance for serum samples was higher than whole blood due to higher concentration of biomarkers and absence of interfering substances in whole blood.[38] In addition, we found higher test sensitivity in studies performed in laboratory settings than in clinic settings. This opens the possibility of using highly sensitive RDT for serum samples in laboratory settings, especially in antenatal syphilis screening, where no cases should be missed for treatment. On the other hand, the lower sensitivity of RDTs in the field may be an acceptable trade-off if RDTs can improve detection and reduce LTFU.

Early testing and treatment for syphilis are critical for pregnant women to prevent congenital disease and other negative pregnancy outcomes.[39] Scaling up the use of these newer dual treponemal–non-treponemal RDTs for syphilis could potentially benefit pregnant women and their babies. A modelling study comparing dual RDT with laboratory RPR+*T. pallidum* haemagglutination (TPHA) estimated that with every 1000 pregnancies, 34 and 26 adverse pregnancy outcomes would be averted, respectively with dual RDT versus RPR+TPHA.[40] Additionally, when RPR+TPHA was used to diagnose maternal syphilis, treatment rates declined from 100% to 67%, indicating that a significant number of clients were LTFU.[40] Hence, the WHO recommends immediate treatment initiation following any reactive syphilis test for pregnant women and their partner(s).[39] While this strategy may result in overtreatment due to false positives for previous syphilis infections, it is preferred to avoid missing syphilis treatment during pregnancy. The ability of the RDT to obtain results and initiate treatment at the same antenatal visit can reduce LTFU, prevent more cases of adverse birth outcomes and interrupt the chain of transmission, thus saving valuable client and provider time and resources.

Priority populations such as MSM and TGW are disproportionately affected by syphilis, and the presence of sociocultural stigma, violence, negative experiences with healthcare systems, prioritisation of hormone therapy by transgender people and frequent life instability place them at a higher risk of LTFU.[41] In a study of MSM and TGW who tested positive with RPR or a single-treponemal rapid screening test, only 37% returned

Table 2 Summary of studies included in the meta-analysis (n=17)

Author	Study site	Population	T1 reference test	T1 reference prevalence (%)	T2 reference test	T2 reference prevalence (%)	Sample type	Sample size	T1 sensitivity (95% CI)	T1 specificity (95% CI)	T2 sensitivity (95% CI)	T2 specificity (95% CI)
Syphilis (DPP-RDT)												
Castro[24]	USA	General	TPPA	40.2	RPR	30.6	Serum	376	0.97 (0.93 to 0.99)*	0.99 (0.97 to 1.00)*	0.97 (0.91 to 0.99)*	0.98 (0.95 to 0.99)*
Castro[23]	USA	General	TPPA	62.9	RPR	52.1	Serum	1601	0.97 (0.95 to 0.98)*	0.95 (0.93 to 0.97)*	0.89 (0.86 to 0.91)*	0.99 (0.97 to 0.99)*
Castro[25]	Portugal	General	TPHA	74.6	RPR	69.8	Serum	248	0.99 (0.97 to 1.00)	0.89 (0.78 to 0.95)	0.99 (0.95 to 1.00)	0.95 (0.87 to 0.98)
Causer[26]	Australia	MSM (majority)	TPPA	73.2	RPR	55	Serum	1005	0.9 (0.87 to 0.92)	0.99 (0.97 to 1.00)	0.94 (0.92 to 0.96)	0.62 (0.58 to 0.67)
Constantine[14]	USA	General	TPPA	31.5	RPR	31.2	Whole blood	1265	0.93 (0.90 to 0.95)	0.99 (0.99 to 1.00)	0.65 (0.60 to 0.70)	1 (0.99 to 1.00)
Guinard[27]	France	General	EIA	57.6	RPR	39.8	Serum, whole blood	T1=144 T2=108	0.9 (0.82 to 0.95)	0.98 (0.91 to 1.00)	0.95 (0.84 to 0.99)	0.92 (0.83 to 0.97)
Hess[28]	USA	Others	TPPA	12.2	RPR	3	Whole blood	T1=765 T2=763	0.53 (0.43 to 0.63)	0.99 (0.97 to 0.99)	0.48 (0.27 to 0.69)	0.99 (0.98 to 1.00)
Langendorf[29]	Burkina Faso	Pregnant women	TPPA	41.7	RPR	25.5	Finger prick, whole blood	T1=242 T2=188	0.95 (0.89 to 0.98)*	0.98 (0.94 to 1.00)*	0.85 (0.72 to 0.92)*	1 (0.97 to 1.00)*
Skinner[30]	Australia	Children	TPPA	75.9	RPR	50.6	Serum	449	0.94 (0.90 to 0.96)*	0.87 (0.79 to 0.93)*	0.96 (0.92 to 0.98)*	0.66 (0.60 to 0.72)*
Yin[31]	China	General	TPPA	49.9	TRUST	35.6	Finger prick, plasma, whole blood	3135	0.96 (0.95 to 0.97)	0.99 (0.99 to 1.00)	0.89 (0.87 to 0.91)	0.91 (0.90 to 0.92)
Zorzi[32]	Italy	MSM	TPPA, CLIA	15.4	RPR	7.1	Finger prick, whole blood	227	0.69 (0.51 to 0.83)	0.99 (0.96 to 1.00)	0.62 (0.35 to 0.85)	1 (0.97 to 1.00)
Yaws (DPP-RDT)												
Ayove[34]	Papua New Guinea	Children	TPHA	55.3	RPR	43.4	Plasma, whole blood	704 500	0.88 (0.85 to 0.91)	0.95 (0.92 to 0.97)	0.88 (0.84 to 0.91)	0.92 (0.89 to 0.95)
					RPR ≤1:4†						0.76 (0.66 to 0.84)	
					RPR ≤1:8†						0.94 (0.90 to 0.97)	
Aziz‡	Ghana	Unknown	TPPA	39.2	RPR	34.9	Finger prick	255	0.97 (0.91 to 0.99)	0.99 (0.96 to 1.00)	0.94 (0.87 to 0.98)	0.99 (0.96 to 1.00)
Marks[33]	Solomon Islands	Children	TPPA	29.6	RPR	28.9	Serum	415	0.59 (0.50 to 0.67)	0.98 (0.95 to 0.99)	0.42 (0.33 to 0.51)	0.95 (0.92 to 0.97)
					RPR≤ 1:4†						0.62 (0.45 to 0.77)	0.93 (0.87 to 0.96)
					RPR ≤ 1:8†						0.72 (0.51 to 0.86)	0.91 (0.8 to 0.95)
					RPR ≤ 1:16†						0.92 (0.67 to 0.99)	0.89 (0.83 to 0.94)
Taleo‡	Vanuatu	Unknown	TPPA	43.7	RPR	34.5	Finger prick	238	0.8 (0.71 to 0.87)	0.93 (0.88 to 0.96)	0.74 (0.64 to 0.83)	0.96 (0.92 to 0.99)

Syphilis (Burnet TP-IgA)

Author	Study site	Population	T1 reference test	T2 reference test	T2 reference prevalence (%)	Sample type	Sample size	Sensitivity (95% CI)	Specificity (95% CI)
Pham[10]	China	General	TPHA	RPR	33.7	Plasma	454	0.96 (0.92 to 0.99)	0.85 (0.80 to 0.89)
Pham[11]	South Africa	Pregnant women	TPAb	RPR	0.6	Whole blood	499	0.88 (0.29 to 1.00)*	0.99 (0.98 to 1.00)

Upper limit of 95% CI above 0.995 is rounded up to 1.00.
*Values are calculated by authors as they were not reported in the original studies.
†Sub-analysis with RPR titre.
‡Unpublished data.
CLIA, chemiluminescence immunoassay; DPP-RDT, Dual Path Platform-rapid diagnostic test; EIA, enzyme immunoassay; MSM, men who have sex with men; RPR, rapid plasma reagin; TPAb, *Treponema pallidum* antibody; TPHA, *Treponema pallidum* haemagglutination; TP-IgA, treponemal IgA-specific assay; TPPA, *Treponema pallidum* passive particle agglutination assay; TRUST, toluidine red unheated serum test.

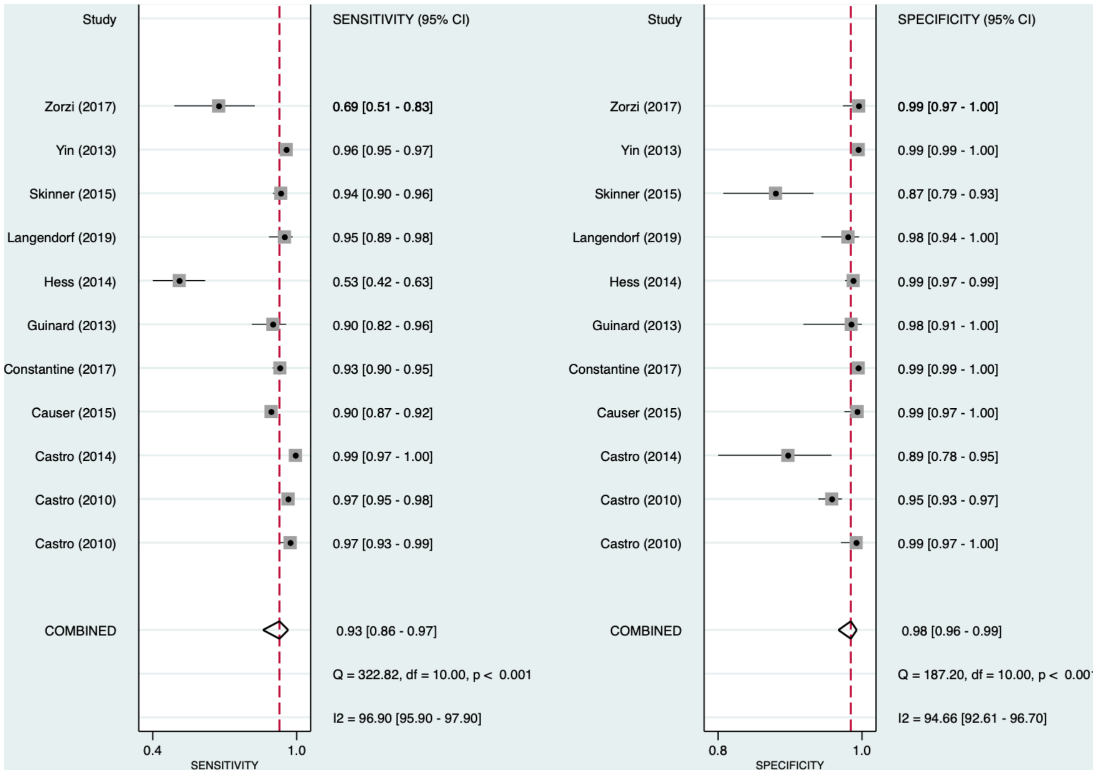


Figure 2 Forest plot of treponemal sensitivity and specificity for syphilis.

for a confirmatory test.[41] Although test performance of RDT is slightly lower in clinical settings than in laboratories, given their high prevalence and LTFU, RDTs could be preferred over conventional laboratory testing. The added value of newer syphilis RDTs, compared with single-treponemal rapid screening tests or conventional laboratory-based testing, lies in facilitating therapy on the same day and reducing overtreatment, particularly among users of HIV pre-exposure prophylaxis and in areas with a high background prevalence of syphilis. Given that they are recommended to undergo syphilis tests every 3–6 months,

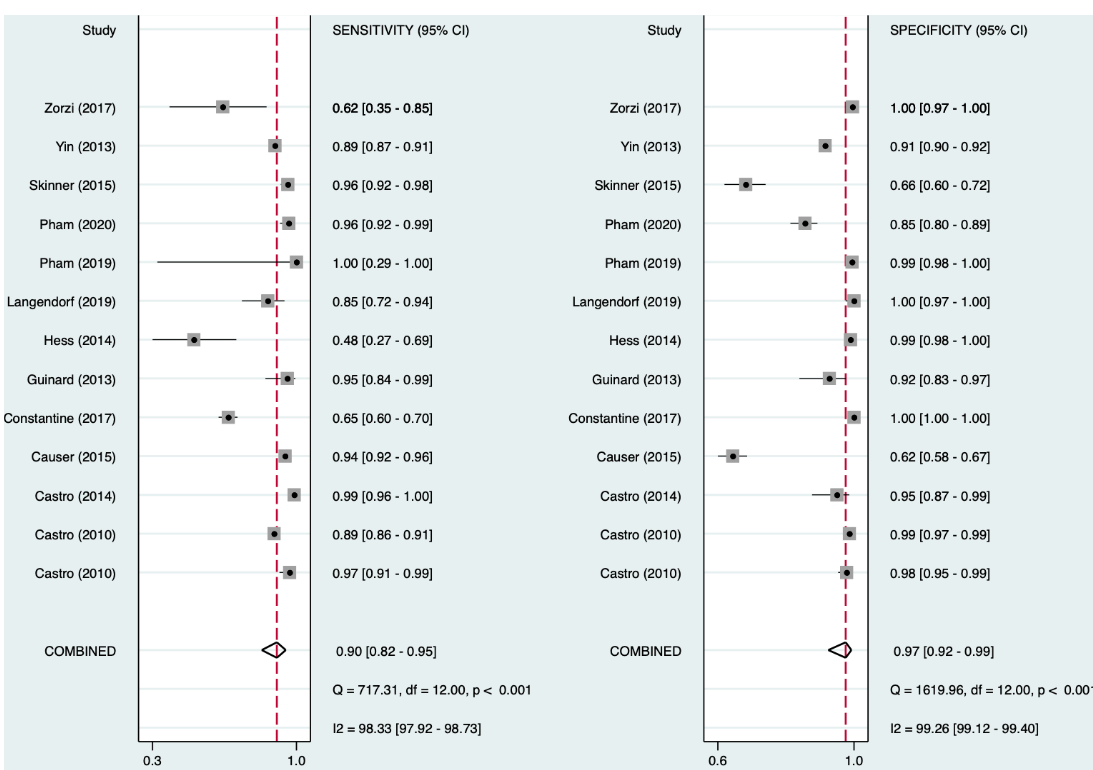


Figure 3 Forest plot of non-treponemal sensitivity and specificity for syphilis.

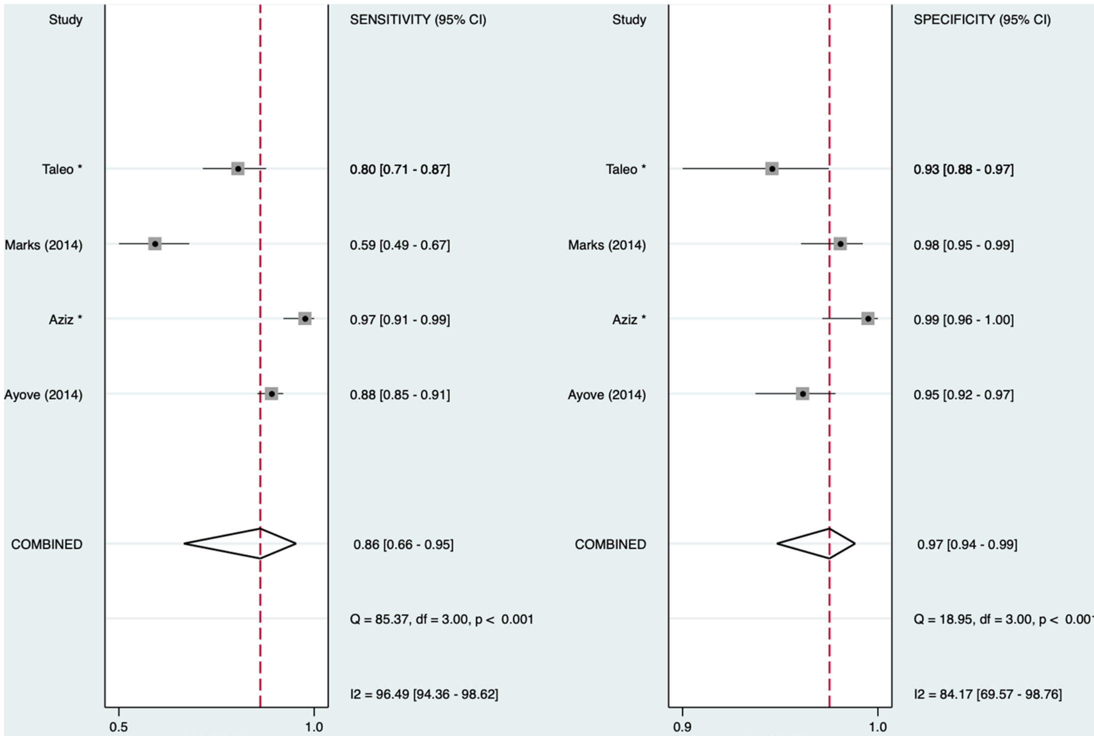


Figure 4 Forest plot of treponemal sensitivity and specificity for yaws. *Unpublished studies.

treatment based solely on a positive single-treponemal rapid test will result in significant overtreatment.

Yaws

Access to quality diagnostics has been identified as a priority in controlling, eliminating and eradicating neglected tropical diseases, and the expanded use of RDTs for yaws is central to WHO's eradication effort. Currently, most countries rely solely on clinical diagnosis, which is not sufficiently accurate and leads to unreliable surveillance data. RDTs allow easier identification of cases of latent yaws in the community who potentially represent an important disease reservoir.[42] As most yaws-endemic countries lack sufficient laboratory capacity for traditional serological assays, these novel RDTs play a pivotal role in supporting

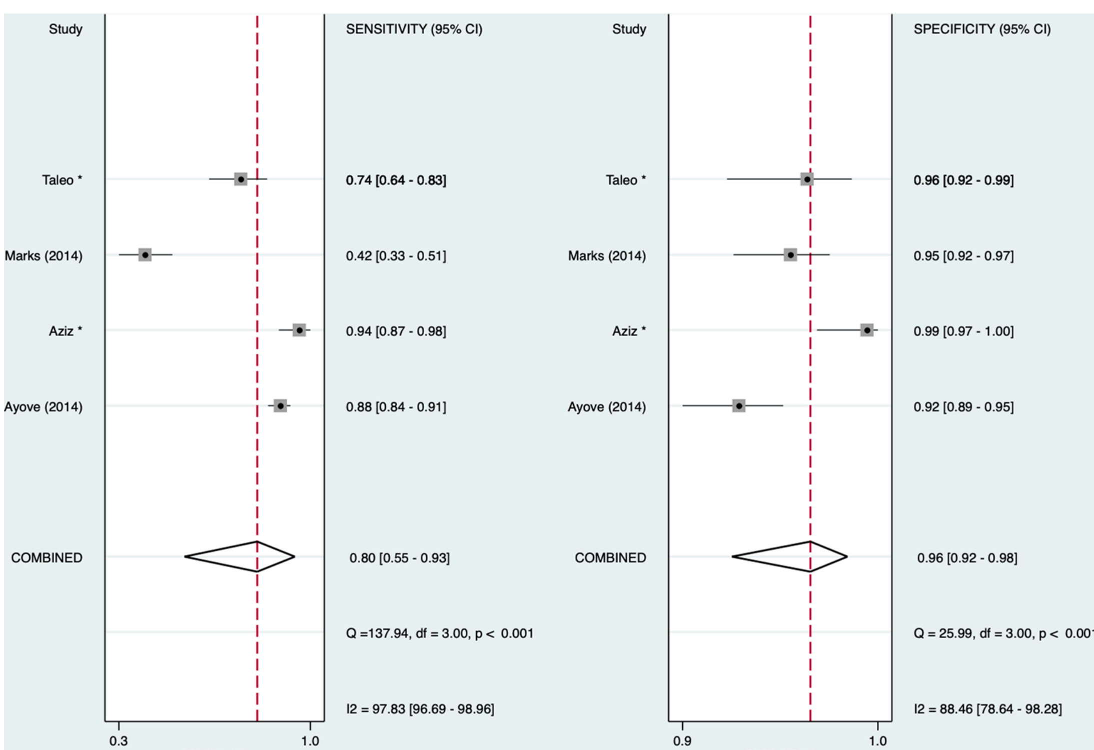


Figure 5 Forest plot of non-treponemal sensitivity and specificity for yaws. *Unpublished studies.

yaws eradication efforts. The use of additional automatic readers can potentially monitor changes in the quantity of the non-treponemal antibodies, thereby assisting in the diagnosis of new infections or monitoring treatment response. In Papua New Guinea, children with yaws were followed up using a DPP-RDT automatic reader to measure optical density after treatment.[34] At 6 months, 95% had attained a fourfold reduction in optical density (serological cure) or seroconversion.[34] This demonstrates that post-treatment serological follow-up might be done in the same way that reference RPR testing is used without relying on laboratory facilities. In a community surveillance study, Marks *et al* reported the sensitivity of the DPP-RDT against *T. pallidum* passive particle agglutination assay and RPR was 47.1%, with the sensitivity of the DPP-RDT being strongly related to the RPR titre. This reduced sensitivity compared with other studies reflects a greater population of asymptomatic latent yaws cases where lower antibody titres contribute to lower sensitivity compared with those with active clinical disease and higher titres.[33] This is important, particularly in antenatal settings, as pregnant women with yaws and lower RPR titres may be less likely to transmit the infection to their infants.

Our review has several limitations. First, many studies were performed in a laboratory setting and included samples with different patterns of serological reactivity but unknown clinical stages of infection. Further comparative studies are needed in syphilis and yaws, where the clinical stages of infection are documented together with direct detection of treponemes (in primary and secondary disease), clinical and treatment histories (including information about serofast status) so that active disease can be ascertained with greater certainty. Second, we did not have information on coinfection status, re-infection status or other diseases in subjects providing samples that might have affected the results. Third, we did not search grey literature, so we may have missed other relevant data. Lastly, we tried to use meta-regression to explain the heterogeneity in our results but was limited by the small number of studies and not enough information to account for other important factors such as the clinical stages of syphilis and yaws, and treatment histories of patients.

CONCLUSIONS

RDTs that can differentiate between active and previously treated infections could optimise management by providing same-day treatment and reducing unnecessary treatment. This systematic review and meta-analysis found that current RDTs for syphilis and yaws had slightly lower sensitivity but a very high specificity than laboratory-based testing. If distributed widely with appropriate training, these tests can potentially decrease the incidence of both adult and congenital syphilis and contribute to the global eradication of yaws.

Author affiliations
[1]School of Public Health, The University of Sydney, Campertown, New South Wales, Australia
[2]Melbourne Sexual Health Centre, Melbourne, Victoria, Australia
[3]Global HIV, Hepatitis and STI Programmes, WHO, Geneva, Switzerland
[4]Clinical Research Department, London School of Hygiene and Tropical Medicine, London, UK
[5]Hospital for Tropical Diseases, University College London Hospital, London, UK
[6]Division of Infection and Immunity, University College London, London, UK
[7]Burnet Institute, Melbourne, Victoria, Australia
[8]School of Public Health and Preventive Medicine, Monash University Faculty of Medicine, Nursing and Health Sciences, Melbourne, Victoria, Australia
[9]Central Clinical School, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Victoria, Australia
[10]Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia
[11]Fight AIDS and Infectious Diseases Foundation, Catalonia, Spain
[12]Department of Sexual and Reproductive Health and Research, WHO, Geneva, Switzerland

Handling editor Laith J Abu-Raddad

Twitter Ying Zhang @lovie_sally, Eric P F Chow @EricPFChow and Jason J Ong @DrJasonJOng

Contributors MM, TW, RB, CJ and JJO conceived the idea. YZ and SMG did the screening and data extraction. YZ and JJO conducted the statistical analysis. All authors contributed to the interpretation of the results and subsequent edits of the manuscript and had final responsibility for the decision to submit for publication.

Funding The WHO supported this work through a grant from the Ministry for Development Cooperation and Humanitarian Affairs, Luxembourg. JJO and EPFC are each supported by the Australian National Health and Medical Research Council (NHMRC) Emerging Leadership Investigator Grant (grant number GNT1193955 for JJO; GNT1172873 for EPFC). CKF is supported by an Australian NHMRC Leadership Investigator Grant (grant number GNT1172900).

Disclaimer WHO technical staff were involved in the study design and interpretation of results as part of ongoing guideline development.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval No ethical clearance was required.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

ORCID iDs
Ying Zhang http://orcid.org/0000-0001-7717-5691
Michael Marks http://orcid.org/0000-0002-7585-4743
Minh D Pham http://orcid.org/0000-0002-5932-3491
Christopher K Fairley http://orcid.org/0000-0001-9081-1664
Eric P F Chow http://orcid.org/0000-0003-1766-0657
Jason J Ong http://orcid.org/0000-0001-5784-7403

REFERENCES

1 World Health Organization. Yaws Geneva; 2021. https://www.who.int/news-room/fact-sheets/detail/yaws [Accessed 30 Oct 2021].
2 World Health Organization. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021, 2021. Available: https://www.who.int/publications/i/item/9789240027077 [Accessed 30 Dec 2021].
3 Gomez GB, Kamb ML, Newman LM, *et al*. Untreated maternal syphilis and adverse outcomes of pregnancy: a systematic review and meta-analysis. *Bull World Health Organ* 2013;91:217–26.
4 Tsuboi M, Evans J, Davies EP, *et al*. Prevalence of syphilis among men who have sex with men: a global systematic review and meta-analysis from 2000-20. *Lancet Glob Health* 2021;9:e1110–8.
5 World Health Organization. Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030; 2021. https://www.who.int/publications/i/item/9789240010352 [Accessed 10 Nov 2021].
6 Unemo M, Ballard R, Ison C. Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. Geneva World Health Organization; 2013: xi, 228 p.
7 World Health Organization. Who guidelines for the treatment of Treponema pallidum (syphilis). Geneva World Health Organization; 2016. https://www.who.int/reproductivehealth/publications/rtis/syphilis-treatment-guidelines/en/ [Accessed 30 Dec 2021].
8 World Health Organization. Who list of prequalified in vitro diagnostic products, 2021. Available: https://extranet.who.int/pqweb/sites/default/files/documents/210827_prequalified_product_list.pdf [Accessed 01 Mar 2022].

9 Marks M, Yin Y-P, Chen X-S, *et al*. Metaanalysis of the performance of a combined treponemal and nontreponemal rapid diagnostic test for syphilis and yaws. *Clin Infect Dis* 2016;63:627–33.
10 Pham MD, Wise A, Garcia ML, *et al*. Improving the coverage and accuracy of syphilis testing: the development of a novel rapid, point-of-care test for confirmatory testing of active syphilis infection and its early evaluation in China and South Africa. *EClinicalMedicine* 2020;24:100440.
11 Pham MD, Wise A, Garcia ML. Novel rapid test for improved diagnosis of active syphilis at the point of care. *Sex Transm Infect* 2019;95:A319.
12 Laksanasopin T, Guo TW, Nayak S, *et al*. A smartphone dongle for diagnosis of infectious diseases at the point of care. *Sci Transl Med* 2015;7:273re1.
13 Pham MD, Ong JJ, Anderson DA, *et al*. Point-of-care diagnostics for diagnosis of active syphilis infection: needs, challenges and the way forward. *Int J Environ Res Public Health* 2022;19. doi:10.3390/ijerph19138172. [Epub ahead of print: 04 07 2022].
14 Constantine NT, Sill AM, Gudesblat E, *et al*. Assessment of two rapid assays for diagnostic capability to accurately identify infection by treponema pallidum. *J Appl Lab Med* 2017;1:346–56.
15 Deeks J, Bossuyt P, Leeflang M. *Cochrane Handbook for systematic reviews of diagnostic test accuracy (version 2)*. Cochrane, 2022. https://training.cochrane.org/handbook-diagnostic-test-accuracy
16 McInnes MDF, Moher D, Thombs BD, *et al*. Preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies: the PRISMA-DTA statement. *JAMA* 2018;319:388–96.
17 The World Bank Groups. World bank country and lending groups. Available: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups [Accessed 19 July 2022].
18 Whiting PF, Rutjes AWS, Westwood ME, *et al*. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;155:529–36.
19 Schünemann HJ, Mustafa RA, Brozek J, *et al*. Grade guidelines: 21 part 1. study design, risk of bias, and indirectness in rating the certainty across a body of evidence for test accuracy. *J Clin Epidemiol* 2020;122:129–41.
20 Harbord RM, Whiting P. Metandi: meta-analysis of diagnostic accuracy using hierarchical logistic regression. *Stata J* 2009;9:211–29.
21 Dwamena B. Midas: stata module for meta-analytical integration of diagnostic test accuracy studies. *Statistical Software Components* 2007 https://ideas.repec.org/c/boc/bocode/s456880.html
22 van Enst WA, Ochodo E, Scholten RJPM, *et al*. Investigation of publication bias in meta-analyses of diagnostic test accuracy: a meta-epidemiological study. *BMC Med Res Methodol* 2014;14:70.
23 Castro AR, Esfandiari J, Kumar S, *et al*. Novel point-of-care test for simultaneous detection of nontreponemal and treponemal antibodies in patients with syphilis. *J Clin Microbiol* 2010;48:4615–9.
24 Castro AR, Mody HC, Parab SY, *et al*. An immunofiltration device for the simultaneous detection of non-treponemal and treponemal antibodies in patients with syphilis. *Sex Transm Infect* 2010;86:532–6.
25 Castro R, Lopes Ângela, da Luz Martins Pereira F. Evaluation of an immunochromatographic point-of-care test for the simultaneous detection of nontreponemal and treponemal antibodies in patients with syphilis. *Sex Transm Dis* 2014;41:467–9.
26 Causer LM, Kaldor JM, Conway DP, *et al*. An evaluation of a novel dual treponemal/nontreponemal point-of-care test for syphilis as a tool to distinguish active from past treated infection. *Clin Infect Dis* 2015;61:184–91.
27 Guinard J, Prazuck T, Péré H, *et al*. Usefulness in clinical practice of a point-of-care rapid test for simultaneous detection of nontreponemal and treponema pallidum-specific antibodies in patients suffering from documented syphilis. *Int J STD AIDS* 2013;24:944–50.
28 Hess KL, Fisher DG, Reynolds GL. Sensitivity and specificity of point-of-care rapid combination syphilis-HIV-HCV tests. *PLoS One* 2014;9:e112190.
29 Langendorf C, Lastrucci C, Sanou-Bicaba I, *et al*. Dual screen and confirm rapid test does not reduce overtreatment of syphilis in pregnant women living in a non-venereal treponematoses endemic region: a field evaluation among antenatal care attendees in burkina faso. *Sex Transm Infect* 2019;95:402–4.
30 Skinner L, Robertson G, Norton R. Evaluation of the dual path platform syphilis point of care test in North Queensland. *Pathology* 2015;47:718–20.
31 Yin Y-P, Chen X-S, Wei W-H, *et al*. A dual point-of-care test shows good performance in simultaneously detecting nontreponemal and treponemal antibodies in patients with syphilis: a multisite evaluation study in China. *Clin Infect Dis* 2013;56:659–65.
32 Zorzi A, Cordioli M, Gios L, *et al*. Field evaluation of two point-of-care tests for syphilis among men who have sex with men, Verona, Italy. *Sex Transm Infect* 2017;93:S51–8.
33 Marks M, Goncalves A, Vahi V, *et al*. Evaluation of a rapid diagnostic test for yaws infection in a community surveillance setting. *PLoS Negl Trop Dis* 2014;8:e3156.
34 Ayove T, Houniei W, Wangnapi R, *et al*. Sensitivity and specificity of a rapid point-of-care test for active yaws: a comparative study. *Lancet Glob Health* 2014;2:e415–21.
35 Shields M, Guy RJ, Jeoffreys NJ, *et al*. A longitudinal evaluation of treponema pallidum PCR testing in early syphilis. *BMC Infect Dis* 2012;12:353.
36 Knaute DF, Graf N, Lautenschlager S, *et al*. Serological response to treatment of syphilis according to disease stage and HIV status. *Clin Infect Dis* 2012;55:1615–22.
37 Tuddenham S, Katz SS, Ghanem KG. Syphilis laboratory guidelines: performance characteristics of nontreponemal antibody tests. *Clin Infect Dis* 2020;71:S21–42.
38 Jafari Y, Peeling RW, Shivkumar S, *et al*. Are treponema pallidum specific rapid and point-of-care tests for syphilis accurate enough for screening in resource limited settings? evidence from a meta-analysis. *PLoS One* 2013;8:e54695.
39 World Health Organization. Who guideline on syphilis screening and treatment for pregnant women. Geneva; 2017. https://www.who.int/reproductivehealth/publications/rtis/syphilis-ANC-screenandtreat-guidelines/en/ [Accessed 30 Dec 2021].
40 Owusu-Edusei K, Gift TL, Ballard RC. Cost-effectiveness of a dual non-treponemal/treponemal syphilis point-of-care test to prevent adverse pregnancy outcomes in sub-Saharan Africa. *Sex Transm Dis* 2011;38:997–1003.
41 Tang EC, Segura ER, Clark JL, *et al*. The syphilis care cascade: tracking the course of care after screening positive among men and transgender women who have sex with men in Lima, Peru. *BMJ Open* 2015;5:e008552.
42 World Health Organization. Report of a global meeting on yaws eradication surveillance, monitoring and evaluation Geneva, 29–30 January 2018; 2018. https://www.who.int/publications/i/item/WHO-CDS-NTD-IDM-2018.08 [Accessed 01 Feb 2022].

Appendix 1: Literature Search

A comprehensive literature search was carried out on October 11, 2021, and updated on July 19, 2022. Five databases were searched to look for information on dual treponemal and non-treponemal test in the diagnosis of syphilis and yaws.

1.1 Search methodology
The search strategy was initially developed in Ovid and adapted for the other databases. The search terms were built around overarching terms like "RDT", "point of care test", "treponemal", "non-treponemal", "syphilis" and "yaws"; relevant terms were included as well. The search limits were from 2010 to current. The search strategy was refined with the research team until the results retrieved reflected the scope of the project.

The following database were searched:
1. OvidSP Medline® All, 1946 to July 19, 2022
2. OvidSP Embase Classic + Embase, 1974 to July 19, 2022
3. OvidSP Global Health, 1973 to July 19, 2022
4. EBSCO CINAHL Complete, complete database
5. Web of Science, All Database,
    a) Web of Science Core Collections
    b) *Current Contents Connect*
    c) *BIOSIS Previews*
    d) *CAB Abstracts*
    e) *MEDLINE*
6. Preprints (MedRxiv, bioRxiv, SSRN)

1.2 Search results

Database name	Number of references before removal of duplicates
OvidSP Medline® + Embase + Global Health	530
CINAHL complete	109
Web of Science	111
Total	750

1.3.1 OvidSP Medline® + Embase + Global Health

Database name	Medline, Embase, Global Health
Database platform	OvidSP
Dates of database coverage	1946 to July 19, 2022 1974 to July 19, 2022 1973 to July 19, 2022
Date searched	July 19, 2022
Searched by	YZ
Number of hits	530

#	Query	Results from July 19, 2022
1	exp syphilis/	90772
2	syphilis.mp.	110496
3	exp yaws/	5120
4	yaws.mp.	5625
5	exp Treponema pallidum/	31646
6	treponema*.mp.	50740
7	(non-treponema* or nontreponema*).mp.	1519
8	1 or 2 or 3 or 4	116245
9	5 and 6 or 7	51534
10	8 and 9	36717
11	(RDT or RST).mp.	14403
12	(rapid adj2 diagnos* adj2 test*).mp.	17859
13	(rapid adj2 screening adj2 test*).mp	2717
14	(point-of-care adj3 test*).mp.	38901
15	(point adj1 of adj1 care adj3 test*).mp.	4571
16	11 or 12 or 13 or 14 or 15	66150
17	10 and 16	618
18	Limit 2010 to current	530

1.3.2 EBSCO CINAHL

Database name	CINAHL complete
Database platform	EBSCOhost
Dates of database coverage	2000 to July 19, 2022
Date searched	July 19, 2022
Searched by	YZ
Number of hits	109

#	Query	Results from July 19, 2022
1	TX syphilis OR TX yaws	16,231
2	TX treponema* OR (TX non-treponema* or nontreponema*)	2,348
3	1 AND 2	1,454
4	(TX rapid N2 diagnos* N2 test*) OR (TX rapid N2 screening N2 test*) OR (TX RDT OR TX RST) OR (TX point-of-care N3 test*)	47,143

	OR (TX point N1 of N1 care N3 test*)	
7	3 AND 4	141
8	Limit from 2010 to current	109

1.3.3 Web of Science

Database name	Web of Science All Database
Database platform	Clarivate Web of Science
Dates of database coverage	Complete to July 19, 2022
Date searched	July 19, 2022
Searched by	YZ
Number of hits	111

#	Query	Results from July 19, 2022
1	ALL=(syphilis OR yaws)	47,459
2	(ALL=(treponema*) OR ALL=(non-treponema* OR nontreponema*))	9,951
3	1 AND 2	3,748
4	AB=(rapid NEAR/2 diagnos* NEAR/2 test* OR rapid NEAR/2 screening NEAR/2 test* OR RDT OR RST OR point-of-care NEAR/3 test* OR point NEAR/1 of NEAR/1 care NEAR/3 test*) OR TI= (rapid NEAR/2 diagnos*NEAR/2 test* OR rapid NEAR/2 screening NEAR/2 test* OR RDT OR RST OR point-of-care NEAR/3 test* OR point NEAR/1 of NEAR/1 care NEAR/3 test*) OR TS=(rapid NEAR/2 diagnos* NEAR/2 test* OR rapid NEAR/2 screening NEAR/2 test* OR RDT OR RST OR point-of-care NEAR/3 test* OR point NEAR/1 of NEAR/1 care NEAR/3 test*)	25,687
5	4 AND 3	127
6	Limit from 2010 to current	111

1.3.4 MedRxiv

Database name	MedRvix

Database platform	Science, Nature, The BMJ, The Scientist
Dates of database coverage	Complete to July 19, 2022
Date searched	July 19, 2022
Searched by	YZ
Number of hits	32

#	Query	Results from July 19, 2022
1	Terms & Keywords =(syphilis OR yaws)	326
2	(Terms & Keywords =(treponema*) OR Terms & Keywords =(non-treponema* OR nontreponema*))	57
3	1 AND 2	32

1.3.5 bioRxiv

Database name	bioRvix
Database platform	bioRvix
Dates of database coverage	Complete to July 19, 2022
Date searched	July 19, 2022
Searched by	YZ
Number of hits	71

#	Query	Results from July 19, 2022
1	Terms & Keywords =(syphilis OR yaws)	854
2	(Terms & Keywords =(treponema*) OR Terms & Keywords =(non-treponema* OR nontreponema*))	370
3	1 AND 2	71

1.3.6 SSRN

Database name	SSRN
Database platform	SSRN All
Dates of database coverage	Complete to July 19, 2022
Date searched	July 19, 2022

Searched by	YZ
Number of hits	4

#	Query	Results from July 19, 2022
1	Title, Abstract, Keywords, Authors = syphilis	73
2	1 AND treponema	3
3	1 AND non-treponema	1
4	1 AND non-treponemal	2
5	1 AND nontreponema	0
6	1 AND nontreponemal	1
7	Title, Abstract, Keywords, Authors = yaws	3

Appendix 2: Summary of secondary outcomes

Acceptability

Two studies assessed the stakeholder acceptability of the dual syphilis RDT in the diagnosis of syphilis – one of them was the DPP-RDT and the other was a smartphone dongle Triplex test. The DPP-RDT for the diagnosis of syphilis and yaws was perceived by most healthcare workers (16/20) in a study in the Solomon Islands to be reliable, and this perception was reinforced by concordance with reference laboratory results.[1] The healthcare workers found the DPP-RDT more favourable in comparison to standard testing which may take a week for results to come back.[1] Healthcare workers in Rwanda also reported satisfaction for the smartphone triplex test as they did not have to rely on user interpretation for results. In terms of client acceptability, overall high levels of satisfaction were reported. The vast majority of patients in the Rwandan study (97%) would recommend the Triplex Test to others, mainly due to the rapid turnaround time but also for the simplicity of the test and the ability to diagnose both HIV and syphilis in one test.[2] Almost all patients (98%) also preferred the RDT testing over conventional venepuncture, as generally only one fingerprick was needed and they cited various benefits including that it was less painful, faster than that compared to venepuncture, healthcare workers would have less difficulty in obtaining a blood sample.[2]

Feasibility

The smartphone dongle test was found to be viewed favourably by healthcare workers in terms of feasibility. As it does not require external power to operate, it would be useful in field settings or in the case of power outages in clinics.[2] In general, healthcare workers found the DPP-RDT also improved access to testing in settings where testing at the clinic level was advantageous as distance and cost of getting to hospital were deemed to be barriers to testing.[1]

Usability

Healthcare workers generally found the DPP-RDT to be easy to perform. All healthcare workers in one study in the Solomon Islands reported that familiarity with using the Malaria point-of-care test (POCT) helped them conduct the DPP POCT for syphilis and yaws, although some noted mistakes made with the timing of the test and volume of buffer had the potential to result in testing errors.[1] Only one healthcare worker out of 20, reported that the withdrawal of blood for fingerprick testing was difficult. Four studies assessed and compared digital and visual reading of the DPP-RDT for either syphilis or yaws.[3-6] Three studies suggested there was a high level of concordance with visual and digital results for both treponemal and non-treponemal tests.[3 4 6] However, one study in Botswana suggested that visual reading missed three out of five active syphilis infections, classifying them as past infections, and therefore suggested that the digital reader should be used to avoid missing cases with confirmed high titre non-treponemal test results.[5]

Appropriate treatment following testing

The rationale for using RDTs to better identify active infection is to eliminate lost to follow-up and reduce unnecessary treatment in clients with previously treated syphilis and yaws. In a modelling study using pilot data from three antenatal screening centres, it was discovered that the single treponemal-only test resulted in much more missed instances of syphilis infection and overtreatment in pregnant women than the dual RDT.[7] According to Owusu-Edusei Jr's study, when RPR+TPHA was used to diagnose maternal syphilis, treatment rates declined from 100% to 67%, indicating that a large proportion of clients were loss to follow-up due to delay with the provision of test results.[8] This lost to follow-up is concerning since these untreated mothers are at an elevated risk of congenital syphilis and adverse birth outcomes. In another study conducted on pregnant women in Burkina Faso, of the women with RPR titres ≥ 1:8, 16% would not be treated if they were only screened with the DPP-RDT compared to the treponemal-only rapid test.[9] There was an unexpectedly high proportion of pregnant women who were found to be treponemal and non-treponemal positive based on reference tests (37.6%). A high proportion had high level RPR titres ≥ 1:8 (19%), suggesting they had either been incompletely treated for untreated bejel (an endemic treponematosis), or untreated active syphilis. The study highlighted the importance of establishing baseline treponemal/non-treponemal seroprevalence in any population when identifying the most effective strategy to screen and treat for treponemal diseases.

Thus, the utility of this test will depend on the proportion of people treated for syphilis and background prevalence of syphilis. As the first syphilis infection can be detected using the cheaper single treponemal-only test, the value of the DPP-RDT is to identify individuals with syphilis or yaws with confirmed high-titre non-treponemal tests. Moreover, Yin reported that the single-treponemal test will result in overtreatment and counselling, particularly in populations with high prevalence of syphilis such as MSM.[6]

In a study on diagnosis of yaws, Avoye advocated for the use of the DPP-RDT before mass treatment to identify clients with active yaws and during resurvey to support detecting new active cases.[3] Clients who were tested dually positive were given immediate treatment, and those who tested negative but had lesions were given syndromic treatment and followed up further.[3] The use of DPP-RDT would potentially reduce overtreatment in mass treatment compared to the standard single-treponemal rapid test, making it a suitable tool to support diagnosis in the renewed eradication effort for yaws-endemic countries.

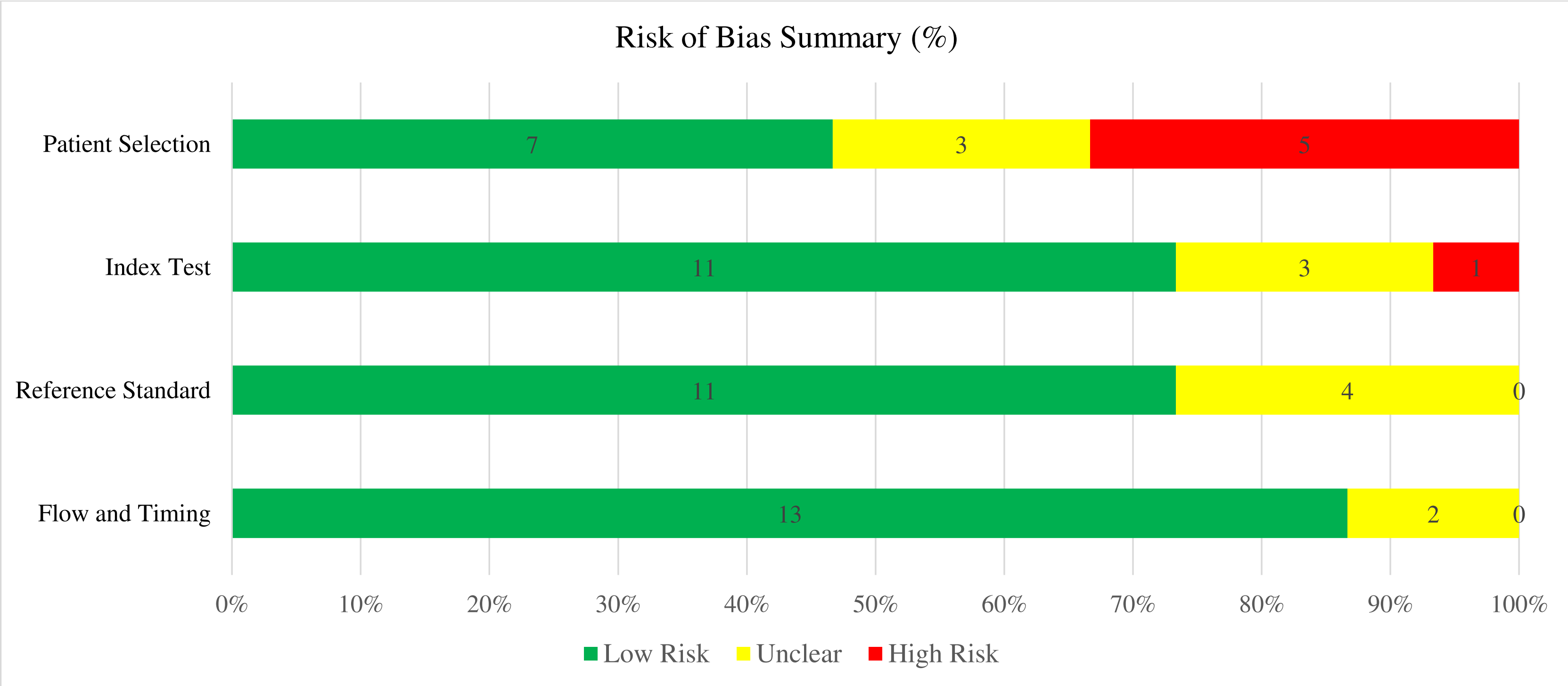
Cost-effectiveness

In a modelling study where several antenatal syphilis screening and treatment strategies were compared, the incremental cost-effectiveness ratio (ICER) of the clinical RPR approach (ICER: US$23−138 per DALY averted) was dominated by the single treponemal-only rapid test (ICER: US$16−53 per DALY averted), the dual treponemal–nontreponemal RDT (ICER: US$18−76 per DALY averted) and the sequential approach (single rapid test followed by dual RDT) (ICER: US$19−62 per DALY averted).[7] Although the dual RDT detected more true cases of syphilis and reduced overtreatment compared to the other three strategies, the cost of per woman screened with the dual RDT was highest, with the exception of Peru where labour cost for RPR testing was high. Further univariate sensitivity analysis showed that the cost of the dual test kit had to be reduced by approximately 38% from the assumed baseline unit price of US$2.50 to achieve the same cost per DALY averted as the treponemal-only rapid test [7]. Even though the single treponemal-only rapid test was most cost-effective among the four strategies, it may lead to overtreatment.[7]

Yet, in another cost-effectiveness study on yaws, the sequential screening strategy (single rapid test followed by dual RDT) versus was concluded to be more cost-effective than the dual RDT for both individual diagnosis and community surveillance of yaws.[10]

Despite the fact that the dual RDT is more expensive than the single rapid test and RPR (excluding labor costs), Owusu-Edusei Jr discovered that test performance had a significant impact on the cost-effectiveness of antenatal syphilis screening.[8] The greatest cost savings occurred when the sensitivity of the dual RDT was increased to 0.97 and this conclusion held true when the unit price was varied from US$0.50 to $5.00, indicating that test performance has a bigger impact on cost-effectiveness than the RDT's price.[8]

Supplementary Figure 1. Risk of bias summary as percentage



Supplementary Table 1. Risk of bias of studies included for meta-analysis

	RISK OF BIAS				APPLICABILITY CONCERNS		
STUDY	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Ayove (2014)[3]	−	+	+	+	−	+	+
Castro (2010)[11]	−	?	+	+	−	+	+
Castro (2010)[12]	+	+	+	+	+	+	+
Castro (2014)[13]	−	?	?	?	−	+	+
Causer (2015)[14]	+	+	+	+	+	+	+
Constantine (2017)[4]	+	+	+	+	+	+	+
Guinard (2013)[15]	?	+	+	+	?	+	+
Hess (2014)[16]	+	+	+	+	+	+	+
Langendorf (2019)[9]	+	+	+	+	+	+	+
Marks (2014)[17]	+	+	+	+	+	+	+
Pham (2020)[18]	−	−	+	+	−	+	+
Pham (2019)[19]	?	?	?	?	?	?	+
Skinner (2015)[20]	?	+	+	+	?	+	+
Yin (2013)[6]	−	+	?	+	−	+	+
Zorzi (2017)[21]	+	+	?	+	+	+	+

+ Low risk − High Risk ? Unclear Risk

N.B. Quality assessment was not conducted for the two unpublished papers (Aziz et al. and Taleo et al.).

Supplementary Table 2. A) Summary of findings for treponemal test component for syphilis; B) Summary of findings for nontreponemal test component for syphilis, C) Summary of findings for treponemal test component for yaws; D) Summary of findings for nontreponemal test component for yaws

A)

T1 Sensitivity	0.93 (95% CI: 0.86 to 0.97)
T1 Specificity	0.98 (95% CI: 0.96 to 0.99)

Prevalence	0%	10%	20%

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested			Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 0%	pre-test probability of 10%	pre-test probability of 20%	
True positives (patients with syphilis)	11 studies 4695 patients	cross-sectional	not serious [a,b,c,d]	not serious	serious [e]	not serious	none	0 (0 to 0)	93 (86 to 97)	186 (172 to 194)	⊕⊕⊕◯ MODERATE
False negatives (patients incorrectly classified as not having syphilis)								0 (0 to 0)	7 (4 to 13)	14 (8 to 26)	
True negatives (patients without syphilis)	11 studies 4762 patients	cross-sectional	not serious [a,b,c,d]	not serious	serious [e]	not serious	none	983 (964 to 992)	884 (868 to 893)	786 (771 to 794)	⊕⊕⊕◯ MODERATE
False positives (patients incorrectly classified as having syphilis)								17 (8 to 36)	15 (7 to 32)	14 (6 to 29)	

Explanations

a. Most studies had low risk of patient selection bias[4 9 12 14 16 21] and was scored “low” for risk of bias using the QUADAS checklist in the patient selection criterion. Two studies [15 20] were unclear in their description of random sampling of patients. Three studies[6 11 13] were at high risk of bias in patient selection, as samples were not selected at random[11 13], or subjects deemed at higher risk for syphilis were oversampled.[6]

b. All the studies had low potential of index test bias except two unclear studies.[11 13] In one study, all the patient identifiers were removed before receipt at the CDC but it did not specify if the assay was performed blinded to the results.[11]
c. All the studies had low potential of reference test bias except three studies which were unclear.[6 13 21]
d. All the studies had low potential for flow and timing bias,[4 6 9 11 12 14-16 20 21] except one. Castro[13] did not present a clear description of the patient flow.
e. There is considerable heterogeneity (p< 0.001), I^2= 96.9% and 94.7% for sensitivity and specificity, respectively, with some overlap in confidence intervals

B)

T2 Sensitivity	0.90 (95% CI: 0.82 to 0.95)
T2 Specificity	0.97 (95% CI: 0.92 to 0.99)

Prevalence	0%	10%	20%

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested			Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 0%	pre-test probability of 10%	pre-test probability of 20%	
True positives (patients with syphilis)	13 studies [a] 3699 patients	cross-sectional	not serious [b,c,d,e]	not serious	serious [f]	not serious	none	0 (0 to 0)	90 (82 to 95)	180 (164 to 190)	⊕⊕⊕◯ MODERATE
False negatives (patients incorrectly classified as not having syphilis)								0 (0 to 0)	10 (5 to 18)	20 (10 to 36)	
True negatives (patients without syphilis)	13 studies [a] 6619 patients	cross-sectional	not serious	not serious	serious [f]	not serious	none	974 (920 to 992)	876 (828 to 893)	779 (735 to 794)	⊕⊕⊕◯

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested			Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 0%	pre-test probability of 10%	pre-test probability of 20%	
False positives (patients incorrectly classified as having syphilis)			b,c,d,e					26 (8 to 80)	23 (7 to 72)	21 (6 to 64)	MODERATE

Explanations

a. There were 2 additional studies for the nontreponemal component.[18 19]

b. Most studies had low risk of patient selection bias[4 9 12 16 21] and was scored "low" for risk of bias using the QUADAS checklist in the patient selection criterion. Four studies[13 15 19 20] were unclear in their description of random sampling of patients. Four studies[6 11 13 18] were at high risk of bias in patient selection, as samples were not selected at random[11 13 18], or subjects deemed at higher risk for syphilis were oversampled.[6]

c. Most of the studies had low potential of index test bias except three unclear studies.[11 13 19] In one study, all the patient identifiers were removed before receipt at the CDC but it did not specify if the assay was performed blinded to the results.[11] Pham[18] had high risk of bias.

d. Four studies had unclear risk of bias for reference standard.[6 13 19 21]

e. All the studies had low potential for flow and timing bias,[4 6 9 11 12 14-16 20 21] except two. Two studies[13 19] did not present a clear description of the patient flow.

f. There is heterogeneity observed in the studies, I^2= 98.3% and 99.3% for sensitivity and specificity, respectively, with some overlap in confidence intervals.

C)

T1 Sensitivity	0.86 (95% CI: 0.66 to 0.95)
T1 Specificity	0.97 (95% CI: 0.94 to 0.99)

Prevalence	0%	10%	20%

Outcome	№ of studies (№	Study design	Factors that may decrease certainty of evidence	Effect per 1,000 patients tested	Test accuracy

	of patients)		Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 0%	pre-test probability of 10%	pre-test probability of 20%	CoE
True positives (patients with yaws)	4 studies [a] 716 patients	cross-sectional	not serious [b,c]	not serious	serious [d]	not serious	none	0 (0 to 0)	86 (66 to 95)	171 (132 to 190)	⨁⨁⨁◯ MODERATE
False negatives (patients incorrectly classified as not having yaws)								0 (0 to 0)	14 (5 to 34)	29 (10 to 68)	
True negatives (patients without yaws)	4 studies [a] 895 patients	cross-sectional	not serious [b,c]	not serious	serious [d]	not serious	none	969 (935 to 985)	872 (841 to 886)	775 (748 to 788)	⨁⨁⨁◯ MODERATE
False positives (patients incorrectly classified as having yaws)								31 (15 to 65)	28 (14 to 59)	25 (12 to 52)	

Explanations

a. Of the 4 studies, there were 2 unpublished studies where assessment of certainty of evidence was not possible.
b. One study[17] was at low risk of bias for patient selection, while another study[3] presented high risk of bias as it was a community-based survey and no comment on randomisation or further detail on recruitment was reported.
c. Both studies[3 17] reported low risk of bias for index test, reference standard and patient flow and timing.
d. There is some heterogeneity observed in the studies for sensitivity (I^2= 96.4%, p<0.001) and specificity (I^2= 84.2%, p< 0.001).

D)

T2 Sensitivity	0.80 (95% CI: 0.55 to 0.93)
T2 Specificity	0.96 (95% CI: 0.92 to 0.98)

Prevalence	0%	10%	20%

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested			Test accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 0%	pre-test probability of 10%	pre-test probability of 20%	
True positives (patients with yaws)	4 studies [a] 597 patients	cross-sectional	not serious [b,c]	not serious	serious [d]	not serious	none	0 (0 to 0)	80 (55 to 93)	160 (110 to 186)	⊕⊕⊕◯ MODERATE
False negatives (patients incorrectly classified as not having yaws)								0 (0 to 0)	20 (7 to 45)	40 (14 to 90)	
True negatives (patients without yaws)	4 studies [a] 1015 patients	cross-sectional	not serious [b,c]	not serious	serious [d]	not serious	none	963 (920 to 983)	867 (828 to 885)	770 (736 to 786)	⊕⊕⊕◯ MODERATE
False positives (patients incorrectly classified as having yaws)								37 (17 to 80)	33 (15 to 72)	30 (14 to 64)	

Explanations

a. Of the 4 studies, there were 2 unpublished studies where assessment of certainty of evidence was not possible.
b. One study[17] was at low risk of bias for patient selection, while another study[3] presented high risk of bias as it was a community-based survey and no comment on randomisation or further detail on recruitment was reported.
c. Both studies[3 17] reported low risk of bias for index test, reference standard and patient flow and timing.
d. There is some heterogeneity observed in the studies for sensitivity (I^2= 97.8%, p<0.001) and little heterogeneity for specificity (I^2= 88.5%, p<0.001).

Supplementary Table 3. A) Meta-regression of treponemal test component for syphilis; B) Meta-regression of nontreponemal test component for syphilis

A)

Variable	Number of studies	Univariate				Multivariable		Joint model	
		Sensitivity	p-value	Specificity	p-value	Sensitivity	Specificity	I^2 (95% CI)	p-value
Study setting								87 (74 - 100)	< 0.001
General Practice/Clinic	5	0.91 (0.82 - 1.00)	0.22	0.98 (0.95 - 1.00)	0.59	0.91 (0.82 - 1.00)	0.98 (0.97 - 1.00)		
Laboratory	5	0.95 (0.89 - 1.00)	1.00	0.98 (0.97 - 1.00)	0.14	0.95 (0.89 - 1.00)	0.98 (0.95 - 1.00)		
Field/Non-clinical facility	0	NA		NA		NA	NA		
Sample type								70 (34 - 100)	0.03
Serum	5	0.96 (0.93 - 1.00)	0.83	0.96 (0.93 - 0.99)	<0.001	0.96 (0.93 - 1.00)	0.96 (0.93 - 0.99)		
Finger-prick	1	NA*		NA*		NA*	NA*		
Whole blood	5	0.88 (0.79 - 0.97)	<0.001	0.99 (0.98 - 1.00)	0.47	0.88 (0.79 - 0.97)	0.99 (0.98 - 1.00)		
Plasma	0	NA		NA		NA	NA		
RDT reading method								60 (9 - 100)	0.08
Human eye	9	0.92 (0.87 - 0.98)	0.56	0.98 (0.96 - 0.99)	<0.001	0.92 (0.87 - 0.98)	0.98 (0.96 - 0.99)		
Digital reader	2	0.95 (0.86 - 1.00)	0.83	0.99 (0.99 - 1.00)	0.56	0.95 (0.86 - 1.00)	0.99 (0.99 - 1.00)		

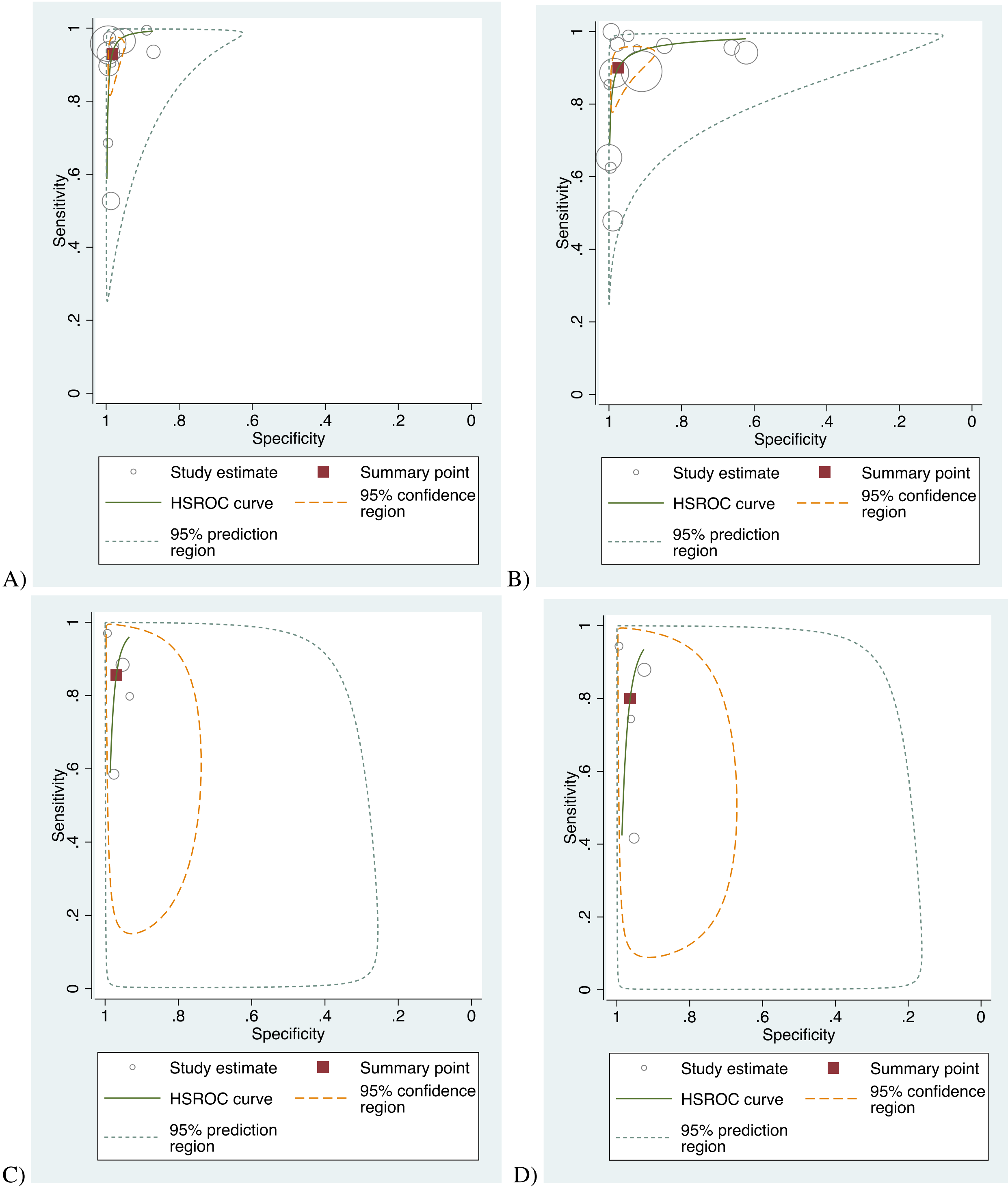
Abbreviations: EIA= enzyme immunoassay, TPHA= *Treponema pallidum* hemagglutination, TPPA= *Treponema pallidum* passive particle agglutination assay
* fingerprick combined with whole blood, TPHA combined with TPPA

B)

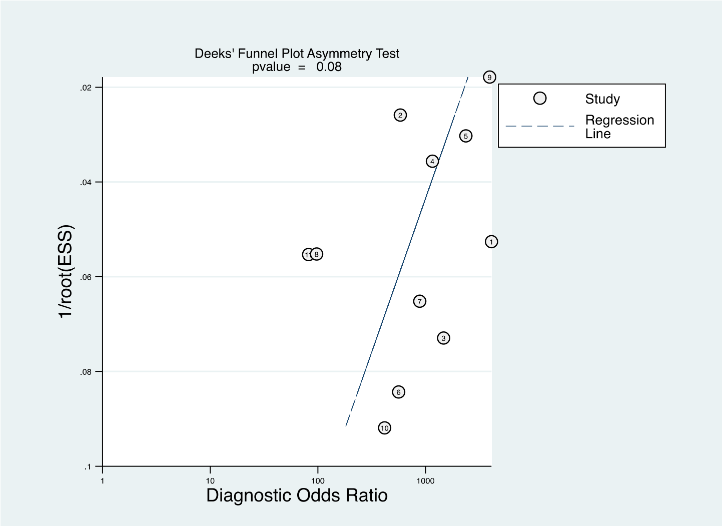
Variable	Number of studies	Univariate				Multivariable		Joint model	
		Sensitivity	p-value	Specificity	p-value	Sensitivity	Specificity	I^2 (95% CI)	p-value
Brand of RDT								0 (0 - 100)	0.74
DPP	11	0.89 (0.83 - 0.96)	0.55	0.98 (0.94 - 1.00)	0.12	0.89 (0.83 - 0.96)	0.98 (0.94 - 1.00)		
Burnet's	2	0.95 (0.85 - 1.00)	0.37	0.97 (0.88 - 1.00)	0.25	0.95 (0.85 - 1.00)	0.97 (0.88 - 1.00)		
Study setting								92 (85 - 99)	< 0.001
General Practice/Clinic	6	0.85 (0.72 - 0.98)	0.05	0.99 (0.97 - 1.00)	0.18	0.85 (0.72 - 0.98)	0.99 (0.97 - 1.00)		
Laboratory	6	0.93 (0.86 - 0.99)	0.94	0.97 (0.92 - 1.00)	0.56	0.93 (0.86 - 0.99)	0.97 (0.92 - 1.00)		
Field/ non-clinical facility	0					NA	NA		
Sample type								64 (20 - 100)	0.05
Serum	5	0.95 (0.92 - 0.99)	0.94	0.92 (0.79 - 1.00)	0.09	0.95 (0.92 - 0.99)	0.92 (0.79 - 1.00)		
Finger-prick	1	NA*		NA*		NA*	NA*		
Whole blood	7	0.83 (0.74 - 0.93)	<0.001	0.99 (0.97 - 1.00)	0.03	0.83 (0.74 - 0.93)	0.99 (0.97 - 1.00)		
Plasma	0	NA		NA		NA	NA		
RDT reading method								0 (0 - 100)	0.48
Human eye	11	0.92 (0.86 - 0.97)	0.48	0.97 (0.93 - 1.00)	0.64	0.92 (0.86 - 0.97)	0.97 (0.93 - 1.00)		
Digital reader	2	0.80 (0.56 - 1.00)	0.13	0.99 (0.97 - 1.00)	<0.001	0.80 (0.56 - 1.00)	0.99 (0.97 - 1.00)		

Abbreviations: RPR= rapid plasma reagin, TRUST= toluidine red unheated serum test
* fingerprick combined with whole blood

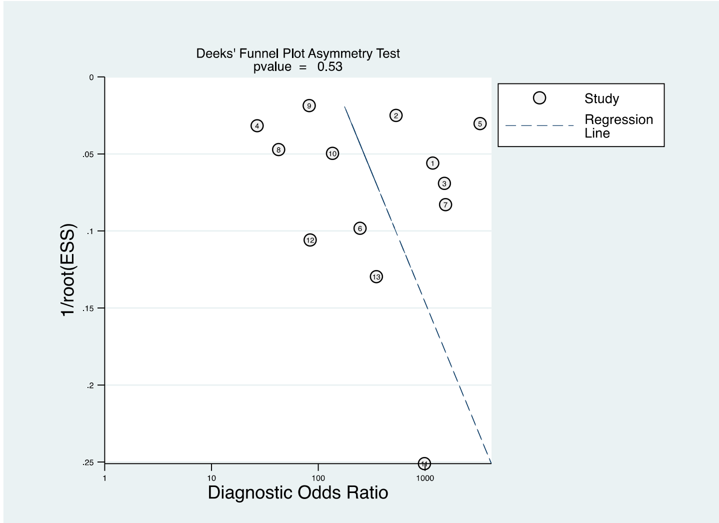
Supplementary Figure 2. A) Hierarchical summary receiver operating characteristic (HSROC) plot for treponemal test component for syphilis; B) HSROC plot for nontreponemal test component for syphilis; C) HSROC plot for treponemal test component for yaws; D) HSROC plot for nontreponemal test component for yaws



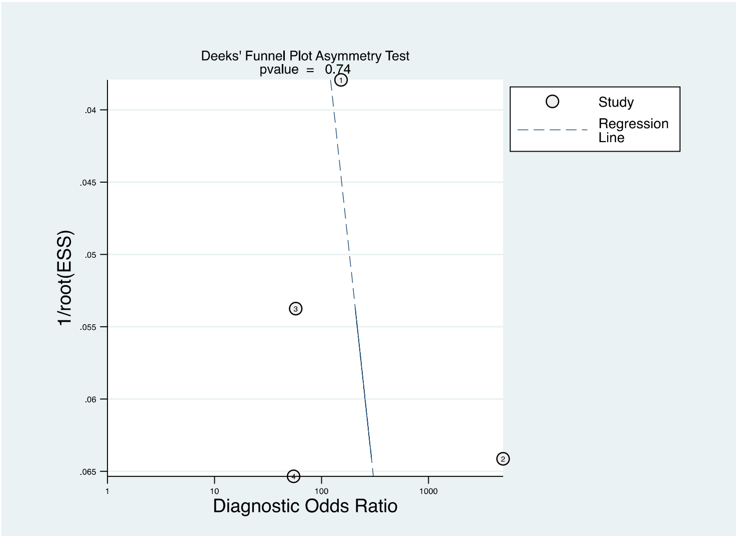
Supplementary Figure 3. A) Deeks' plot for treponemal test component for syphilis; B) Deeks' plot for nontreponemal test component for syphilis; C) Deeks' plot for treponemal test component for yaws; D) Deeks' plot for nontreponemal test component for yaws



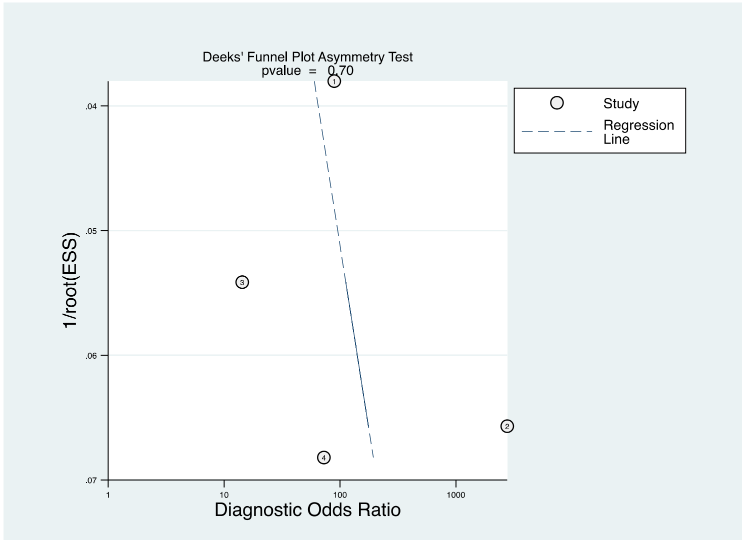
A)



B)



C)



D)

Supplementary Table 4. A) The positive predictive value (PPV) and negative predictive value (NPV) for treponemal component of syphilis, over a range of background prevalence of syphilis; B) PPV and NPV for nontreponemal component of syphilis, over a range of background prevalence of syphilis; C) PPV and NPV for treponemal component of yaws, over a range of background prevalence of yaws; D) PPV and NPV for nontreponemal component of yaws, over a range of background prevalence of yaws

A)

Prevalence	Sensitivity	Specificity	PPV	NPV	Number of cases	Missed cases	False Positive (Overtreated)
0.05	0.930	0.983	0.742	0.996	50	4	16
0.1	0.930	0.983	0.859	0.992	100	7	15
0.15	0.930	0.983	0.906	0.988	150	11	14
0.2	0.930	0.983	0.932	0.983	200	14	14
0.25	0.930	0.983	0.948	0.977	250	18	13
0.3	0.930	0.983	0.959	0.970	300	21	12
0.35	0.930	0.983	0.967	0.963	350	25	11
0.4	0.930	0.983	0.973	0.955	400	28	10
0.45	0.930	0.983	0.978	0.945	450	32	9
0.5	0.930	0.983	0.982	0.934	500	35	9
0.55	0.930	0.983	0.985	0.920	550	39	8
0.6	0.930	0.983	0.988	0.903	600	42	7
0.65	0.930	0.983	0.990	0.883	650	46	6
0.7	0.930	0.983	0.992	0.858	700	49	5
0.75	0.930	0.983	0.994	0.824	750	53	4
0.8	0.930	0.983	0.995	0.778	800	56	3
0.85	0.930	0.983	0.997	0.712	850	60	3
0.9	0.930	0.983	0.998	0.609	900	63	2
0.95	0.930	0.983	0.999	0.425	950	67	1
1	0.930	0.983	1.000	0.000	1000	70	0

B)

Prevalence	Sensitivity	Specificity	PPV	NPV	Number of cases	Missed cases	False Positive (Overtreated)
0.05	0.900	0.974	0.646	0.995	50	5	25
0.1	0.900	0.974	0.794	0.989	100	10	23
0.15	0.900	0.974	0.859	0.982	150	15	22

0.2	0.900	0.974	0.896	0.975	200	20	21
0.25	0.900	0.974	0.920	0.967	250	25	20
0.3	0.900	0.974	0.937	0.958	300	30	18
0.35	0.900	0.974	0.949	0.948	350	35	17
0.4	0.900	0.974	0.958	0.936	400	40	16
0.45	0.900	0.974	0.966	0.923	450	45	14
0.5	0.900	0.974	0.972	0.907	500	50	13
0.55	0.900	0.974	0.977	0.889	550	55	12
0.6	0.900	0.974	0.981	0.867	600	60	10
0.65	0.900	0.974	0.985	0.840	650	65	9
0.7	0.900	0.974	0.988	0.807	700	70	8
0.75	0.900	0.974	0.990	0.765	750	75	7
0.8	0.900	0.974	0.993	0.709	800	80	5
0.85	0.900	0.974	0.995	0.632	850	85	4
0.9	0.900	0.974	0.997	0.520	900	90	3
0.95	0.900	0.974	0.998	0.339	950	95	1
1	0.900	0.974	1.000	0.000	1000	100	0

C)

Prevalence	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Number of cases**	**Missed cases**	**False Positive (Overtreated)**
0.05	0.856	0.969	0.592	0.992	50	7	29
0.1	0.856	0.969	0.754	0.984	100	14	28
0.15	0.856	0.969	0.830	0.974	150	22	26
0.2	0.856	0.969	0.873	0.964	200	29	25
0.25	0.856	0.969	0.902	0.953	250	36	23
0.3	0.856	0.969	0.922	0.940	300	43	22
0.35	0.856	0.969	0.937	0.926	350	50	20
0.4	0.856	0.969	0.948	0.910	400	58	19
0.45	0.856	0.969	0.958	0.892	450	65	17
0.5	0.856	0.969	0.965	0.871	500	72	16
0.55	0.856	0.969	0.971	0.846	550	79	14
0.6	0.856	0.969	0.976	0.818	600	86	12
0.65	0.856	0.969	0.981	0.784	650	94	11
0.7	0.856	0.969	0.985	0.743	700	101	9
0.75	0.856	0.969	0.988	0.692	750	108	8

0.8	0.856	0.969	0.991	0.627	800	115	6
0.85	0.856	0.969	0.994	0.543	850	122	5
0.9	0.856	0.969	0.996	0.428	900	130	3
0.95	0.856	0.969	0.998	0.262	950	137	2
1	0.856	0.969	1.000	0.000	1000	144	0

D)

Prevalence	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Number of cases**	**Missed cases**	**False Positive (Overtreated)**
0.05	0.800	0.963	0.532	0.989	50	10	35
0.1	0.800	0.963	0.706	0.977	100	20	33
0.15	0.800	0.963	0.792	0.965	150	30	31
0.2	0.800	0.963	0.844	0.951	200	40	30
0.25	0.800	0.963	0.878	0.935	250	50	28
0.3	0.800	0.963	0.903	0.918	300	60	26
0.35	0.800	0.963	0.921	0.899	350	70	24
0.4	0.800	0.963	0.935	0.878	400	80	22
0.45	0.800	0.963	0.946	0.855	450	90	20
0.5	0.800	0.963	0.956	0.828	500	100	19
0.55	0.800	0.963	0.964	0.798	550	110	17
0.6	0.800	0.963	0.970	0.762	600	120	15
0.65	0.800	0.963	0.976	0.722	650	130	13
0.7	0.800	0.963	0.981	0.674	700	140	11
0.75	0.800	0.963	0.985	0.616	750	150	9
0.8	0.800	0.963	0.989	0.546	800	160	7
0.85	0.800	0.963	0.992	0.459	850	170	6
0.9	0.800	0.963	0.995	0.349	900	180	4
0.95	0.800	0.963	0.998	0.202	950	190	2
1	0.800	0.963	1.000	0.000	1000	200	0

REFERENCES

1. Marks M, Esau T, Asugeni R, et al. Point-of-care tests for syphilis and yaws in a low-income setting– A qualitative study of healthcare worker and patient experiences. *PLoS Negl Trop Dis* 2018;12(4):e0006360.
2. Laksanasopin T, Guo TW, Nayak S, et al. A smartphone dongle for diagnosis of infectious diseases at the point of care. *Sci Transl Med* 2015;7(273):273re1.
3. Ayove T, Houniei W, Wangnapi R, et al. Sensitivity and specificity of a rapid point-of-care test for active yaws: A comparative study. *Lancet Glob Health* 2014;2(7):E41-421.
4. Constantine NT, Sill AM, Gudesblat E, et al. Assessment of two rapid assays for diagnostic capability to accurately identify infection by treponema pallidum. *J Appl Lab Med* 2017;1(4):346-56.
5. Maan I, Lawrence DS, Tlhako N, et al. Using a dual antibody point-of-care test with visual and digital reads to diagnose syphilis among people living with HIV in Botswana. *Int J STD AIDS* 2021;32(5):453-61.
6. Yin YP, Chen XS, Wei WH, et al. A dual point-of-care test shows good performance in simultaneously detecting nontreponemal and treponemal antibodies in patients with syphilis: A multisite evaluation study in China. *Clin Infect Dis* 2013;56(5):659-65.
7. Terris-Prestholt F, Vickerman P, Torres-Rueda S, et al. The cost-effectiveness of 10 antenatal syphilis screening and treatment approaches in Peru, Tanzania, and Zambia. *Int J Gynaecol Obstet* 2015;130:S73-S80.
8. Owusu-Edusei Jr K, Gift TL, Ballard RC. Cost-effectiveness of a dual non-treponemal/treponemal syphilis point-of-care test to prevent adverse pregnancy outcomes in Sub-Saharan Africa. *Sex Transm Dis* 2011;38(11):997-1003.
9. Langendorf C, Lastrucci C, Sanou-Bicaba I, et al. Dual screen and confirm rapid test does not reduce overtreatment of syphilis in pregnant women living in a non-venereal treponematoses endemic region: A field evaluation among antenatal care attendees in Burkina Faso. *Sex Transm Infect* 2019;95(6):402-04.
10. Fitzpatrick C, Asiedu K, Sands A, et al. The cost and cost-effectiveness of rapid testing strategies for yaws diagnosis and surveillance. *PLoS Negl Trop Dis* 2017;11(10):e0005985.
11. Castro AR, Mody HC, Parab SY, et al. An immunofiltration device for the simultaneous detection of non-treponemal and treponemal antibodies in patients with syphilis. *Sex Transm Infect* 2010;86(7):532-36.
12. Castro AR, Kikkert SE, Ballard RC, et al. Novel point-of-care test for simultaneous detection of nontreponemal and treponemal antibodies in patients with syphilis. *J Clin Microbiol* 2010;48(12):4615-19.
13. Castro R, Lopes Â, da Luz Martins Pereira F. Evaluation of an immunochromatographic point-of-care test for the simultaneous detection of nontreponemal and treponemal antibodies in patients with syphilis. *Sex Transm Infect* 2014;41(8):467-9.
14. Causer LM, Kaldor JM, Conway DP, et al. An evaluation of a novel dual treponemal/nontreponemal point-of-care test for syphilis as a tool to distinguish active from past treated infection. *Clin Infect Dis* 2015;61(2):184-91.
15. Guinard J, Prazuck T, Pere H, et al. Usefulness in clinical practice of a point-of-care rapid test for simultaneous detection of nontreponemal and Treponema pallidum-specific antibodies in patients suffering from documented syphilis. *Int J STD AIDS* 2013;24(12):944-50.

16. Hess KL, Fisher DG, Reynolds GL. Sensitivity and specificity of point-of-care rapid combination syphilis-HIV-HCV tests. *PLoS One* 2014;9(11):e112190.
17. Marks M, Goncalves A, Mabey D, et al. Evaluation of a rapid diagnostic test for yaws infection in a community surveillance setting. *PLoS Negl Trop Dis* 2014;8(9):e3156.
18. Pham MD, Wise A, Garcia ML, et al. Improving the coverage and accuracy of syphilis testing: The development of a novel rapid, point-of-care test for confirmatory testing of active syphilis infection and its early evaluation in China and South Africa. *EClinicalMedicine* 2020;24:100440.
19. Pham MD, Wise A, Garcia ML, et al. Novel rapid test for improved diagnosis of active syphilis at the point of care. *Sex Transm Infect* 2019;95:A319.
20. Skinner L, Robertson G, Norton R. Evaluation of the dual path platform syphilis point of care test in North Queensland. *Pathology* 2015;47(7):718-20.
21. Zorzi A, Cordioli M, Gios L, et al. Field evaluation of two point-of-care tests for syphilis among men who have sex with men, Verona, Italy. *Sex Transm Infect* 2017;93:S51-S58.