Laboratory Recommendations for Syphilis Testing in the United States – Supplementary Material

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Overview

In 2017, the Association of Public Health Laboratories (APHL) assisted with the literature review through an independent work group formed to evaluate the scientific literature for CDC to consider in the development of evidence-based recommendations for syphilis testing in the United States. APHL work group members were selected based on expertise in the field of syphilis and represented public health and commercial laboratory directors, public- and private-sector providers, and academic researchers. The workgroup leads were experienced in conducting systematic reviews of the literature. Potential conflicts of interest were disclosed to APHL and are listed at the end of the work group section (Supplementary Appendix 1).

CDC identified key questions regarding syphilis testing in the United States that should be addressed during the literature review process and shared these questions with the APHL work group members in March 2017. Work

group members were assigned key questions to review (Supplementary Appendix 1) and, with the assistance of CDC and APHL staff, conducted an extensive literature search on Medline, Embase, Scopus, Cochrane Library, and CINAHL; combinations of search terms for each key question were used to search for literature published during 1960–June 30, 2017. In November 2017, work group members presented their reviews to CDC and APHL staff. Key questions and pertinent publications were reviewed for strengths, weaknesses, and relevance and were openly discussed by individual work group members. The discussions were informal and not designed to reach consensus; no formal rating system was used.

Following the meeting, the APHL work group was disbanded, and CDC staff reviewed the scientific evidence and ranked the evidence as high, medium, and low, based on each study's strengths and weaknesses as outlined by the U.S. Preventive Services Task Force Ratings (https://www.uspreventiveservicestaskforce.org/uspstf/us-preventive-services-task-force-ratings). The tables of evidence reviewed and ranked are available at (https://www.cdc.gov/std/syphilis/lab/testing/lab-recs-for-testing.htm). Publications were rated as an "A" if they were high quality using clinically characterized specimens, stratified by stage, larger sample size, prospective or a well-done cross-sectional or retrospective study. "B" rated studies were good to moderate quality with large sample sizes, clinically characterized but not stratified by stage, or characterized but unclear exactly how it was done, mild methodological issues. A fair, "C" rated study included those with small sample sizes, moderate methodological issues, single lab test as gold standard, or descriptive. Poor, "D" rated studies were those with major methodological issues or small sample sizes. Case reports or small case studies were rated as "I." Studies that were not relevant to the key question were assigned as "NR" and not further rated. Laboratory Recommendations for Syphilis Testing in the United States were developed by CDC staff based on high-ranking scientific evidence published in peer-reviewed scientific journals (Supplementary Tables 1-7).

Draft recommendations were peer reviewed as defined by the Office of Management and Budget for influential scientific information. In February 2022, draft recommendations were peer reviewed by four experts in the field of syphilis who were not United States federal employees, were not funded by CDC for syphilis research, and were not involved in the development of these recommendations (Supplementary Appendix 3).

Supplementary Table 1. Performance characteristics of nontreponemal (lipoidal antigen) serologic tests used for the diagnosis of syphilis

Assay	Study summary and reference standard	Performance characteristics*	Reference
AIX1000 Gold Standard Diagnostics 2851 Spafford St Davis, CA 95618	Retrospective cross-sectional clinical trial study for submission to FDA Reference standard: ASI RPR card Clinically characterized samples: Primary syphilis: genital lesion, positive for spirochetes on darkfield microscopy (if performed), and reactive treponemal serologic test Secondary syphilis: rash or mucous patches or condyloma lata with reactive treponemal serologic test Latent syphilis reactive treponemal and nontreponemal serologic test with a nonreactive nontreponemal serologic test for more than a year or unknown duration	Prospective serum samples (N = 765) PPA: 95.5% (95% CI: 77.2%–99.9%) PNA: 99.9% (95% CI: 99.3%–100%) Retrospective serum from patients referred for syphilis testing (N = 2,246) PPA: 97.2% (95% CI: 95.5%–98.4%) PNA: 99.1% (95% CI: 98.5%–99.5%) Samples from HIV+ patients (n = 250 non-treponemal test negative; n = 30 nontreponemal test positive) PPA: 100% (95% CI: 90.5%–100%) PNA: 100% (95% CI: 98.8%–100%) Clinically characterized samples: All samples positive on AIX1000 and comparator; 100% sensitive at all stages. Primary treated (n = 13): 100% agreement (95% CI: 79.4%–100%) Primary untreated (n = 12): 100% agreement (95% CI: 77.9% –100%) Secondary treated (n = 25): 100% agreement (95% CI: 88.7%–100%) Secondary untreated (n = 25): 100% agreement (95% CI: 88.7%–100%) Latent treated (n = 25): 100% agreement (95% CI: 88.7%–100%) Latent untreated (n = 25): 100% agreement (95% CI: 88.7%–100%)	(*1*) †
ASI Evolution	Prospective and retrospective cross-sectional clinical trial study for submission to FDA	Prospective serum samples (N = 1,068) PPA: 99.1% (95% CI: 95.2%–99.9%)	(*2*) †

Assay	Study summary and reference standard	Performance characteristics*	Reference
Arlington Scientific 1840 N Technology Dr Springville, UT 84663	Prospective serum samples: 1,068 Retrospective serum samples: 10 Retrospective plasma samples: 1003 Clinically diagnosed syphilis patients: 143 Pregnant women: 250 Reference standard: ASI RPR card Clinical characteristics not defined beyond the stage of syphilis being diagnosed by a licensed physician	PNA: 99.9% (95% CI: 99.4%–100%) Retrospective serum samples (N = 10) PPA: 100% (95% CI: 59%–100%) PNA: 100% (95% CI: 29.2%–100%) Retrospective plasma samples (N = 1,003) PPA: 100% (95% CI: 69.2%–100%) PNA: 100% (95% CI: 99.6%–100%) Clinically diagnosed syphilis patients (N = 143) Primary treated (n = 25): 100% agreement (95% CI: 81.5%–100%) Primary untreated (n = 18): 100% agreement (95% CI: 86.3%–100%) Secondary treated (n = 25): 100% agreement (95% CI: 86.3%–100%) Secondary untreated (n = 25): 100% agreement (95% CI: 86.3%–100%) Latent treated (n = 25): 100% agreement (95% CI: 86.3%–100%) Latent untreated (n = 25): 100% agreement (95% CI: 86.3%–100%) All phases treated (n = 75): 100% agreement (95% CI: 95.1%–100%) All phases untreated (n = 25): 100% agreement (95% CI: 94.7%–100%) Pregnant women (N = 250) PPA: 100% (95% CI: 88.7%–100%) PNA: 100% (95% CI: 98.5%–100%)	
Rapid Plasma Reagin (RPR) §	Retrospective cross-sectional study Patients with primary syphilis: 106	Primary syphilis (n = 106) Sensitivity: 72.5%	(*3*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Reference standard: Darkfield positive chancre and no signs of secondary syphilis (signs and symptoms not reported in the paper)		
	Cross-sectional study Patients with primary syphilis: 109 Reference standard: Darkfield positive chancre and no signs of secondary syphilis	Primary syphilis (n = 109) Sensitivity: 92.7%	(*4*)
	Retrospective cross-sectional study based on stored serum from clinically classified patients Patients with primary syphilis: 119 Patients with secondary syphilis: 98 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 119) Sensitivity: 72.3% Secondary syphilis (n = 98) Sensitivity: 100%	(*5*)
	Cross-sectional study Patients with primary syphilis: 111 Patients with secondary syphilis: 56 Reference standard: (1) Primary syphilis—darkfield positive chancre and no signs of secondary syphilis; (2) secondary syphilis—darkfield positive secondary lesions or at least two symptoms of secondary syphilis, such as condylomata lata, alopecia, and lymphadenopathy	Primary syphilis (n = 111) Sensitivity: 64.8% Secondary syphilis (n = 56) Sensitivity: 100%	(*6*)
	Cross-sectional study	Primary syphilis (n = 80) Sensitivity: 62.5%	(*7*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Patients with primary syphilis: 80 Patients with secondary syphilis: 29 Reference standard: (1) Primary syphilis—darkfield positive chancre and no signs of secondary syphilis; (2) secondary syphilis—darkfield positive secondary lesions or at least two symptoms of secondary syphilis, such as condylomata lata, alopecia, and lymphadenopathy	Secondary syphilis (n = 29) Sensitivity: 100%	
	Cross-sectional study Patients with primary syphilis: 134 Patients with secondary syphilis: 217 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 134) Sensitivity: 76.1% Secondary syphilis (n = 217) Sensitivity: 91.2%	(*8*)
	Cross-sectional study Patients with primary syphilis: 21 Reference standard: Darkfield positive chancre and no signs of secondary syphilis	Primary syphilis (n = 21) Sensitivity: 71%	(*9*)
	Retrospective cross-sectional study Patients with primary syphilis: 76 Patients with secondary syphilis: 100 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 76) Sensitivity: 48.7% Secondary syphilis (n = 100) Sensitivity: 91%	(*10*)
	Prospective cross-sectional study	Secondary syphilis (n = 23) Sensitivity: 100%	(*11*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Patients with secondary syphilis: 23 Reference standard: Positive FTA-ABS serology plus clinical findings		
	Cross-sectional study Patients with secondary syphilis: 31 Reference standard: Positive VDRL plus clinical findings	Secondary syphilis (n = 31) Sensitivity: 100%	(*12*)
	Retrospective case series Patients with late latent syphilis: 1,303 Reference standard: Positive FTS-ABS or MHA-TP serologic tests plus a diagnosis of late latent syphilis	Late latent syphilis (n = 1,303) Sensitivity: 63.6%	(*13*)
	Retrospective cross-sectional study Patients with neurosyphilis: 25 (24 patients were considered to have neurosyphilis, from which 8 had symptomatic neurosyphilis [disease meningovascular = 6; meningitis = 1; cranial neuritis =1], 16 asymptomatic neurosyphilis [no neurologic symptoms or signs], and 1 patient with all clinical and laboratory criteria of neurosyphilis, except increased proteins; all 25 were living with HIV) Syphilis positive control patients: 163 patients with syphilis based on serology and no signs of neurosyphilis Syphilis negative control patients with other neurologic disorders: 126 Reference standard: Reactive FTA-ABS, increased CSF protein ≥45 mg/dL and CSF pleocytosis ≥10 cell/mm^3	Combined data from asymptomatic and symptomatic neurosyphilis patients (n = 25) Sensitivity: 75% Specificity: 99.3% Asymptomatic neurosyphilis patients (n = 16) Sensitivity: 68.8% Symptomatic neurosyphilis patients (n = 8) Sensitivity: 100%	(*14*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
Unheated Serum Reagin (USR) §	Retrospective cross-sectional study based on stored serum from clinically classified patients Patients with primary syphilis: 119 Patients with secondary syphilis: 98 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 119) Sensitivity: 71.4% Secondary syphilis (n = 98) Sensitivity: 100%	(*5*)
Venereal Disease Research Laboratory (VDRL) §	Retrospective cross-sectional study Patients with primary syphilis: 106 Reference standard: Darkfield positive chancre and no signs of secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 106) Sensitivity: 72.6%	(*3*)
	Cross-sectional study Patients with primary syphilis: 109 Reference standard: Darkfield microscopy	Primary syphilis (n = 109) Sensitivity: 72.5%	(*4*)
	Retrospective cross-sectional study based on stored serum from clinically classified patients Patients with primary syphilis: 119 Patients with secondary syphilis: 98 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 119) Sensitivity: 66.4% Secondary syphilis (n = 98) Sensitivity: 100%	(*5*)
	Cross-sectional study Patients with primary syphilis: 111	Primary syphilis (n = 111) Sensitivity: 63.1%	(*6*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Patients with secondary syphilis: 56 Reference standard: (1) Primary syphilis—darkfield positive chancre and no signs of secondary syphilis; (2) secondary syphilis—darkfield positive secondary lesions or at least two symptoms of secondary syphilis, such as condylomatalata, alopecia, and lymphadenopathy	Secondary syphilis (n = 56) Sensitivity: 100%	
	Cross-sectional study Patients with primary syphilis: 80 Patients with secondary syphilis: 29 Reference standard: (1) Primary syphilis - darkfield positive chancre and no signs of secondary syphilis; (2) Secondary syphilis - darkfield positive secondary lesions or at least two symptoms of secondary syphilis such as condylomata lata, alopecia, and lymphadenopathy	Primary syphilis (n = 80) Sensitivity: 62.5% Secondary syphilis (n = 29) Sensitivity: 100%	(*7*)
	Cross-sectional study Patients with primary syphilis: 134 Patients with secondary syphilis: 217 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 134) Sensitivity: 78.4% Secondary syphilis (n = 217) Sensitivity: 100%	(*8*)
	Cross-sectional study Patients with primary syphilis: 63 Patients with secondary syphilis: 23 Reference standard: (1) Primary syphilis—darkfield positive chancre and no signs of secondary syphilis; (2) secondary	Primary syphilis (n = 63) Sensitivity: 76.2% Secondary syphilis (n = 23) Sensitivity: 100%	(*15*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	syphilis—darkfield positive secondary lesions or at least two symptoms of secondary syphilis, such as condylomata lata, alopecia, and lymphadenopathy		
	Cross-sectional study Patients with primary syphilis: 130 Reference standard: Darkfield positive chancre and no signs of secondary syphilis	Primary syphilis (n = 130) Sensitivity: 68.5%	(*16*)
	Cross-sectional study Patients with primary syphilis: 13 Patients with secondary syphilis: 16 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 13) Sensitivity: 76.9% Secondary syphilis (n =16) Sensitivity: 100%	(*17*)
	Cross-sectional study Patients with primary syphilis: 62 Reference standard: Darkfield positive chancre and no signs of secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 62) Sensitivity: 63%	(*18*)
	Retrospective cross-sectional study Patients with primary syphilis: 322 Reference standard: Darkfield positive chancre and no signs of secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 322) Sensitivity: 73.3%	(*19*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Retrospective cross-sectional study Patients with primary syphilis: 76 Patients with secondary syphilis: 100 Reference standard: Darkfield positive lesions consistent with primary and secondary syphilis (signs and symptoms not reported in the paper)	Primary syphilis (n = 76) Sensitivity: 50% Secondary syphilis (n = 100) Sensitivity: 100%	(*10*)
	Retrospective cross-sectional study Patients with early latent syphilis: 6 Patients with late latent syphilis: 12 Reference standard: Reactive TPPA, FTA-ABS tests and Western blot plus a diagnosis of syphilis (signs and symptoms not reported in the paper)	Early latent syphilis (n = 6) Sensitivity: 100% Late latent syphilis (n = 12) Sensitivity: 75%	(*20*)
	Retrospective cross-sectional study Patients with early latent syphilis: 23 Patients with late latent syphilis: 44 Reference standard: Reactive FTA-ABS, TPHA, and VDRL serologic tests plus a diagnosis of syphilis (signs and symptoms not reported in the paper). Early latent was defined as <1 year and late latent syphilis defined as >1 year	Early latent syphilis (n = 23) Sensitivity: 82.1% Late latent syphilis (n = 12) Sensitivity: 65.9%	(*21*)
	Cross-sectional study Patients with recent secondary syphilis: 17 Patients with recurrent secondary syphilis: 44 Patients with early latent syphilis: 34 Patients with late latent syphilis: 44	Recent secondary syphilis (n = 17) Sensitivity: 100% Recurrent secondary syphilis (n = 44) Sensitivity: 100% Early latent syphilis (n = 34) Sensitivity: 100%	(*22*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Reference standard: Positive FTA-ABS, TPHA, and CAPTIA Syphilis M serologic tests plus clinical findings consistent with secondary syphilis	Late latent syphilis (n = 44) Sensitivity: 63.6%	
	Prospective study Patients with secondary syphilis: 68 Patients with early latent syphilis: 72 Reference standard: (1) Secondary syphilis—based on clinical features consistent with secondary syphilis (lab confirmation and clinical features not reported in the paper); (2) early latent syphilis—reactive antitreponemal EIA, TPPA, or antitreponemal IgM EIA in the absence of clinical signs of infection in patients who had had nonreactive serology within the preceding 2 years or were known to have had recent sexual contact with an individual infected with syphilis.	Secondary syphilis (n = 68) Sensitivity: 100% Early latent syphilis (n = 72) Sensitivity: 100%	(*23*)

Abbreviations: FDA = Food and Drug Administration; PPA = percent positive agreement; PPN = percent negative agreement; PA = percent agreement; CI = confidence interval; FTA-ABS = fluorescent treponemal antibody-absorption; VDRL = Venereal Disease Research Laboratory; MHA-TP = microhemaggluntination assay for antibodies to *T. pallidum*; CSF = cerebral spinal fluid; TPPA = *T. pallidum* particle agglutination; TPHA = *T. pallidum* hemagglutination assay; EIA = enzyme immunoassay; RPR = rapid plasma reagin; IgG = immunoglobulin G; IgM = immunoglobulin M; N/A = not applicable

*Performance characteristics are stratified by syphilis stage if available. Otherwise, the performance characteristics are derived from data that did not specify the stage of syphilis.

†Unpublished data from the FDA 510(k) Substantial Equivalence Determination Decision Summary.

§Data reported from peer-reviewed studies are based on the methodology and not specific tests marketed in the United States. Unpublished data the FDA 510(k) Substantial Equivalence Determination Decision Summary for specific tests are not reported.

Supplementary Table 2. Performance characteristics of treponemal serologic tests used for the diagnosis of syphilis

Assay	Study summary and reference standard	Performance characteristics*	Reference
ADVIA Centaur† Siemens Medical Solutions USA, Inc 40 Liberty Blvd Malvern, PA 19355	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy plus reactive treponemal and nontreponemal serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no sexual contact with an individual with early latent syphilis in the past 12 months	Overall sensitivity (N = 262): 97.3% (95% CI: 94.6%– 98.9%) Overall specificity (N = 403): 95.5% (95% CI: 93%–97.3%) Primary syphilis (n = 55) Sensitivity: 94.5% (95% CI: 84.9%–98.9%) Secondary syphilis (n = 98) Sensitivity: 100% (95% CI: 96.2%–100%) Early latent syphilis (n = 41) Sensitivity: 100% (95% CI: 90.7%–100%) Late latent syphilis (n = 68) Sensitivity: 94.1% (95% CI: 85.6%–98.4%)	(*24*)
ADVIA Centaur Syphilis and Atellica IM Syphilis (Syph)	Prospective and retrospective cross-sectional clinical trial study for submission to FDA§ Patient samples collected from total study population: 2108	Patient samples collected from total study population (N = 2108) PPA: 97.9% (95% CI: 96.6%–98.8%) PNA: 99.4% (95% CI: 98.8%–99.7%)	(*25*)¶

Assay	Study summary and reference standard	Performance characteristics*	Reference
Siemens	Apparently healthy individuals: 806 (including 399 non-pregnant people, 332 pregnant people, and 75 pediatric patients) Expected positive population: 561 (including 272 TPPA reactive and 285 from patients who had been medically diagnosed with syphilis) Intended use population: 741 Reference standard: Commercially available syphilis assay (not reported) and previous laboratory testing. Stage of syphilis was not reported.	Apparently healthy individuals (N = 806) Non-pregnant people (n = 399) PPA: Not applicable PNA: 98.2% (389/396; 3 samples were reactive on both tests) Pregnant people (n = 332) PPA: Not applicable PNA: 99.7% (329/330; 1 sample was reactive on both tests and 1 sample was excluded because it was indeterminate on the predicate device) Pediatric patients (n = 75) PPA: Not applicable PNA: 98.6% (73/74; 1 sample was reactive on both tests) Expected positive population (N = 561) PPA: 99.4% (95% CI: 98.4%–99.9%) PNA: 100% (95% CI: 85.2%–100%) Intended use population (N=741) PPA: 98.2% (95% CI: 94.7%–99.6%) PNA: 98.4% (95% CI: 97.1%–99.3%)	
Architect Syphilis TP Abbott Laboratories 100 Abbott Park Rd Abbott Park, IL 60064	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Patient samples collected from intended use population: 1145 Preselected patient samples reactive in treponemal serologic tests: 406 (including 20 pregnant women) Apparently healthy individuals: 480 Patients with primary treated syphilis: 44 Patients with primary untreated syphilis: 25 Patients with secondary treated syphilis: 29 Patients with secondary untreated syphilis: 27	Samples from intended use population (N = 1145) PPA: 96.2% (95% CI: 92%–98.3%) PNA: 99% (95% CI: 98.1%–99.4%) Preselected patient samples (N = 406) Patients with reactive serology for syphilis (n = 386) PPA: 98.9% (95% CI: 97.2%–99.6%) PNA: 92.3% (95% CI: 75.9%–97.9%) Pregnant women with reactive serology for syphilis (n = 20) PPA: 100% (95% CI: 83.9%–100%) PNA: Not applicable	(*26*) §

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Patients with latent treated syphilis: 25 Patients with latent untreated syphilis: 29 Reference standard: Chemiluminescent immunoassay, RPR, and TPPA. Two out of three tests must be reactive for a sample to be considered reactive Stage of syphilis determined by a licensed physician based on the clinical symptoms, medical history, and laboratory test results at the time of diagnosis	Clinically diagnosed syphilis patients (N = 179) Primary treated (n = 44): 75% agreement Primary untreated (n = 25): 100% agreement Secondary treated (n = 29): 100% agreement Secondary untreated (n = 27): 100% agreement Latent treated (n = 25): 100% agreement Latent untreated (n = 25): 100% agreement All phases treated (n = 29): 100% agreement	
AtheNA Multi-Lyte *T. pallidum* IgG Plus Test System ZEUS Scientific 199 & 200 Evans Way Branchburg, NJ 08876	Retrospective cross-sectional clinical trial study for submission to the FDA Patient serum samples: 280 Previously characterized serum samples by syphilis stage Primary treated syphilis: 11 Secondary treated syphilis: 39 Secondary untreated syphilis: 43 Latent treated syphilis: 52 Latent untreated syphilis: 11 Congenital syphilis: 3 Reference standard for patient serum samples: Reactive RPR and TPPA Reference standard for clinically characterized serum sample: CDC specimen bank	Patient serum samples (N = 280) PPA: 96.3% (95% CI: 81%–99.9%) PNA: 96% (95% CI: 92.8%–98.1%) Primary treated (n = 11): 90.9% agreement (95% CI: 58.7%–99.8%) Secondary treated (n = 39): 100% agreement (95% CI: 92.6%–100%) Secondary untreated (n = 43): 93% agreement (95% CI: 80.8%–98.5%) Latent treated (n = 52): 86.5% agreement (95% CI: 74.2%–94.4%) Latent untreated (n = 11): 54.5% agreement (95% CI: 23.4%–83.3%) Congenital syphilis (n = 3): 66.7% agreement (95% CI: 9.4%–99.2%)	(*27*) ¶
CAPTIA Syphilis-G Assay** Trinity Biotech USA Inc 2823 Girts Rd Jamestown, NY 14701	Cross-sectional study Unselected screening specimens: 1,617 Known specimen panel: 114 Reference standard: VDRL reactive	Unselected screening specimens (N = 1,617) Sensitivity: 92.1% Specificity: 99.2% Retesting of unselected screening specimens Sensitivity: 92.1% Specificity: 99.2% Primary treated (n = 8): 100% agreement	(*28*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
		Primary untreated (n = 6): 100% agreement Secondary treated (n = 23): 95.7% agreement Secondary untreated (n = 3): 100% agreement Early latent treated (n = 11): 90.9% agreement Early latent untreated (n = 4): 100% agreement Late latent treated (n = 19): 94.7% agreement Late latent untreated (n = 13): 92.3% agreement Neurosyphilis treated (n = 5): 100% agreement Neurosyphilis untreated (n = 5): 100% agreement Cardiovascular syphilis treated (n = 1): 100% agreement Congenital syphilis treated (n = 1): 100% agreement Unknown syphilis stage treated (n = 2): 100% agreement Unknown treatment status (n = 13): 84.6% agreement	
	Cross-sectional study Unselected screening specimens: 1,184 Known specimen panel: 101 (89 were classified as primary, secondary, early latent, or late latent) Unselected screening serum samples reference standard: ICE Syphilis immunoassay (DiaSorin Molecular LLC), CDRL, TPHA, and FTA-ABS Clinical stage reference standard: Medical diagnosis and syphilis serology. Early latent and late latent cutoff was at two years, not one year	Unselected screening specimens (N = 1,184) Sensitivity: 91.4% Retesting of unselected screening specimens Sensitivity: 92.4% Known specimen panel classified as primary, secondary, early latent, and late latent (N = 89) Primary treated (n = 17): 88.2% agreement Primary untreated (n = 7): 100% agreement Secondary treated (n = 21): 90.5% agreement Secondary untreated (n = 2): 100% agreement Early latent treated (n = 9): 88.9% agreement Early latent untreated (n = 2): 100% agreement Late latent treated (n = 19): 100% agreement Late latent untreated (n = 12): 91.7% agreement	(*29*)
	Retrospective cross-sectional study Patients with untreated syphilis: 96 Patients with old syphilis: 63	Patient serum samples (N = 169) Primary syphilis (n = 17) Sensitivity: 82.3%	(*30*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Neonatal serum samples from mothers treated for syphilis: 10 Reference standard: Reactive MHA-TA, FTA-ABS, and chart review for clinical characterization	Secondary syphilis (n = 13) Sensitivity: 100% Early latent syphilis (n = 14) Sensitivity: 100% Late latent syphilis (n = 33) Sensitivity: 100% Neurosyphilis (n = 3) Sensitivity: 100% Congenital syphilis (n = 1) Sensitivity: 100% Reinfection (n = 15) Sensitivity: 100% Patients with old syphilis (n = 63) Sensitivity: 100% Neonatal serum from mothers treated for syphilis (n = 10) Sensitivity: 100%	
Elecsys Syphilis Roche Diagnostics 9115 Hague Rd Indianapolis, IN 46256	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Patient samples collected from intended use population: 2,282 (including 1,524 routine syphilis, 457 patients living with HIV, and 301 pregnant women) Preselected patient samples reactive in treponemal serologic tests: 169 (including 15 pregnant women) Apparently healthy individuals: 209	Samples from intended use population (N = 2,282) Overall PPA: 100% (95% CI: 98.4%–100%) Overall PNA: 99.2% (95% CI: 98.7%–99.5%) Routine syphilis (N = 1,524) PPA: 100% (95% CI: 94.6%–100%) PNA: 99.8% (95% CI: 99.4%–100%) Patients living with HIV (N = 457) PPA: 100% (95% CI: 97.8%–100%)	(*31*)¶

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Patients with primary treated syphilis: 29 Patients with primary untreated syphilis: 25 Patients with secondary treated syphilis: 25 Patients with secondary untreated syphilis: 25 Patients with latent treated syphilis: 25 Patients with latent untreated syphilis: 25 Reference standard: Chemiluminescent immunoassay, RPR, and TPPA. Two out of three tests must be reactive for a sample to be considered reactive Stage of syphilis determined by a licensed physician based on clinical symptoms, medical history, and laboratory test results at the time of diagnosis	PNA: 95.6% (95% CI: 92.6%–97.6%) Pregnant women (N = 301) PPA: Not applicable PNA: 100% (95% CI: 98.8%–100%) Preselected patient samples (N =169) PPA: 98.7% (95% CI: 95.5%–99.9%) PNA: 100% (95% CI: 73.5%–99.6%) Clinically diagnosed syphilis patients (N = 154) Primary treated (n = 29): 55.2% agreement Primary untreated (n = 25): 100% agreement Secondary treated (n = 25): 96% agreement Secondary untreated (n = 25): 100% agreement Latent treated (n = 25): 100% agreement Latent untreated (n = 25): 100% agreement	
Fluorescent Treponemal Antibody-Absorption Test (FTA-ABS) ††	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy (or if darkfield microscopy is not performed) plus reactive treponemal and nontreponemal serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests	Overall sensitivity (N = 262): 90.8% (95% CI: 86.7%–94%) Overall specificity (N = 403): 98% (95% CI: 96.1%–99.1%) Primary syphilis (n = 55) Sensitivity: 78.2% (95% CI: 65%–88.2%) Secondary syphilis (n = 98) Sensitivity: 92.8% (95% CI: 85.7%–97%) Early latent syphilis (n = 41) Sensitivity: 100% (95% CI: 90.7%–100%) Late latent syphilis (n = 68) Sensitivity: 92.6% (95% CI: 83.7%–97.6%)	(*24*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal (EIA or TPPA) and nontreponemal (RPR) serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no sexual contact with an individual with early latent syphilis in the past 12 months Reference standard for specificity (no syphilis): No diagnosis of syphilis on the day of testing or in the 6 months after the day of specimen collection, no syphilis in the past medical history, no reactive prior syphilis serology (all available lab records reviewed), and at least 4 out of 7 treponemal serologic tests were negative (after testing by CDC reference laboratory)		
	Retrospective cross-sectional study Patients with primary syphilis: 50 Patients with secondary syphilis: 43 Patients with latent syphilis: 47 Patients with neurosyphilis: 11 Reference standard for primary syphilis: Presence of a lesion or chancre plus presence of spirochetes in lesion or lymph node (method to visualize spirochetes was not described) and/or reactive serologic tests	Primary syphilis (n = 50) Sensitivity: 90% Secondary syphilis (n = 43) Sensitivity: 100% Latent syphilis (n = 47) Sensitivity: 100% Results for neurosyphilis presented in Supplementary Table 2	(*32*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Reference standard for secondary syphilis: Presence of spirochetes in generalized skin lesions or lymph node (method to visualize spirochetes was not described) and/or reactive serologic tests Reference standard for latent syphilis: Absence of symptoms or a history of syphilis plus reactive serologic tests Reference standard for neurosyphilis: Reactive FTA or TPHA plus reactive CSF VDRL or mononuclear cell count of >5 cell per µl of CSF		
	Retrospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 39 Patients with latent syphilis: 54 Patients with yaws: 15 Reference standard for new and old syphilis: Prior clinical diagnosis of syphilis	Primary syphilis (n = 55) Sensitivity: 84% Secondary syphilis (n = 39) Sensitivity: 100% Latent syphilis (n = 54) Sensitivity: 100% Yaws (n = 15) Sensitivity: 93%	(*33*)
	Prospective cross-sectional study Patients with primary syphilis: 63 Patients with secondary syphilis: 3 Reference standard for new and old syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy and/or reactive serologic tests or a four-fold increase in a quantitative RPR	Primary and secondary syphilis combined (n = 66) Sensitivity: 93% Specificity: 87%	(*34*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
Immulite 2000 Syphilis Screen Siemens Medical Solutions USA, Inc 40 Liberty Blvd Malvern, PA 19355	Prospective cross-sectional clinical trial study for submission to FDA Patient samples collected from intended use population: 1,286 (including 281 from patients medically diagnosed with syphilis of unknown stage, 420 patients living with HIV, and 924 samples submitted to laboratories for routine syphilis testing; some samples might overlap categories) Reference standard: Results compared with a commercially available assay	Retrospective serum samples (N = 1,286) Medically diagnosed syphilis of unknown stage (n = 281) PPA: 99.3% (95% CI: 97.4%–99.9%) PNA: 75% (95% CI: 34.9%–96.8%) Patients living with HIV (N = 420) PPA: 99.6% (95% CI: 97.9%–100%) PNA: 95.6% (95% CI: 91.1%–98.2%) Routine syphilis testing (N = 924) PPA: 99.4% (95% CI: 98%–99.9%) PNA: 99.1% (95% CI: 97.9%–99.7%)	(*35*)¶
LIAISON DiaSorin Molecular LLC 11331 Valley View St Cypress, CA 90630	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy plus reactive treponemal and nontreponemal serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal and nontreponemal serologic tests Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an	Overall sensitivity (N = 262): 96.9% (95% CI: 94.1%– 98.7%) Overall specificity (N = 403): 94.5% (95% CI: 91.8%–96.5%) Primary syphilis (n = 55) Sensitivity: 96.4% (95% CI: 94.5%–98.2%) Secondary syphilis (n = 98) Sensitivity: 100% (95% CI: 96.2%–100%) Early latent syphilis (n = 41) Sensitivity: 97.6% (95% CI: 87.4%–99.9%) Late latent syphilis (n = 68) Sensitivity: 96.2% (95% CI: 83.7%–97.6%)	(*24*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no sexual contact with an individual with early latent syphilis in the past 12 months Reference standard for specificity (no syphilis): No diagnosis of syphilis on the day of testing or in the 6 months after the day of specimen collection, no syphilis in the past medical history, no reactive prior syphilis serology (all available lab records reviewed), and at least 4 out of 7 treponemal serologic tests were negative (after testing by CDC reference laboratory)		
	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Apparently healthy non-pregnant people: 992 Pregnant people: 200 People living with HIV: 200 People diagnosed with syphilis: 51 Intended use population: 999 Reference standard: Trinity Captia Syphilis – G assay.	Apparently healthy non-pregnant people (N=992) PPA: 62.7% (95% CI: 51.7%–93.0%) PNA: 99.3% (95% CI: 98.5%–99.8%) Pregnant people (N=200) PPA: 100% (95% CI: 39.8%–100%) PNA: 100% (95% CI: 98.1%–100%) People living with HIV (N=200) PPA: 75.8% (95% CI: 65.8%–83.5%) PNA: 96.2% (95% CI: 90.4%–98.9%)	(*36*)¶

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Stage of syphilis was not reported.	People diagnosed with syphilis (N=51) PPA: 97.9% (95% CI: 89.0%–99.9%) PNA: 100% (95% CI: 2.5%–100%) Intended use population (N=999) PPA: 55% (95% CI: 38.9%–70.7%) PNA: 98.9% (95% CI: 98.0%–99.5%)	
Lumipulse G TP-N Fujirebio US, Inc 205 Great Valley Pkwy Malvern, PA 19355	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Patient samples collected from intended use population: 1,290 Retrospective samples: 1,472 (including 379 pregnant women, 520 patients living with HIV, 130 samples known to be reactive in treponemal serologic tests, 68 samples from a research facility from patients clinically diagnosed with syphilis, and 375 samples submitted to laboratories for routine syphilis testing) Apparently healthy individuals: 474 Patients with primary treated syphilis: 2 Patients with primary untreated syphilis: 27 Patients with secondary treated syphilis: 25 Patients with secondary untreated syphilis: 30 Patients with latent treated syphilis: 5 Patients with latent untreated syphilis: 200 Reference standard: Treponemal EIA, RPR, and TPPA. Two out of three tests must be reactive for a sample to be considered reactive	Samples from intended use population (N = 1,290) PPA: 92.7% (95% CI: 88.6%–95.4%) PNA: 99.6% (95% CI: 99%–99.9%) Retrospective serum samples (N = 1,472) Pregnant women (N = 379) PPA: 96.8% (95% CI: 91.1%–98.9%) PNA: 96.8% (95% CI: 94.1%–98.3%) Patients living with HIV (N = 520) PPA: 90.3% (95% CI: 85.9%–93.4%) PNA: 97.5% (95% CI: 95%–98.8%) Reactive by previous laboratory testing (n = 130) PPA: 99.2% (95% CI: 94.6%–99.8%) PNA: 100% (95% CI: 67.6%–100%) Routine syphilis (N = 375) PPA: 91.2% (95% CI: 77%–97%) PNA: 99.7% (95% CI: 98.4%–99.9%) Medically diagnosed syphilis of unknown stage (N = 68)	(*37*)¶

Assay	Study summary and reference standard	Performance characteristics*	Reference
		PPA: 98.2% (95% CI: 90.6%–99.7%) PNA: 91.7% (95% CI: 64.6%–98.5%)	
	Stage of syphilis determined by a licensed physician based on clinical symptoms, medical history, and laboratory test results at the time of diagnosis	Clinically diagnosed syphilis patients (N = 289) Primary treated (n = 2): 100% agreement Primary untreated (n = 27): 100% agreement Secondary treated (n = 25): 100% agreement Secondary untreated (n = 30): 100% agreement Latent treated (n = 5): 100% agreement Latent untreated (n = 200): 91.5% agreement	
Microhemagglun-tination Assay for Antibodies to *Treponema pallidum* (MHA-TP)††	Cross-sectional study Patients with primary syphilis: 109 Reference standard: Darkfield microscopy	Sensitivity: 72.5%	(*4*)
	Prospective cross-sectional study Patient serum samples: 510 (including 128 from patients with primary syphilis, 243 with secondary syphilis, and 139 with early latent syphilis) Reference standard: Darkfield microscopy, RPR, FTA-ABS	Primary syphilis (n = 128) Sensitivity: 88.6% Secondary syphilis (n = 243) Sensitivity: 98.8% Early latent syphilis (n = 139) Sensitivity: 100%	(*38*)
	Retrospective cross-sectional study Serum from patients with syphilis: 328 (including 78 from patients with primary syphilis, 89 with secondary syphilis, 103 with early latent syphilis, 10 from neurosyphilis, 21 from cardiovascular syphilis, and 25 from patients with old syphilis)	Primary syphilis (n = 78) Sensitivity: 88.6% Secondary syphilis (n = 89) Sensitivity: 100% Early latent syphilis (n = 103) Sensitivity: 99%	(*39*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Reference standard: Hemagglutination treponemal test for syphilis, MHA-TP, FTA-ABS, and VDRL. Darkfield microscopy.	Cardiovascular syphilis (n = 21) Sensitivity: 89.5% Old syphilis (n = 25) Sensitivity: 100% Results for neurosyphilis presented in Supplementary Table 2	
	Retrospective cross-sectional study Serum from patients with syphilis: 75 (including 24 from patients with primary syphilis, 20 with secondary syphilis, 27 with latent syphilis, 3 from neurosyphilis, and 1 from cardiovascular syphilis) Serum from patients without syphilis: 222 Reference standard: FTA-ABS	Primary syphilis (n = 24) Sensitivity: 45.9% Secondary syphilis (n = 20) Sensitivity: 90% Latent syphilis (n = 31) Sensitivity: 90.3% Cardiovascular syphilis (n = 1) Sensitivity: 100% Results for neurosyphilis presented in Supplementary Table 2	(*40*)
	Retrospective cross-sectional study Serum from patients with syphilis based on clinical history and laboratory findings: 312 (including 63 from patients with primary syphilis, 43 with secondary syphilis, 53 with early latent syphilis, 87 with late latent syphilis, and 66 from late symptomatic syphilis) Reference standard: VDRL, FTA-ABS, MHA-TP, and *T. pallidum* immobilization (TPI) test	Primary syphilis (n = 63) Percent reactive: MHA-TP 64%, VDRL 73%, FTA-ABS 82%, and TPI 67% Secondary syphilis (n = 43) Percent reactive: MHA-TP 96%, VDRL 100%, FTA-ABS 100%, and TPI 100% Early latent syphilis (n = 53) Percent reactive: MHA-TP 96%, VDRL 100%, FTA-ABS 98%, and TPI 96%	(*41*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
		Late latent syphilis (n = 87) Percent reactive: MHA-TP 97%, VDRL 93%, FTA-ABS 98%, and TPI 97% Early symptomatic syphilis (n = 66) Percent reactive: MHA-TP 98%, VDRL 94%, FTA-ABS 100%, and TPI 98%	
Treponema pallidum Passive Particle Agglutination (TPPA)††	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy plus reactive treponemal and nontreponemal serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal and nontreponemal serologic tests Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past	Overall sensitivity (N = 262): 95.4% (95% CI: 92.1%–97.6%) Overall specificity (N = 403): 100% (95% CI: 99%–100%) Primary syphilis (n = 55) Sensitivity: 94.5% (95% CI: 84.9%–98.9%) Secondary syphilis (n = 98) Sensitivity: 100% (95% CI: 96.2%–100%) Early latent syphilis (n = 41) Sensitivity: 100% (95% CI: 90.7%–100%) Late latent syphilis (n = 68) Sensitivity: 86.8% (95% CI: 76.4%–93.8%)	(*24*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	12 months, and no sexual contact with an individual with early syphilis in the past 12 months Reference standard for specificity (no syphilis): No diagnosis of syphilis on the day of testing or in the 6 months after the day of specimen collection, no syphilis in the past medical history, no reactive prior syphilis serology (all available lab records reviewed), and at least 4 out of 7 treponemal serologic tests were negative (after testing by CDC reference laboratory)		
	Prospective observational study Patients with primary syphilis: 50 Patients with secondary syphilis: 26 Patients with early latent syphilis: 8 Patients with late latent syphilis: 21 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes and reactive serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions and reactive serologic tests Reference standard for early latent syphilis: Reactive serologic tests and nonreactive serologic test in the past 2 years Reference standard for late latent syphilis: Reactive serologic tests and nonreactive serologic test in the past 2 years or no serologic tests within the past 2 years	Primary syphilis (n = 50) Sensitivity: 96% Secondary syphilis (n = 26) Sensitivity: 100% Early latent syphilis (n = 8) Sensitivity: 100% Late latent syphilis (n = 21) Sensitivity: 100%	(*42*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Prospective cross-sectional study Patients with primary syphilis: 39 Patients with secondary syphilis: 20 Patients with early latent syphilis: 18 Patients with late latent syphilis: 58 Reference standard for primary syphilis: Presence of a lesion or chancre and reactive serologic tests Reference standard for secondary syphilis: Mucocutaneous lesions and reactive serologic tests Reference standard for early latent syphilis: no symptoms or signs together with reactive syphilis serology results and nonreactive syphilis serology results within past 12 months Reference standard for late latent syphilis: no symptoms or signs together with reactive syphilis serology results and no nonreactive syphilis serology results within the past 12 months.	Primary syphilis (n = 39) TPPA sensitivity: 94.9% (95% CI: 83.1%–98.6%) FTA-ABS sensitivity: 84.6% (95% CI: 70.3%–92.8%) Secondary syphilis (n = 20) TPPA sensitivity: 100% (95% CI: 83.9%–100%) FTA-ABS sensitivity: 95% (95% CI: 76.4%–99.1%) Early latent syphilis (n = 18) TPPA sensitivity: 94.4% (95% CI: 74.2%–99.0%) FTA-ABS sensitivity: 94.4% (95% CI: 74.2%–99.0%) Late latent syphilis (n = 58) TPPA sensitivity: 91.4% (95% CI: 81.4%–96.3%) FTA-ABS sensitivity: 84.5% (95% CI: 73.1%–91.6%) Specificity: 100% (95% CI: 91.8%–100%) for all tests	(*43*)
Trep-Sure Trinity Biotech USA Inc 2823 Girts Rd Jamestown, NY 14701	Prospective cross-sectional study Patients with primary syphilis: 55 Patients with secondary syphilis: 98 Patients with early latent syphilis: 41 Patients with late latent syphilis: 68 Reference standard for primary syphilis: Presence of a lesion or chancre with visible spirochetes on darkfield microscopy or the absence of spirochetes on darkfield microscopy plus reactive treponemal and nontreponemal serologic tests	Overall sensitivity (N = 262): 98.5% (95% CI: 96.1%–99.6%) Overall specificity (N = 403): 82.6% (95% CI: 78.4%–86.1%) Primary syphilis (n = 55) Sensitivity: 94.5% (95% CI: 84.9%–98.9%) Secondary syphilis (n = 98) Sensitivity: 100% (95% CI: 96.2%–100%) Early latent syphilis (n = 41) Sensitivity: 100% (95% CI: 90.7%–100%)	(*24*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Reference standard for secondary syphilis: Mucocutaneous lesions with reactive treponemal and nontreponemal serologic tests Reference standard for early latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests and no history of prior syphilis or prior sexual contact with an individual with early syphilis within the past 12 months or prior nonreactive serology within the past 12 months Reference standard for late latent syphilis: Absence of symptoms plus reactive treponemal and nontreponemal serologic tests or two reactive treponemal serologic tests, no history of prior syphilis, no serologic test results on the past 12 months, and no sexual contact with an individual with early syphilis in the past 12 months	Late latent syphilis (n = 68) Sensitivity: 98.5% (95% CI: 92.1%–99.9%)	
	Retrospective cross-sectional study Patients with primary syphilis: 52 Reference standard for primary syphilis: Presence of a lesion or chancre, reactive serologic tests, and no reported history of syphilis	Primary syphilis (n = 52) Trep-Sure sensitivity: 53.8% (95% CI: 39.5%–67.8%) RPR sensitivity: 76.9% (95% CI: 63.2%–87.5%)	(*44*)
	Prospective and retrospective cross-sectional clinical trial study for submission to FDA. Apparently healthy non-pregnant people: 1,655 People suspected of or diagnosed with syphilis: 636 Reference standard: TPPA or TPHA. Stage of syphilis was not reported.	Apparently healthy non-pregnant people (N=1,655) PPA: 100% (95% CI: 79.4%–100%) PNA: 99.8% (95% CI: 99.4%–100%) People suspected of or diagnosed with syphilis (N=636) PPA: 99.5% (95% CI: 98.4%–99.9%) PNA: 91.9% (95% CI: 87.1%–95.3%)	(*45*)§

Assay	Study summary and reference standard	Performance characteristics*	Reference
Zeus Scientific *T. pallidum* IgG Test System ZEUS Scientific 199 & 200 Evans Way Branchburg, NJ 08876	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Specimens submitted for routine syphilis testing: 500 Specimens from pregnant women submitted for routine syphilis testing: 500 Unselected specimens from hospitalized patients: 1,000 Retrospective specimens from patients living with HIV: 223 Retrospective specimens known to be reactive to RPR and TPPA: 280 Retrospective specimens from pregnant persons known to have been previously tested by RPR and TPPA: 250 nonreactive both tests and 27 reactive both tests CDC specimen panel: 157 (clinically staged) Reference standard: Phoenix Bio-Tech Syphilis Trep-Check Test	Specimens submitted for routine syphilis testing (N = 500) PPA: 80% (95% CI: 28.4%–99.5%) PNA: 99.2% (95% CI: 97.9%–99.8%) Specimens from pregnant women submitted for routine syphilis testing (N = 500) PPA: 75% (95% CI: 19.4%–99.4%) PNA: 100% (95% CI: 99.4%–100%) Unselected specimens from hospitalized patients (N = 1,000) PPA: 61.9% (95% CI: 38.4%–81.9%) PNA: 97.1% (95% CI: 95.9%–98.1%) Retrospective specimens from patients living with HIV (N = 223) PPA: 85.4% (95% CI: 72.2%–93.9%) PNA: 99.4% (95% CI: 96.9%–100%) Retrospective specimens known to be reactive to RPR and TPPA (N = 280) PPA: 98.5% (95% CI: 96.2%–99.6%) PNA: 70.6% (95% CI: 46.9%–98.7%) Retrospective specimens from pregnant persons known to have been previously tested by RPR and TPPA (n = 250 nonreactive both tests and N=27 reactive both tests) PPA: 92.9% (95% CI: 76.5%–99.1%) PNA: 99.6% (95% CI: 97.8%–100%) CDC specimen panel (N = 157)	(*46*)¶

Assay	Study summary and reference standard	Performance characteristics*	Reference
		Primary treated (n = 11): 100% agreement (95% CI: 76.2%–100%) Secondary treated (n = 39): 100% agreement (95% CI: 92.6%–100%) Secondary untreated (n = 43): 95.3% agreement (95% CI: 84.2%–99.4%) Latent treated (n = 50): 96% agreement (95% CI: 86.3%–99.5%) Latent untreated (n = 11): 54.5% agreement (95% CI: 23.4%–83.3%) Congenital syphilis (n = 3): 33.3% agreement (95% CI: 0.84%–90.6%) Late latent untreated (n = 12): 91.7% agreement	

Abbreviations: FDA = Food and Drug Administration; PPA = percent positive agreement; PPN = percent negative agreement; PA = percent agreement; CI = confidence interval; FTA-ABS = fluorescent treponemal antibody-absorption; VDRL = Venereal Disease Research Laboratory; MHA-TP = microhemaggluntination assay for antibodies to *T. pallidum*; CSF = cerebral spinal fluid; TPPA = *T. pallidum* particle agglutination; TPHA = *T. pallidum* hemagglutination assay; EIA = enzyme immunoassay; RPR = rapid plasma reagin; IgG = immunoglobulin G; IgM = immunoglobulin M; N/A = not applicable

*Performance characteristics are stratified by syphilis stage if available. Otherwise, the performance characteristics are derived from data that did not specify the stage of syphilis.

†The study stated data from the Advia Centaur Syphilis immunoassay but did not specify if the assay used was Advia Centaur Syphilis CP or Advia Centaur XP/XPT Syphilis System.

§The FDA 510(k) Substantial Equivalence Determination Decision Summary covers the reagents and calibrators for the Advia Centaur Syphilis CP/ XP/XPT and Atellica IM Syphilis (Syph) analyzers.

¶Unpublished data from the FDA 510(k) Substantial Equivalence Determination Decision Summary.

**Unpublished data the FDA 510(k) Substantial Equivalence Determination Decision Summary for specific tests are not available.

††Data reported from peer-reviewed studies are based on the methodology and not specific tests marketed in the United States. Unpublished data the FDA 510(k) Substantial Equivalence Determination Decision Summary for specific tests are not reported.

Supplementary Table 3. Performance characteristics of combined nontreponemal (lipoidal antigen) and treponemal serologic assays used for the diagnosis of syphilis

Assay	Study summary and reference standard	Performance characteristics*	Reference
BioPlex 2200 Syphilis Total & RPR Biorad, 2000 Alfred Nobel Dr Hercules, CA 94547	Prospective and retrospective cross-sectional clinical trial study for submission to FDA Prospective samples: 1,001 (including 401 samples submitted for syphilis testing, 295 from pregnant women, and 305 patients living with HIV) Retrospective samples: 546 (including 412 reactive by RPR and treponemal serologic test, 32 syphilis-positive pregnant women, 45 pregnant women with a history of STD infection, and 57 HIV/syphilis dual-positive patients) Apparently healthy individuals: 301 Clinically diagnosed patients: 156 Reference standard: Treponemal IgG/IgM assay, a nontreponemal serologic test, and TPPA. Two out of three tests must be reactive for a sample to be considered reactive. Bioplex 2200 RPR results compared with BD Macro-Vue RPR card Tests. Stage of syphilis determined by a licensed physician based on clinical symptoms, medical history, and laboratory test results at the time of diagnosis	BioPlex Total testing of prospective samples compared two of three tests being reactive (N = 1,001) PPA: 92.5% (95% CI: 87.3%–95.6%) PNA: 97.9% (95% CI: 96.7%–98.6%) BioPlex RPR component testing of prospective samples compared with BD Macro-Vue RPR Card Tests (N = 1,001) PPA: 81.5% (95% CI: 72.4%–88.1%) PNA: 96.5% (95% CI: 95.1%–97.5%) BioPlex Total testing of retrospective samples compared two of three tests being reactive (n = 546) PPA: 99.6% (95% CI: 98.5%–99.9%) PNA: 100% (95% CI: 93.6%–100%) BioPlex RPR component testing of retrospective samples compared with BD Macro-Vue RPR Card Tests (n = 546) PPA: 98.1% (95% CI: 96.4%–99.1%) PNA: 80.7% (95% CI: 72.5%–86.9%) BioPlex Total testing of samples pregnant women compared two of three tests being reactive (n = 372) PPA: 100% (95% CI: 89.3%–100%) PNA: 98.8% (95% CI: 97%–99.5%) BioPlex RPR component testing of samples pregnant women compared with BD Macro-Vue RPR Card Tests (n = 372) PPA: 100% (95% CI: 86.7%–100%)	(*47*) †

Assay	Study summary and reference standard	Performance characteristics*	Reference
		PNA: 98.3% (95% CI: 96.3%–99.2%) BioPlex Total testing of samples from patients living with HIV compared two of three tests being reactive (n = 362) PPA: 93.3% (95% CI: 88.2%–96.3%) PNA: 93.9% (95% CI: 89.8%–96.4%) BioPlex RPR component testing of samples from patients living with HIV compared with BD Macro-Vue RPR Card Tests (N=362) PPA: 85.7% (95% CI: 72.2%–93.3%) PNA: 90.6% (95% CI: 86.9%–93.4%) BioPlex Total reactivity compared two of three tests being reactive in medically diagnosed syphilis patients (n = 156) Primary treated (n = 29): BioPlex Total reactivity 86.2%; comparator algorithm reactivity 86.2% Primary untreated (n = 26): BioPlex Total reactivity 96.2%; comparator algorithm reactivity 100% Secondary treated (n = 26): BioPlex Total reactivity 100%; comparator algorithm reactivity 100% Secondary untreated (n = 25): BioPlex Total reactivity 100%; comparator algorithm reactivity 100% Latent treated (n = 27): BioPlex Total reactivity 100%; comparator algorithm reactivity 100% Latent untreated (n = 23): BioPlex Total reactivity 100%; comparator algorithm reactivity 100% All phases treated (n = 82): BioPlex Total reactivity 95.1%; comparator algorithm reactivity 95.1% All phases untreated (n = 74): BioPlex Total reactivity 98.6%; comparator algorithm reactivity 100%	

Assay	Study summary and reference standard	Performance characteristics*	Reference
		BioPlex Total testing of samples from apparently healthy individuals compared two of three tests being reactive (n = 301) PPA: 75% (95% CI: 30.1%–95.5%) PNA: 99% (95% CI: 97.1%–95.7%) BioPlex RPR component testing of samples from apparently healthy individuals compared with BD Macro-Vue RPR Card Tests (N = 301) PPA: 0% (95% CI: 0%–49%) PNA: 98% (95% CI: 95.7%–99.1%) BioPlex RPR reactivity compared with BD Macro-Vue RPR Card Tests in medically diagnosed syphilis patients (N = 156) Primary treated (n =29): BioPlex RPR reactivity 65.5%; RPR card reactivity 75.9% Primary untreated (n = 26): BioPlex RPR reactivity 92.3%; RPR card reactivity 88.5% Secondary treated (n = 26): BioPlex RPR reactivity 88.5%; RPR card reactivity 80.8% Secondary untreated (n = 25): BioPlex RPR reactivity 100%; RPR card reactivity 100% Latent treated (n = 27): BioPlex RPR reactivity 66.7%; RPR card reactivity 66.7% Latent untreated (n = 23): BioPlex RPR reactivity 95.7%; RPR card reactivity 95.7% All phases treated (n = 82): BioPlex RPR reactivity 73.2%; RPR card reactivity 74.4% All phases untreated (n = 74): BioPlex RPR reactivity 95.9%; RPR card reactivity 95%	

Abbreviations: FDA = Food and Drug Administration; PPA = percent positive agreement; PPN = percent negative agreement; PA = percent agreement; CI = confidence interval; FTA-ABS = fluorescent treponemal antibody-absorption; VDRL = Venereal Disease Research Laboratory; MHA-TP = microhemaggluntination assay for antibodies to *T. pallidum*; CSF = cerebral spinal fluid; TPPA = *T. pallidum* particle agglutination; TPHA = *T. pallidum* hemagglutination assay; EIA = enzyme immunoassay; RPR = rapid plasma reagin; IgG = immunoglobulin G; IgM = immunoglobulin M; N/A = not applicable

*Performance characteristics are stratified by syphilis stage if available. Otherwise, the performance characteristics are derived from data that did not specify the stage of syphilis.
†Unpublished data from the FDA 510(k) Substantial Equivalence Determination Decision Summary.

Supplementary Table 4. Performance characteristics of nontreponemal (lipoidal antigen) tests used to detect syphilis reactive antibodies in the cerebral spinal fluid

Assay	Study summary and reference standard	Performance characteristics	Reference
Rapid Plasma Reagin (RPR)	Retrospective cross-sectional study Patients with neurosyphilis: 25 (24 patients were considered to have neurosyphilis, from which 8 had symptomatic neurosyphilis [disease meningovascular = 6; meningitis = 1; cranial neuritis = 1], 16 asymptomatic neurosyphilis [no neurologic symptoms or signs], and 1 patient with all clinical and laboratory criteria of neurosyphilis, except increased proteins; all 25 were living with HIV) Syphilis-positive control patients: 163 patients with syphilis based on serology and no signs of neurosyphilis Syphilis-negative control patients with other neurologic disorders: 126 Reference standard: Reactive FTA-ABS, increased CSF protein ≥45 mg/dL, and CSF pleocytosis ≥10 cell/mm^3	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 25) CSF RPR sensitivity: 75% CSF RPR specificity: 99.3% Asymptomatic neurosyphilis patients (n = 16) CSF RPR sensitivity: 68.8% Symptomatic neurosyphilis patients (n = 8) CSF RPR sensitivity: 100%	(*14*)
	Prospective cross-sectional study Patients with asymptomatic neurosyphilis: 56 Patients with symptomatic neurosyphilis: 154 Asymptomatic neurosyphilis reference standard: ≥10 white blood cells in the CSF and reactive CSF TPPA with no blood contamination	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 210) CSF RPR sensitivity: 76.2% (95% CI: 70.2%–82.2%) CSF RPR specificity: 93.4% (95% CI: 91.4%–95.4%) CSF RPR-V* sensitivity: 79.2% (95% CI: 73.5%–85.5%) CSF RPR-V* specificity: 92.7% (95% CI: 90.7%–94.7%) Asymptomatic neurosyphilis patients (n = 56)	(*48*)

Assay	Study summary and reference standard	Performance characteristics	Reference
	Symptomatic neurosyphilis reference standard: Reactive CSF TPPA with no blood contamination and with clinical signs and symptoms	CSF RPR sensitivity: 60.7% (95% CI: 50.7%–70.7%) CSF RPR specificity: 82.6% (95% CI: 80.6%–84.6%) CSF RPR-V* sensitivity: 69.6% (95% CI: 59.6%–79.6%) CSF RPR-V* specificity: 87.8% (95% CI: 79.8%–83.8%) Symptomatic neurosyphilis patients (n = 154) CSF RPR sensitivity: 81.8% (95% CI: 75.8%–87.8%) CSF RPR specificity: 90.2% (95% CI: 88.2%–92.2%) CSF RPR-V* sensitivity: 83.1% (95% CI: 77.1%–89.1%) CSF RPR-V* specificity: 89.1% (95% CI: 87.1%–91.1%)	
	Retrospective cross-sectional study Patients with neurosyphilis: 149 Patients with symptomatic neurosyphilis: 33 Neurosyphilis reference standard: Reactive CSF FTA-ABS and >20 white blood cells in the CSF Symptomatic neurosyphilis reference standard: Vision or hearing loss with clinical or serologic evidence of neurosyphilis	Neurosyphilis patients (N = 149) CSF RPR sensitivity: 56.4% (95% CI: 40.8%–72%) CSF RPR specificity: 100% (95% CI: 100%–100%) CSF RPR-V* sensitivity: 59% (95% CI: 43.6%–74.4%) CSF RPR-V* specificity: 98.4% (95% CI: 95%–100%) Symptomatic neurosyphilis patients (n = 33) CSF RPR sensitivity: 51.5% (95% CI: 34.4%–68.6%) CSF RPR specificity: 89.7% (95% CI: 84.2%–95.2%) CSF RPR-V* sensitivity: 57.6% (95% CI: 40.7%–74.5%) CSF RPR-V* specificity: 84.5% (95% CI: 77.9%–91.1%)	(*49*)

Assay	Study summary and reference standard	Performance characteristics	Reference
Toluidine Red Unheated Serum Test (TRUST)	Prospective cross-sectional study Patients with asymptomatic neurosyphilis: 56 Patients with symptomatic neurosyphilis: 154 Asymptomatic neurosyphilis reference standard: ≥10 white blood cells in the CSF and reactive CSF TPPA with no blood contamination Case classification: Symptomatic neurosyphilis reference standard: Reactive CSF TPPA with no blood contamination and with clinical signs and symptoms	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 210) CSF TRUST sensitivity: 76.2% (95% CI: 70.2%–82.2%) CSF TRUST specificity: 93.1% (95% CI: 91.1%–95.1%) Asymptomatic neurosyphilis patients (n = 56) CSF TRUST sensitivity: 58.9% (95% CI: 48.9%–68.9%) CSF TRUST specificity: 82.1% (95% CI: 80.1%–84.1%) Symptomatic neurosyphilis patients (n = 154) CSF TRUST sensitivity: 82.5% (95% CI: 76.5%–88.5%) CSF TRUST specificity: 90.1% (95% CI: 76.5%–88.5%)	(*48*)
Venereal Disease Research Laboratory (VDRL)	Retrospective cross-sectional study Patients with neurosyphilis: 25 (24 patients were considered to have neurosyphilis, from which 8 had symptomatic neurosyphilis [disease meningovascular = 6; meningitis = 1; cranial neuritis =1], 16 asymptomatic neurosyphilis [no neurologic symptoms or signs], and 1 patient with all clinical and laboratory criteria of neurosyphilis, except increased proteins; all 25 were living with HIV) Syphilis positive control patients: 163 patients with syphilis based on serology and no signs of neurosyphilis Syphilis negative control patients with other neurologic disorders: 126	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 25) CSF VDRL sensitivity: 70.8% CSF VDRL specificity: 99% Asymptomatic neurosyphilis patients (n = 16) CSF VDRL sensitivity: 62.5% Symptomatic neurosyphilis patients (n = 8) CSF VDRL sensitivity: 87.5%	(*14*)

Assay	Study summary and reference standard	Performance characteristics	Reference
	Reference standard: Reactive FTA-ABS, increased CSF protein ≥45 mg/dL, and CSF pleocytosis ≥10 cell/mm^3		
	Prospective cross-sectional study Patients with asymptomatic neurosyphilis: 56 Patients with symptomatic neurosyphilis: 154 Asymptomatic neurosyphilis reference standard: ≥10 white blood cells in the CSF and reactive CSF TPPA with no blood contamination Symptomatic neurosyphilis reference standard: Reactive CSF TPPA with no blood contamination and with clinical signs and symptoms	Combined data from asymptomatic and symptomatic neurosyphilis patients (N = 210) CSF VDRL sensitivity: 81.4% (95% CI: 75.4%–87.4%) CSF VDRL specificity: 90.3% (95% CI: 88.3%–92.3%) Asymptomatic neurosyphilis patients (n = 56) CSF VDRL sensitivity: 69.6% (95% CI: 59.6%–79.6%) CSF VDRL specificity: 79.4% (95% CI: 77.4%–81.4%) Symptomatic neurosyphilis patients (n = 154) CSF VDRL sensitivity: 85.7% (95% CI: 79.7%–91.7%) CSF VDRL specificity: 86.7% (95% CI: 84.7%–88.7%)	(*48*)
	Retrospective cross-sectional study Patients with neurosyphilis: 149 Patients with symptomatic neurosyphilis: 33 Neurosyphilis reference standard: Reactive CSF FTA-ABS and >20 white blood cells in the CSF Symptomatic neurosyphilis reference standard: Vision or hearing loss with clinical or serologic evidence of neurosyphilis	Neurosyphilis patients (n = 149) CSF VDRL sensitivity: 71.8% (95% CI: 57.7%–85.9%) CSF VDRL specificity: 98.3% (95% CI: 95%–100%) Symptomatic neurosyphilis patients (n = 33) CSF VDRL sensitivity: 66.7% (95% CI: 50.6%–82.8%) CSF VDRL specificity: 80.2% (95% CI: 72.9%–87.5%)	(*49*)

Abbreviations: CSF = cerebral spinal fluid; RPR = rapid plasma reagin; FTA-ABS = fluorescent treponemal antibody-absorption; CI = confidence interval; TPPA = *T. pallidum* particle agglutination; TRUST = Toluidine Red Unheated Serum Test; VDRL = Venereal Disease

Research Laboratory; TPHA = *T. pallidum* hemagglutination assay; MHA-TP = microhemaggluntination assay for antibodies to *T. pallidum*; NAAT = nucleic acid amplification test
*CSF RPR-V is a modified RPR by diluting it 1:2 in 10% saline to account for the lower concentration of immunoglobulin in CSF compared with serum.

Supplementary Table 5. Performance characteristics of treponemal tests used to detect syphilis reactive antibodies in the cerebral spinal fluid

Assay	Study summary and reference standard	Performance characteristics	Reference
Fluorescent Treponemal Antibody-Absorption Test (FTA-ABS)	Retrospective cross-sectional study Patients with primary syphilis: 50 Patients with secondary syphilis: 43 Patients with latent syphilis: 47 Patients with neurosyphilis: 11 Reference standard for primary syphilis: Presence of a lesion or chancre plus presence of spirochetes in lesion or lymph node (method to visualize spirochetes was not described) and/or reactive serologic tests Reference standard for secondary syphilis: Presence of spirochetes in generalized skin lesions or lymph node (method to visualize spirochetes was not described) and/or reactive serologic tests Reference standard for latent syphilis: Absence of symptoms or a history of syphilis plus reactive serologic tests Reference standard for neurosyphilis: Reactive FTA-ABS or TPHA plus reactive CSF VDRL or mononuclear cell count of >5 cell per µl of CSF	Neurosyphilis (n = 11) CSF FTA-ABS sensitivity: 100% Results for syphilis other than neurosyphilis presented in Supplementary Table 1	(*32*)

Assay	Study summary and reference standard	Performance characteristics	Reference
Microhemagglutination Assay for Antibodies to *Treponema pallidum* (MHA-TP)	Retrospective cross-sectional study Serum from patients with syphilis: 75 (including 24 from patients with primary syphilis, 20 with secondary syphilis, 27 with latent syphilis, 3 with neurosyphilis, and 1 with cardiovascular syphilis) Serum from patients without syphilis: 222 Reference standard: CSF FTA-ABS	Neurosyphilis (n = 3) CSF MHA-TP sensitivity: 66.7% Results for syphilis other than neurosyphilis presented in Supplementary Table 1	(*40*)
Treponema pallidum Passive Particle Agglutination (TPPA)	Prospective cross-sectional study Two data sets Training data set (CSF samples from individuals enrolled in a study of CSF abnormalities in syphilis; n = 191), including 45 with *T. pallidum* detected in CSF by NAAT and 40 with symptoms Validation data set (study participants enrolled after the last training sample was collected; n = 380), including 41 with *T. pallidum* detected in CSF by NAAT and 95 with symptoms Reference standard: CSF VDRL positive or *T. pallidum* detected in CSF or new vision or hearing loss with clinical or serologic evidence of syphilis	Training dataset compared with *T. pallidum* detected in CSF by NAAT CSF TPPA sensitivity: 75.6% (95% CI: 63.0%–88.1%) CSF TPPA specificity with a titer ≥1:160: 63.0% (95% CI: 55.2%–70.8%) CSF TPPA specificity with a titer ≥1:320: 73.3% (95% CI: 66.1%–80.5%) CSF TPPA specificity with a titer ≥1:640: 81.5% (95% CI: 75.2%–87.8%) CSF FTA-ABS sensitivity: 66.7% (95% CI: 52.9%–80.4%) CSF VDRL sensitivity: 58.9% (95% CI: 34.3%–63.5%) Training dataset compared with new vision or hearing loss CSF TPPA sensitivity: 77.5% (95% CI: 64.6%–90.4%) CSF TPPA specificity with a titer ≥1:160: 63.4% (95% CI: 55.5%–71.3%) CSF TPPA specificity with a titer ≥1:320: 75.4% (95% CI: 68.3%–82.5%) CSF TPPA specificity with a titer ≥1:640: 85.2% (95% CI: 79.4%–91.0%)	(*50*)

Assay	Study summary and reference standard	Performance characteristics	Reference
		CSF FTA-ABS sensitivity: 77.5% (95% CI: 64.6%–90.4%) CSF VDRL sensitivity: 67.5% (95% CI: 53.0%–82.0%) Training dataset compared with reactive CSF VDRL CSF TPPA sensitivity: 95.0% (95% CI: 89.5%–100%) CSF TPPA specificity with a titer ≥1:160: 75.6% (95% CI: 68.2%–83.0%) CSF TPPA specificity with a titer ≥1:320: 86.3% (95% CI: 80.4%–92.2%) CSF TPPA specificity with a titer ≥1:640: 93.9% (95% CI: 89.8%–98.0%) CSF FTA-ABS sensitivity: 98.3% (95% CI: 95.0%–100%) Validation dataset compared with *T. pallidum* detected in CSF by NAAT CSF TPPA specificity with a titer ≥1:640: 93.8% (95% CI: 91.2%–96.4%) CSF VDRL specificity: 91.2% (95% CI: 88.1%–94.2%) Validation dataset compared with new vision or hearing loss CSF TPPA specificity with a titer ≥1:640: 93.3% (95% CI: 90.4%–96.2%) CSF VDRL specificity: 90.2% (95% CI: 86.7%–93.6%) Validation dataset compared with reactive CSF VDRL	

Assay	Study summary and reference standard	Performance characteristics	Reference
		CSF TPPA specificity with a titer ≥1:640: 97.0% (95% CI: 95.2%–98.8%) No difference in sensitivity or specificity based on HIV status	

Abbreviations: CSF = cerebral spinal fluid; RPR = rapid plasma reagin; FTA-ABS = fluorescent treponemal antibody-absorption; CI = confidence interval; TPPA = *T. pallidum* particle agglutination; TRUST = Toluidine Red Unheated Serum Test; VDRL = Venereal Disease Research Laboratory; TPHA = *T. pallidum* hemagglutination assay; MHA-TP = microhemaggluntination assay for antibodies to *T. pallidum*; NAAT = nucleic acid amplification test

Supplementary Table 6. Performance characteristics of tests for the direct detection of *T. pallidum*

Direct Detection Test	Study Summary and Reference Standard	Performance Characteristics	Reference
Darkfield microscopy	Prospective cross-sectional study Patients with primary syphilis: 63 Patients with secondary syphilis: 3 Patients without syphilis: 62 Syphilitic patients with genital lesion(s): 63 Syphilitic patients with anogenital lesion(s): 3 Non-syphilitic patients with genital lesion(s): 59 Non-syphilitic patients with anogenital lesion(s): 3 Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy, direct fluorescence microscopy using H9-1 monoclonal antibody to 47-58kDa tp protein, RPR serology	Patients with primary or secondary syphilis (n = 66) Positive by darkfield microscopy: 78.8% Positive by direct fluorescence microscopy: 72.7% Non-syphilitic patients with genital or anogenital lesions (n = 62) Positive by darkfield microscopy: 0% Positive by direct fluorescence microscopy: 0% Results were not grouped by stage of syphilis or anatomic site of lesion	(*34*)

Syphilis diagnosis: Clinical presentation and RPR serology		
Prospective cross-sectional study Patients with secondary syphilis: 12 Patients with non-syphilitic lesions: 24 Specimen types: Lesion exudate and biopsy Tests performed: Darkfield microscopy, PCR tp47 (amplicons detected by Southern blot for 25bp region and sequenced), IHC on FFPE using avidin-biotin peroxidase complex technique with polyclonal antibodies (BioCare) Syphilis diagnosis: Clinical presentation, RPR, and TPHA serology	Patients with secondary syphilis (n = 12) Positive by darkfield microscopy: 58% Positive by PCR: 75% Positive by IHC: 91.7% Patients without syphilis (n = 24) Positive by darkfield microscopy: 0% Positive by PCR: 0% Positive by IHC: 0%	(*51*)
Prospective cross-sectional study Two studies with only study A relevant to darkfield microscopy Study A Patients with skin lesion(s): 350 Stage of syphilis not defined Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy, PCR tp47 (amplicons detected by Southern blot for 25bp region and sequenced),	Patients with skin lesions (n = 350) Sensitivity of darkfield microscopy: 73.8% Specificity of darkfield microscopy: 97.4%	(*52*)

immunohistochemistry on FFPE using avidin-biotin peroxidase complex technique with rabbit polyclonal antibodies Syphilis diagnosis: Clinical presentation, VDRL, and FTA-ABS serology Sensitivity and specificity based on clinical diagnosis of syphilis		
Prospective cross-sectional study Patients with primary syphilis: 87 (specimens from 65 patients used to assess darkfield microscopy) Patients with secondary syphilis: 103 (specimens from 44 patients used to assess darkfield microscopy) Patients without syphilis: 35 (specimens from 12 patients used to assess darkfield microscopy) Primary syphilis patients with genital lesions: 35 Primary syphilis patients with anal lesions: 6 Primary syphilis patients with oral lesions: 4 Primary syphilis patients with cutaneous lesions: 2 Primary syphilis patients with lesions from unknown anatomic site: 18 Secondary syphilis patients with genital lesions: 22 Secondary syphilis patients with anal lesions: 3	Patients with primary syphilis assessed by darkfield microscopy (n = 65) Positive by darkfield microscopy: 75.4% Patients with primary syphilis and genital lesions (n = 35) Positive by darkfield microscopy: 88.6% Patients with primary syphilis and anal lesions (n = 6) Positive by darkfield microscopy:66.7% Patients with primary syphilis and oral lesions (n = 4) Positive by darkfield microscopy: 75% Patients with primary syphilis and cutaneous lesions (n = 2) Positive by darkfield microscopy:100% Patients with primary syphilis and lesions from unknown anatomic site (n = 18) Positive by darkfield microscopy:	(*53*)

Secondary syphilis patients with oral lesions: 5 Secondary syphilis patients with cutaneous lesions: 10 Secondary syphilis patients with lesions from unknown anatomic site: 4 Non-syphilitic patients with genital lesions: 8 Non-syphilitic patients with anal lesions: 2 Non-syphilitic patients with oral lesions: 0 Non-syphilitic patients with cutaneous lesions: 0 Non-syphilitic patients with lesions from unknown anatomic site: 2 Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy, PCR tp47 Syphilis diagnosis: Clinical presentation, nontreponemal and treponemal serology (test types not stated)	50% Patients with secondary syphilis and assessed by darkfield microscopy (n = 44) Positive by darkfield microscopy: 70.5% Patients with secondary syphilis and genital lesions (n = 22) Positive by darkfield microscopy: 63.6% Patients with secondary syphilis and anal lesions (n = 3) Positive by darkfield microscopy: 66.7% Patients with secondary syphilis and oral lesions (n = 5) Positive by darkfield microscopy: 100% Patients with secondary syphilis and cutaneous lesions (n = 10) Positive by darkfield microscopy: 80% Patients with secondary syphilis and lesions from unknown anatomic site (n = 4) Positive by darkfield microscopy: 50% Non-syphilitic patients assessed by darkfield microscopy (n = 12) Positive by darkfield microscopy: 0% Non-syphilitic patients with genital lesions (n = 8) Positive by darkfield microscopy: 0% Non-syphilitic patients with anal lesions (n = 2) Positive by darkfield microscopy: 0%

Prospective cross-sectional study Primary syphilis patients: 22 Secondary syphilis patients: 8 Of the 30 patients with syphilis, 24 had genital lesions, 5 had anal lesions and 1 had cutaneous lesions Non-syphilitic patients: 31 Of the 30 patients without syphilis, 20 had genital lesions, 6 had anal lesions and 5 had oral lesions Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy and direct fluorescence microscopy using H9-1 monoclonal antibody to 47-58kDa tp protein Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL) and treponemal serology (FTA-ABS)	Patients with primary or secondary syphilis (N = 30) Positive by darkfield microscopy: 96.7% Non-syphilitic patients (n = 31) Positive by darkfield microscopy: 6.5%	(*54*)
Retrospective cross-sectional study Patients with syphilis: 30 Specimens from patients with primary syphilis: 5 (3 specimens used to assess darkfield microscopy) Specimens from patients with secondary syphilis: 31 (14 specimens used to assess darkfield microscopy)	Patients with primary syphilis assessed by darkfield microscopy (n = 3) Positive by darkfield microscopy: 100% Patients with secondary syphilis assessed by darkfield microscopy (n = 14) Positive by darkfield microscopy: 64.3%	(*55*)

Note: More than one specimen was obtained from a patient, but the number of specimens per patient was not defined Specimen type for darkfield microscopy: Lesion exudate Tests performed: Darkfield microscopy, avidin-biotin-peroxidase complex, indirect immunoperoxidase, and FTA-ABS Complement Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL) and treponemal serology (FTA-ABS, TPHA)		
Prospective cross-sectional study Pregnant women with syphilis: 11 (included in darkfield microscopy assessment) Neonates with probable or suspected congenital syphilis: 20 (not included in darkfield microscopy assessment) Pregnant women with primary syphilis: 4 Pregnant women with secondary syphilis: 3 Pregnant women with early latent syphilis: 4 Specimen type for darkfield microscopy: Amniotic fluid Tests performed: Darkfield microscopy, rabbit infectivity test, PCR for Tp47 gene with Southern blot confirmation	Amniotic fluid from pregnant women with primary syphilis (n = 4) Positive by darkfield microscopy: 25% Amniotic fluid from pregnant women with secondary syphilis (n = 3) Positive by darkfield microscopy: 33.3% Amniotic fluid from pregnant women with early latent syphilis (n = 4) Positive by darkfield microscopy: 100%	(*56*)

	Syphilis diagnosis: Clinical presentation and nontreponemal (VDRL) serology		
	Prospective cross-sectional study Pregnant women with primary syphilis: 6 Pregnant women with secondary syphilis: 12 Pregnant women with early latent syphilis: 6 Specimen type for darkfield microscopy: Amniotic fluid Tests performed: Darkfield microscopy, rabbit infectivity test, PCR for Tp47 gene with Southern blot confirmation Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL), and treponemal (MHA-TP) serology	Amniotic fluid from pregnant women with primary syphilis (n = 6) Positive by darkfield microscopy: 16.7% Amniotic fluid from pregnant women with secondary syphilis and assessed by darkfield microscopy (n = 20) Positive by darkfield microscopy: 20% Amniotic fluid from pregnant women with early latent syphilis and assessed by darkfield microscopy (n = 5) Positive by darkfield microscopy: 60%	(*57*)
Immunofluorescent antibody test staining	Prospective cross-sectional study Two studies with both study A and B relevant to immunofluorescent antibody test staining Study A Patients with skin lesion(s): 350 Study B Patients with skin lesion(s): 95 Stage of syphilis not defined in both studies	Patients with skin lesions (n = 445) Sensitivity of immunofluorescent antibody test stain: 85.9% Specificity of immunofluorescent antibody test stain: 100%	(*52*)

	Specimen type for immunofluorescent antibody test staining (both studies): Lesion exudate Syphilis diagnosis (both studies): Clinical presentation, VDRL, and FTA-ABS serology Sensitivity and specificity based on clinical diagnosis of syphilis in both studies		
	Prospective cross-sectional study Primary syphilis patients: 22 Secondary syphilis patients: 8 Of the 30 patients with syphilis, 24 had genital lesions, 5 had anal lesions and 1 had cutaneous lesions Non-syphilitic patients: 31 Of the 30 patients without syphilis, 20 had genital lesions, 6 had anal lesions and 5 had oral lesions Specimen type for immunofluorescent antibody test staining: Lesion exudate Tests performed: Darkfield microscopy and direct fluorescence microscopy using H9-1 monoclonal antibody to 47-58kDa tp protein Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL) and treponemal serology (FTA-ABS)	Patients with primary or secondary syphilis patients (n = 30) Positive by immunofluorescent antibody test stain: 100% Non-syphilitic patients (n = 31) Positive by immunofluorescent antibody test stain: 0%	(*54*)
Immunohistochemistry staining	Prospective cross-sectional study	Patients with secondary syphilis (n = 12) Positive by immunohistochemistry stain: 91.7%	(*51*)

Patients with secondary syphilis: 12 Patients with non-syphilitic lesions: 24 Specimen types: Lesion exudate and biopsy Tests performed: Darkfield microscopy, PCR tp47 (amplicons detected by Southern blot for 25bp region and sequenced), immunohistochemistry staining on FFPE using avidin-biotin peroxidase complex technique with polyclonal antibodies (BioCare) Syphilis diagnosis: Clinical presentation, RPR, and TPHA serology	Non-syphilitic patients (n = 24) Positive by immunohistochemistry stain: 0%	
Retrospective cross-sectional study Patient with syphilis: 30 Specimens from patients with primary syphilis to assess immunohistochemistry staining: 5 Specimens from patients with secondary syphilis immunohistochemistry staining: 31 Note: More than one specimen was obtained from a patient, but the number of specimens per patient was not defined Specimen type for immunohistochemistry staining: cutaneous lesion that was FFPE Tests performed: Darkfield microscopy, immunohistochemistry using avidin-biotin-peroxidase complex, indirect	Patients with primary syphilis patients (n = 5) Positive by avidin-biotin-peroxidase complex staining: 100% Positive by indirect immunoperoxidase stain: 100% Patients with secondary syphilis (n = 31) Positive by avidin-biotin-peroxidase complex staining: 90.3% Positive by indirect immunoperoxidase stain: 87.1%	(*55*)

immunoperoxidase immunohistochemistry, FTA-ABS, and complement fixation Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL) and treponemal serology (FTA-ABS, TPHA)		
Retrospective cross-sectional study Secondary syphilis patients: 36 (33 confirmed by serology and 3 not serologically tested) Specimen type for immunohistochemistry staining: cutaneous lesion that was FFPE Tests performed: Immunohistochemistry using rabbit polyclonal antibodies, Dieterle silver stain, nested PCR (Tp1; 228 bp) and semi-nested (Tp2; 125 bp) PCR for DNA polymerase I Syphilis diagnosis: Clinical presentation and, in 33/36 patients, syphilis serology (undefined)	Patients with secondary syphilis (n = 35) Positive by indirect immunohistochemistry stain: 48.6%	(*58*)
Retrospective cross-sectional study Secondary syphilis patients: 17 Biopsies from patients without syphilis: 14 (similar histologic pattern to secondary syphilis, including 2 with lichen planus, 3 with psoriasis, 3 with psoriasiform dermatitis, 2 with pityriasis lichenoides et varioliformis acuta, 1 with	Patients with secondary syphilis (n = 17) Positive by avidin-biotin-peroxidase complex immunohistochemistry stain: 70.6% Non-syphilitic patients (n = 14) Positive by avidin-biotin-peroxidase complex immunohistochemistry stain: 0%	(*59*)

	erythema annulare centrifugum, 2 with acne keloidalis, and 1 with folliculitis decalvans Specimen type for immunohistochemistry staining: cutaneous lesion that was FFPE Tests performed: Immunohistochemistry using avidin-biotin-peroxidase complex and Steiner silver stain Syphilis diagnosis: Clinical presentation, nontreponemal (RPR or VDRL), and treponemal (TPPA or FTA-ABS) serology		
Silver stain	Retrospective cross-sectional study Secondary syphilis patients: 36 (33 confirmed by serology and 3 not serologically tested) Specimen type for Dieterle silver staining: cutaneous lesion that was FFPE Tests performed: Immunohistochemistry using rabbit polyclonal antibodies, Dieterle silver stain, nested PCR (Tp1; 228 bp) and semi-nested (Tp2; 125 bp) PCR for DNA polymerase I Syphilis diagnosis: Clinical presentation and, in 33/36 patients, syphilis serology (undefined)	Patients with secondary syphilis (n = 35) Positive by Dieterle silver stain: 25.7%	(*58*)
	Retrospective cross-sectional study	Patients with secondary syphilis (n = 17) Positive by Steiner silver stain: 41.2%	(*59*)

Secondary syphilis patients: 17 Biopsies from patients without syphilis: 14 (similar histologic pattern to secondary syphilis, including 2 with lichen planus, 3 with psoriasis, 3 with psoriasiform dermatitis, 2 with pityriasis lichenoides et varioliformis acuta, 1 with erythema annulare centrifugum, 2 with acne keloidalis, and 1 with folliculitis decalvans Specimen type for Steiner silver staining: cutaneous lesion that was FFPE Tests performed: Immunohistochemistry using avidin-biotin-peroxidase complex and Steiner silver stain Syphilis diagnosis: Clinical presentation, nontreponemal (RPR or VDRL), and treponemal (TPPA or FTA-ABS) serology	Non-syphilitic patients (n = 14) Positive by Steiner silver stain: 0%	
Prospective cross-sectional study Secondary syphilis patients: 57 (only 11 lesion biopsies were microscopically examined after Warthin-Starry silver staining) Specimen type for Warthin-Starry silver staining: cutaneous lesion that was FFPE Tests performed: Warthin-Starry silver stain, nested PCR (Tp1; 228 bp), and RT-PCR for Tp *polA*	Patients with secondary syphilis (n = 11) Positive by Warthin-Starry silver stain: 9.1%	(*60*)

	Syphilis diagnosis: Clinical presentation, nontreponemal (RPR), and treponemal (FTA-ABS) serology		
	Retrospective cross-sectional study Secondary syphilis patients: 6 Tertiary syphilis patients: 7 Non-syphilitic patients: 5 Specimen type for Warthin-Starry silver staining: cutaneous lesion that was FFPE Tests performed: Warthin-Starry silver stain, nested PCR (Tp1; 228 bp), and nested PCR for Tp47 Syphilis diagnosis: Clinical presentation and treponemal (TPHA and FTA-ABS) serology	Patients with secondary or tertiary syphilis (n = 13) Positive by Warthin-Starry silver stain: 0% Non-syphilitic patients (n = 5) Positive by Warthin-Starry silver stain: 0%	(*61*)
NAATs	Prospective cross-sectional study Patients with suspected primary syphilis: 716 Patients with suspected secondary syphilis: 133 Specimen type for RT-PCR: dry swab from anogenital lesion or cutaneous lesion Tests performed: Darkfield microscopy on all anogenital lesions and RT-PCR for polA on all anogenital and cutaneous lesions Primary syphilis diagnosis standard 1: Darkfield microscopy positive	Patients with suspected primary syphilis (n = 716) Positive by RT-PCR: 13% Patients with suspected secondary syphilis (n = 133) Positive by RT-PCR: 25.6% Patients with primary syphilis defined by clinical standard 1 involving darkfield microscopy (n = 716) RT-PCR sensitivity: 87% RT-PCR specificity 93.1%	(*62*)

Primary syphilis diagnosis standard 2: Clinical presentation, darkfield microscopy positive, and syphilis serology (not defined) Primary syphilis diagnosis standard 3: Patients with a positive TPPA result (irrespective of the RPR test result) without a history of syphilis or in patients with an RPR titer of ≥1:8 and a history of syphilis Clinical presentation, darkfield microscopy, and syphilis serology (not defined) Secondary syphilis diagnosis: Clinical presentation with cutaneous or mucosal lesions characteristic of secondary syphilis and RPR titer of ≥1:8	Patients with primary syphilis defined by clinical standard 2 involving clinical history, darkfield microscopy, and serology (n = 716) RT-PCR sensitivity: 72.8% RT-PCR specificity: 98.8% Patients with primary syphilis clinical standard 3 involving clinical history and serology (n = 716) RT-PCR sensitivity: 74.5% RT-PCR specificity: 97.2% Patients with secondary syphilis (n = 133) RT-PCR sensitivity: 42.9% RT-PCR specificity: 98.2%	
Prospective cross-sectional study Case-control nested in prospective cohort Primary syphilis patients: 26 (10 HIV positive and 16 HIV negative) Secondary syphilis patients: 40 (19 HIV positive and 21 HIV negative) Latent syphilis patients: 8 Case control for primary syphilis: 7 patients with genital or oral lesion Case control for secondary syphilis: 5 patients with cutaneous rash Case control for latent syphilis: 3 patients without symptoms	Patients with primary syphilis (n = 26) RT-PCR sensitivity: 65.4% (95% CI: 44%–83%) Patients with secondary syphilis (n = 40) RT-PCR sensitivity: 52.5% (95% CI: 36%–68%) Patients with latent syphilis (n = 8) RT-PCR sensitivity: 0% No difference in performance based on HIV status Lesion swab specimens tested from patients with primary syphilis (n = 10) RT-PCR sensitivity: 80% (95% CI: 44%– 97%)	(*63*)

Specimen types for RT-PCR from primary syphilis patients: 8 dry lesion swab, 18 whole blood, 11 serum, and 7 urine Specimen types for RT-PCR from secondary syphilis patients: 5 dry lesion swab, 31 whole blood, 15 serum, 2 plasma, 6 CSF, and 9 urine Specimen types for RT-PCR from latent syphilis patients: 6 whole blood, 2 serum, 2 CSF, and 2 urine Tests performed: Darkfield microscopy on all anogenital lesions and RT-PCR for tp47 Syphilis diagnosis: Clinical presentation, nontreponemal (VDRL), and treponemal (TPHA) serology to determine stage	Whole blood tested from patients with primary syphilis (n = 18) RT-PCR sensitivity: 28% (95% CI: 10%–53%) Serum tested from patients with primary syphilis (n = 11) RT-PCR sensitivity: 55% (95% CI 23% - 83%) Urine tested from patients with primary syphilis (n = 7) RT-PCR sensitivity: 29% (95% CI: 4%– 71%) All controls negative Lesion swab specimens tested from patients with secondary syphilis (n = 5) RT-PCR sensitivity: 20% (95% CI: 0.5%–72%) Whole blood tested from patients with primary syphilis (n = 31) RT-PCR sensitivity: 36% (95% CI: 19%–55%) Serum tested from patients with primary syphilis (n = 15) RT-PCR sensitivity: 47% (95% CI: 21%–73%) Plasma tested from patients with primary syphilis (n = 2) RT-PCR sensitivity 100% (95% CI: 16%–100%) CSF tested from patients with primary syphilis (n = 6) RT-PCR sensitivity: 50% (95% CI: 12%–88%)

	Urine tested from patients with primary syphilis (n = 7) RT-PCR sensitivity: 29% (95% CI: 4%–71%) All controls negative	
Prospective cross-sectional study Patients with secondary syphilis: 12 Patients with non-syphilitic lesions: 24 Specimen types: Lesion exudate and biopsy Tests performed: Darkfield microscopy, PCR tp47 (amplicons detected by Southern blot for 25bp region and sequenced), immunohistochemistry on FFPE tissue using avidin-biotin peroxidase complex technique with polyclonal antibodies (BioCare) Syphilis diagnosis: Clinical presentation, RPR, and TPHA serology	Patients with secondary syphilis (n = 12) Positive by PCR: 75% PCR limit of detection: 1ng of DNA	(*51*)
Prospective cross-sectional study Study A Patients with primary syphilis: 87 (specimens from 65 patients used to assess PCR) Patients with secondary syphilis: 103 (specimens from 44 patients used to assess PCR) Patients without syphilis: 35 (specimens from 12 patients used to assess PCR)	Study A Patients with primary syphilis (n = 65) Positive by PCR: 80% Patients with primary syphilis and genital lesions (n = 35) Positive by PCR: 82.9% Patients with primary syphilis and anal lesions (n = 6) Positive by PCR: 83.3%	(*53*)

Primary syphilis patients with genital lesions: 35 Primary syphilis patients with anal lesions: 6 Primary syphilis patients with oral lesions: 2 Primary syphilis patients with cutaneous lesions: 2 Primary syphilis patients with lesions from unknown anatomic site: 18 Secondary syphilis patients with genital lesions: 22 Primary syphilis patients with anal lesions: 3 Primary syphilis patients with oral lesions: 5 Primary syphilis patients with cutaneous lesions: 10 Primary syphilis patients with lesions from unknown anatomic site: 4 Non-syphilitic patients with genital lesions: 8 Non-syphilitic patients with anal lesions: 2 Non-syphilitic patients with oral lesions: 0 Non-syphilitic patients with cutaneous lesions: 0 Non-syphilitic patients with lesions from unknown anatomic site: 2 Study B Primary syphilis patients: 81 (not all tested specimen types tested for all patients) Secondary syphilis patients: 97 (not all tested specimen types tested for all patients) Latent syphilis patients: 40 (not all tested specimen types tested for all patients)	Patients with primary syphilis and oral lesions (n = 4) Positive by PCR: 50% Patients with primary syphilis and cutaneous lesions (n = 2) Positive by PCR: 100% Patients with primary syphilis and lesions from unknown anatomic site (n = 18) Positive by PCR: 77.8% Patients with secondary syphilis (n = 44) Positive by PCR: 86.4% Patients with secondary syphilis and genital lesions (n = 22) Positive by PCR: 86.4% Patients with secondary syphilis and anal lesions (n = 3) Positive by PCR: 66.7% Patients with secondary syphilis and oral lesions (n = 5) Positive by PCR: 80% Patients with secondary syphilis and cutaneous lesions (n = 10) Positive by PCR: 100% Patients with secondary syphilis and lesions from unknown anatomic site (n = 4) Positive by PCR: 75%

Specimen types for PCR (both studies): Lesion exudate, whole blood, serum, plasma, and peripheral blood mononuclear cells Tests performed: Darkfield microscopy, PCR tp47 (study A), and PCR tp47 (study B) Syphilis diagnosis (both studies): Clinical presentation, nontreponemal, and treponemal serology (test types not stated)	Non-syphilitic patients (n = 12) Positive by PCR: 0% Non-syphilitic patients with genital lesions (n = 8) Positive by PCR: 0% Non-syphilitic patients with anal lesions (n = 2) Positive by PCR: 0% Study B Whole blood tested from patients with primary syphilis (n = 61) Positive by PCR: 13.1% Serum tested from patients with primary syphilis (n = 63) Positive by PCR: 19% Plasma tested from patients with primary syphilis (n = 67) Positive by PCR: 11.9% Peripheral blood mononuclear cells tested from patients with primary syphilis (n = 72) Positive by PCR: 31.9% Whole blood tested from patients with secondary syphilis (n = 69) Positive by PCR: 37.7% Serum tested from patients with secondary syphilis (n = 65) Positive by PCR: 15.4%

	Plasma tested from patients with secondary syphilis (n = 66) Positive by PCR: 28.8% Peripheral blood mononuclear cells tested from patients with secondary syphilis (n = 83) Positive by PCR: 31.3% Whole blood tested from patients with latent syphilis (n = 28) Positive by PCR: 14.3% Serum tested from patients with latent syphilis (n = 28) Positive by PCR: 3.6% Plasma tested from patients with latent syphilis (n = 29) Positive by PCR: 10.3% Peripheral blood mononuclear cells tested from patients with latent syphilis (n = 31) Positive by PCR: 16.1% Specimens for patients without syphilis were all negative PCR limit of detection: 20 organisms/mL	
Retrospective cross-sectional study Secondary syphilis patients: 36 (33 confirmed by serology and 3 were not serologically tested)	Patients with secondary syphilis (n = 36) Positive by nested PCR: 19.4% Positive by semi-nested PCR: 38.9%	(*58*)

Specimen type for PCR: cutaneous lesion that was FFPE Tests performed: Immunohistochemistry using rabbit polyclonal antibodies, Dieterle silver stain, nested PCR (Tp1; 228 bp), and semi-nested (Tp2; 125 bp) PCR for DNA polymerase I Syphilis diagnosis: Clinical presentation and, in 33/36 patients, syphilis serology (undefined)		
Prospective cross-sectional study Secondary syphilis patients: 57 (only 12 lesion biopsies were tested by PCR and whole blood tested from 26 patients) Specimen type for PCR: cutaneous lesion that was FFPE and whole blood Tests performed: Warthin-Starry silver stain, nested PCR (Tp1; 228 bp), and RT-PCR for Tp polA Syphilis diagnosis: Clinical presentation, nontreponemal (RPR), and treponemal (FTA-ABS) serology	Lesion biopsy from patients with secondary syphilis (n = 12) Positive by PCR: 66.7% Whole blood from patients with secondary syphilis (n = 23) Positive by PCR: 46.2% Limit of detection by PCR: 12–150 spirochetes/mL (one log higher if specimens stored at 4˚C for 26h versus room temperature for 1h)	(*60*)
Retrospective cross-sectional study Secondary syphilis patients: 6 Tertiary syphilis patients: 7 Non-syphilitic patients: 5	Patients with secondary syphilis (n = 6) Positive by PCR: 66.7% Patients with tertiary syphilis (n = 7)	(*61*)

Specimen type for PCR: cutaneous lesion that was FFPE Tests performed: Warthin-Starry silver stain, nested PCR (Tp1; 228 bp), and nested PCR for Tp47 Syphilis diagnosis: Clinical presentation and treponemal (TPHA and FTA-ABS) serology	Positive by PCR: 14.3% (the positive specimen was from a gumma) Non-syphilitic patients (n = 5) Positive by PCR: 0%	
Prospective cross-sectional study Number of patients evaluated: 298 Specimen type for PCR: Genital lesion exudate Tests performed: Darkfield microscopy and multiplex PCR for *T. pallidum* tp47, HSV, and *Haemoplilus ducreyi* Syphilis diagnosis: Clinical presentation, darkfield microscopy, and nontreponemal (RPR or VDRL) serology	Patients with syphilis and tested by multiplex PCR and darkfield microscopy (n = 295) Positive by multiplex PCR and darkfield microscopy: 19.7% Positive by multiplex PCR and negative by darkfield microscopy: 5.8% Negative by multiplex PCR and positive by darkfield microscopy: 2.4% Negative by multiplex PCR and darkfield microscopy: 72.2% Patients with syphilis and tested by multiplex PCR and serology (n = 296) Positive by multiplex PCR and syphilis serology: 21.7% Positive by multiplex PCR and negative by syphilis serology: 3.7% Negative by multiplex PCR and positive by syphilis serology: 8.1% Negative by multiplex PCR and syphilis serology: 66.6%	(*64*)
Prospective cross-sectional study	Patients with primary syphilis (n = 19)	(*65*)

Primary syphilis patients: 19 (4 from anal lesions, 6 from oral lesions, 13 from penial lesions, 1 from a rectal lesion, and 2 lesions from unspecified anatomic site) Secondary syphilis patients: 10 (2 from anal lesions, 6 from oral lesions, 5 from penial lesions, and 1 from a vulval lesion) Patients with HSV: 17 (2 from anal lesions, 9 from penial lesions, 4 from vulval lesions, and 3 lesions from unspecified anatomic site) Non-syphilitic patients: 48 (9 from anal lesions, 11 from oral lesions, 19 from penial lesions, 2 from rectal lesions, 7 from vulval lesions and 1 lesion from unspecified anatomic site) Non-syphilitic patients but with history of syphilis: 6 (2 from anal lesions and 4 from penial lesions) Specimen type for PCR: Dry swab or swab from lesion placed in viral or chlamydia suitable transport medium Tests performed: PCR for *T. pallidum* tp47 Syphilis diagnosis: Clinical presentation, darkfield microscopy (34 specimens), nontreponemal (RPR), and treponemal (TPHA or IgM/IgG EIA) serology	Positive by PCR: 94.7% (anatomic site not specified) Patients with secondary syphilis (n = 10) Positive by PCR: 80% (anatomic site not specified) Patients with HSV (n = 17) Positive by PCR: 0% Non-syphilitic patients with lesions (n = 48) Positive by PCR: 2.1% (anatomic site not specified) Non-syphilitic patients but with history of syphilis (n = 6) Positive by PCR: 0% PCR limit of detection: 1pg *T. pallidum* DNA	
Prospective cross-sectional study	Patients with primary syphilis (n = 19)	(*66*)

Primary syphilis patients: 19 Secondary syphilis patients: 9 Latent syphilis patients: 10 Congenital syphilis patients: 3 Non-syphilitic patients: 27 Specimen type for PCR: Swab from ulcer or cutaneous lesion placed in viral or chlamydia-suitable transport medium, whole blood collected in tube containing EDTA, serum, or CSF Tests performed: Nested PCR for *T. pallidum* bmp, and tp47 nPCR for bmp and tp47, and PCR for tp47 Primary syphilis diagnosis: (1) The identification of *T. pallidum* by darkfield microscopy, fluorescent antibody, or equivalent examination of material from a chancre or a regional lymph node; or (2) the presence of one or more typical lesions (chancres) and reactive treponemal serology, regardless of nontreponemal test reactivity, in individuals with no previous history of syphilis; or (3) the presence of one or more typical lesions (chancres) and at least a fourfold increase in the titer over that of the last known nontreponemal test in individuals with a past history of syphilis treatment Secondary syphilis diagnosis: (1) The identification of *T. pallidum* by microscopy, as in primary syphilis, or equivalent examination	Positive by PCR: 47.4% (9 swab specimens positive, 3 swab specimens negative (β-globin control also negative), and 7 blood specimens negative) Patients with secondary syphilis (n = 9) Positive by PCR: 44.4% (1 swab specimen positive, 2 tissue specimens positive, 4 blood specimens positive, 4 blood specimens negative, and 1 CSF specimen negative [β-globin control also negative]) Patients with congenital syphilis (n = 3) Positive by PCR: 33.3% (1 blood specimen positive and 2 blood specimens negative) Patients with latent syphilis (n = 10) Positive by PCR: 0% Non-syphilitic patients (n = 27) Positive by PCR: 0%

	of mucocutaneous lesions, condylomata lata, and reactive serology (nontreponemal and treponemal); or (2) the presence of typical mucocutaneous lesions, alopecia, loss of eyelashes and the lateral third of eyebrows, iritis, generalized lymphadenopathy, fever, malaise or splenomegaly, and either a reactive serology (nontreponemal and treponemal) or at least a fourfold increase in titer over that of the last known nontreponemal test Early latent syphilis diagnosis: Asymptomatic patient with reactive serology (nontreponemal and treponemal) who within the past 12 months had one of the following: nonreactive serology or symptoms suggestive of primary or secondary syphilis or exposure to a sexual partner with primary, secondary, or early latent syphilis Late latent syphilis diagnosis: Asymptomatic patient with persistently reactive treponemal serology (regardless of nontreponemal serology reactivity) who does not meet the criteria for early latent disease and who has not been previously treated for syphilis		
	Prospective cross-sectional study Patient population: Male (N = 267); 90.6% of whom were living with HIV Primary syphilis patients: 38 (17 had oral lesions)	Oral swabs tested from patient population (N = 267) Positive by PCR: 42.3% Oral swabs tested from patients with primary syphilis and oral lesions (n = 17) Positive: 100%	(*67*)

Secondary syphilis patients: 76 (0 had oral lesions) Early latent syphilis patients: 125 (0 had oral lesions) Late latent syphilis patients: 5 (0 had oral lesions) Congenital syphilis patients: 3 Non-syphilitic patients: 27 Specimen type for PCR: Oral swab from lesion (if present) or upper and lower gingiva, tonsils, hard palate, and soft palate in the absence of a lesion Tests performed: PCR for *T. pallidum* polA and typing using arp, tpr, and tp0548 Syphilis diagnosis and staging: According to the CDC Sexually Transmitted Treatment Guidelines (no additional information provided)	Oral swabs tested from patients with primary syphilis without oral lesions (n= 21) Positive by PCR: 61.9% Patients with secondary syphilis (n = 76) Positive PCR: 64.5% Patients with early latent syphilis (n = 125) Positive by PCR: 28% Patients with late latent syphilis (n = 5) Positive by PCR: 40%

Abbreviations: kDa = kilodaltons; RPR = rapid plasma reagin; PCR = polymerase chain reaction; bp = base pairs; IHC = immunohistochemistry; FFPE = formalin fixed and paraffin embedded tissue; TPHA = *T. pallidum* hemagglutination assay; VDRL = Venereal Disease Research Laboratory; FTA-ABS = fluorescent treponemal antibody-absorption; MHA-TP = microhemaggluntination assay for antibodies to *T. pallidum*; DNA = deoxyribonucleic acid; TPPA = *T. pallidum* particle agglutination; NAAT = nucleic acid amplification test; CI = confidence interval; CSF = cerebral spinal fluid; HSV = herpes simplex virus; IgG = immunoglobulin G; IgM = immunoglobulin M; EIA = enzyme immunoassay; EDTA = ethylenediaminetetraacetic acid

Supplementary Table 7. Performance characteristics of point-of-care syphilis tests

Assay	Study summary and reference standard	Performance characteristics*	Reference
Syphilis Health Check Treponemal Antibody Test Diagnostics Direct LLC 359 9th St, Suite 303 Stone Harbor, NJ 08247	Prospective cross-sectional study Patients enrolled: 562 Specimens tested with Syphilis Health Check: fingerstick whole blood and serum Stage of syphilis was not determined Reference standard: RPR and Trep-Sure EIA	Reactive by RPR and Trep-Sure: 7 Reactive by Trep-Sure: 16 Reactive by Syphilis Health Check using fingerstick whole blood: 31 Reactive by Syphilis Health Check using serum: 18 Syphilis Health Check (fingerstick whole blood) versus RPR and Trep-Sure (N = 562) Sensitivity: 100% (95% CI 59.0%–100%) Specificity: 95.7% (95% CI 93.6%–97.2%) Syphilis Health Check (fingerstick whole blood) versus Trep-Sure (N = 562) Sensitivity: 50.0% (95% CI 24.7%–75.4%) Specificity: 95.9% (95% CI 93.8%–97.4%) Syphilis Health Check (serum) versus RPR and Trep-Sure (N = 562) Sensitivity: 100% (95% CI 59.0%–100%)	(*68*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
		Specificity: 98.0% (95% CI 96.5%–99.2%) Syphilis Health Check (serum) versus Trep-Sure (N = 562) Sensitivity: 43.8% (95% CI 19.8%–70.1%) Specificity: 98.0% (95% CI 96.4%–98.9%)	
	Prospective cross-sectional study Patients enrolled: 202 Stage of syphilis was determined for 6 patients Reference standard: Trep-Sure EIA RPR performed but not included as a comparator test	Nonreactive by all tests: 171 Reactive by RPR: 10 Reactive by Trep-Sure: 10 Reactive by Syphilis Health Check: 26 Primary syphilis: 1 Secondary syphilis: 3 Early latent syphilis: 1 Previously treated syphilis: 1 Syphilis Health Check versus Trep-Sure (N = 202) Sensitivity: 71.4% (95% CI 41.9%–95.1%) Specificity: 91.5% (95% CI 87.5%–95.5%)	(*69*)
	Observational study Patients enrolled: 690 Stage of syphilis was determined for 10 patients Clinical data, including the stage of syphilis, was extracted from the medical record. The criteria used to stage syphilis was not reported in the paper. Reference standard: TPPA and RPR	Nonreactive by all tests: 671 Reactive by TPPA and RPR: 10 Reactive by Syphilis Health Check: 9 Primary syphilis: 0 Secondary syphilis: 1 Early latent syphilis: 2 Late latent syphilis: 3 Neurosyphilis: 2 Unspecified stage: 1 Previously treated syphilis: 1 Syphilis Health Check versus TPPA and RPR (N = 690) Sensitivity: 90.0% (95% CI 55.5%–99.8%) Specificity: 98.5% (95% CI 97.3%–99.3%)	(*70*)

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Prospective cross-sectional study Patients enrolled: 965 Stage of syphilis was not determined Reference standard: TPPA and RPR	Syphilis Health Check versus TPPA and RPR (N = 965) Sensitivity: 76.9% (95% CI 46.2%–95.0%) Specificity: 99.0% (95% CI 98.1%–99.5%) Syphilis Health Check versus TPPA (N = 962; 3 patients excluded from the initial 965 because of a nonreactive RPR and indeterminate TPPA) Sensitivity: 50.0% (95% CI 29.9%–70.1%) Specificity: 99.4% (95% CI 98.6%–99.8%)	(*71*)
	Retrospective study Patients enrolled: 1,406 Stage of syphilis was not determined Reference standard: TPPA, EIA, CIA, and RPR	Syphilis Health Check versus TPPA, EIA, CIA and, RPR (n = 1,237) Sensitivity: 95.7% (95% CI 93.6%–97.2%) Specificity: 93.2% (95% CI 91.0%–95.1%) Syphilis Health Check versus TPPA, EIA, and CIA (N = 1,406) Sensitivity: 88.7% (95% CI 86.2%–90.9%) Specificity: 93.1% (95% CI 91.0%–94.9%)	(*72*)
	Prospective and retrospective cross-sectional clinical trial study for submission to FDA. Prospectively and retrospectively collected samples: 1292 (stage of syphilis not reported) Prospective study population: 783 University clinic site: 39 Hospital clinic site: 50 Study site 1: 400 Study site 2: 89 Study site 3: 205 Retrospective studies with samples from patients suspected of or diagnosed with syphilis: 412	Prospectively and retrospectively collected samples (N=1292) PPA: 98.5% (95% CI: 97.1%–99.4%) PNA: 97.3% (95% CI: 95.9%–98.4%) Prospective study population (N=783) University clinic site (n=39) PPA: 100% (95% CI: 87.2%–100%) PNA: 50% (95% CI: 21.1%–78.9%) Hospital clinic site (n=50) PPA: 100% (95% CI: 54.1%–100%) PNA: 100% (95% CI: 92.0%–100%) Study site 1 (n=400) PPA: 77.8% (95% CI: 57.7%–91.4%) PNA: 97.9% (95% CI: 95.8%–99.1%)	(*73*) §

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Patients diagnosed with syphilis: 315 (stage not reported) Patients suspected of having syphilis: 97 Retrospective studies with samples from patients diagnosed with syphilis and stage reported: 164 Patients clinically diagnosed with primary treated syphilis: 28 Patients clinically diagnosed with primary untreated syphilis: 23 Patients with clinically diagnosed secondary treated syphilis: 26 Patients with clinically diagnosed secondary untreated syphilis: 25 Patients with clinically diagnosed latent treated syphilis and reactive RPR: 18 Patients with clinically diagnosed latent treated syphilis and nonreactive RPR: 19 Patients with clinically diagnosed latent untreated syphilis and reactive RPR: 22 Patients with clinically diagnosed latent treated syphilis and nonreactive RPR: 3 Reference standard: Predicate test was either ELISA, FTA-ABS, TPHA, or TPPA. Stage of syphilis determined by a licensed physician based on the clinical symptoms, medical history, and laboratory test results at the time of diagnosis	Study site 2 (n=89) PPA: 100% (95% CI: 39.8%–100%) PNA: 100% (95% CI: 95.8%–100%) Study site 3 (n=205) PPA: 90% (95% CI: 55.5%–99.7%) PNA: 99% (95% CI: 96.3%–99.9%) Retrospective studies with samples from patients suspected of or diagnosed with syphilis (N=412) Patients diagnosed with syphilis (n=315) PPA: 99.6% (95% CI: 97.9%–100%) PNA: 85.7% (95% CI: 53.7%–97%) Patients suspected of having syphilis (n=97) PPA: 100% (95% CI: 95.8%–100%) PNA: 100% (95% CI: 69.2%–100%) Retrospective studies with samples from patients diagnosed with syphilis and stage reported (N=164) Patients clinically diagnosed with primary treated syphilis (n=28) PA: 100% (95% CI: 87.8%–100%) Patients clinically diagnosed with primary untreated syphilis: 23 PA: 100% (95% CI: 85.2%–100%) Patients with clinically diagnosed secondary treated syphilis: 26 PA: 100% (95% CI: 86.8%–100%) Patients with clinically diagnosed secondary untreated syphilis: 25 PA: 100% (95% CI: 86.3%–100%) Patients with clinically diagnosed latent treated syphilis and reactive RPR: 18 PA: 100% (95% CI: 81.5%–100%) Patients with clinically diagnosed latent treated syphilis and nonreactive RPR: 19 PA: 100% (95% CI: 82.4%–100%)	

Assay	Study summary and reference standard	Performance characteristics*	Reference
		Patients with clinically diagnosed latent untreated syphilis and reactive RPR: 22 PA: 100% (95% CI: 84.6%–100%) Patients with clinically diagnosed latent treated syphilis and nonreactive RPR: 3 PA: 100% (95% CI: 29.2%–100%)	
DPP HIV-Syphilis Assay Chembio Diagnostic Systems, Inc 555 Wireless Blvd Hauppauge, NY, 11788	Retrospective study Patients enrolled: 150 Stage of syphilis was not determined Reference standard: TPPA	DPP HIV-Syphilis Assay versus TPPA (N = 150) Sensitivity: 95.3% (95% CI 87.9%–98.5%) Specificity: 100% (95% CI 92.9%–100%)	(*74*)
	Retrospective study Patients enrolled: 450 Stage of syphilis was not determined Reference standard: TPPA	DPP HIV-Syphilis Assay versus TPPA (N = 450) Sensitivity: 100% (95% CI 97.6%–100%) Specificity: 98.7% (95% CI 96.6%–99.6%)	(*75*)
	Prospective and retrospective cross-sectional clinical trial study for submission to FDA. Prospectively collected fingerstick samples: 1282 (stage of syphilis not reported) Patients being screened for syphilis: 704 People living with HIV: 171 Pregnant people: 407 Prospectively collected venous whole blood samples: 1280 (stage of syphilis not reported) Patients being screened for syphilis: 704 People living with HIV: 171	Prospectively collected fingerstick samples (N=1282) Patients being screened for syphilis (n=704) PPA: 92.5% (95% CI: 52.1%–97%) PNA: 97.1% (95% CI: 95.5%–98.1%) People living with HIV (n=171) PPA: 96.6% (95% CI: 88.5%–99.1%) PNA: 95.5% (95% CI: 90%–98.1%) Pregnant people (n=407) PPA: 100% (95% CI: N/A) PNA: 93.1% (95% CI: 90.2%–95.2%) Prospectively collected venous whole blood samples (N=1280)	(*76*) †

Assay	Study summary and reference standard	Performance characteristics*	Reference
	Pregnant people: 405 Prospectively collected plasma samples: 1163 (stage of syphilis not reported) Patients being screened for syphilis: 688 People living with HIV: 68 Pregnant people: 407 Retrospective studies with samples from pregnant people presumed positive for syphilis: 164 Pregnant people with primary treated syphilis: 0 Pregnant people with primary untreated syphilis: 3 Pregnant people with secondary treated syphilis: 0 Pregnant people with secondary untreated syphilis: 1 Pregnant people with early latent treated syphilis: 0 Pregnant people with early latent untreated syphilis: 5 Pregnant people with latent treated syphilis: 0 Pregnant people with latent treated syphilis: 3 Pregnant people with unknown stage of syphilis and unknown treatment status: 22 Retrospective studies with samples from patients diagnosed with syphilis and stage reported: 163 Patients with primary treated syphilis: 18 Patients with primary untreated syphilis: 10 Patients diagnosed secondary treated syphilis: 33 Patients diagnosed secondary untreated syphilis: 30 Patients with latent treated syphilis: 42 Patients with latent treated syphilis: 30 Reference standard: RPR, EIA, and TPPA. Stage of syphilis determined by a licensed physician based on the clinical symptoms, medical history, and laboratory test results at the time of diagnosis	Patients being screened for syphilis (n=704) PPA: 96.2% (95% CI: 87.2%–99%) PNA: 96.3% (95% CI: 94.6%–97.5%) People living with HIV (n=171) PPA: 96.6% (95% CI: 88.5%–99.1%) PNA: 95.5% (95% CI: 90%–98.1%) Pregnant people (n=405) PPA: 100% (95% CI: N/A) PNA: 90.8% (95% CI: 87.6%–93.3%) Prospectively collected plasma samples (N=1163) Patients being screened for syphilis (n=688) PPA: 94.9% (95% CI: 83.1%–98.6%) PNA: 95.1% (95% CI: 93.1%–96.5%) People living with HIV (n=68) PPA: 100% (95% CI: 84.5%–100%) PNA: 97.9% (95% CI: 88.9%–99.6%) Pregnant people (n=407) PPA: 100% (95% CI: N/A) PNA: 91.6% (95% CI: 88.5%–93.9%) Retrospective studies with samples from pregnant people presumed positive for syphilis (N=164) Pregnant people with primary treated syphilis (n=0) Percent reactive: N/A Pregnant people with primary untreated syphilis (n=3) Percent reactive: 100% Pregnant people with secondary treated syphilis (n=0) Percent reactive: N/A Pregnant people with secondary untreated syphilis (n=1) Percent reactive: 100% Pregnant people with early latent treated syphilis (n=0) Percent reactive: N/A	

Assay	Study summary and reference standard	Performance characteristics*	Reference
		Pregnant people with early latent untreated syphilis (n=5) Percent reactive: 100% Pregnant people with latent treated syphilis (n=0) Percent reactive: N/A Pregnant people with latent treated syphilis (n=3) Percent reactive: 100% Pregnant people with unknown stage of syphilis and unknown treatment status (n=22) Percent reactive: N/A Retrospective studies with samples from patients diagnosed with syphilis and stage reported (N=163) Patients with primary treated syphilis (n=18) Percent reactive: 100% Patients with primary untreated syphilis (n=10) Percent reactive: 100% Patients diagnosed secondary treated syphilis (n=33) Percent reactive: 100% Patients diagnosed secondary untreated syphilis (n=30) Percent reactive: 100% Patients with latent treated syphilis (n=42) Percent reactive: 100% Patients with latent treated syphilis (n=30) Percent reactive: 100%	

Abbreviations: FDA = Food and Drug Administration; PPA = percent positive agreement; PPN = percent negative agreement; PA = percent agreement; CI = confidence interval; FTA-ABS = fluorescent treponemal antibody-absorption; VDRL = Venereal Disease Research Laboratory; MHA-TP = microhemaggluntination assay for antibodies to *T. pallidum*; CSF = cerebral spinal fluid; TPPA = *T. pallidum* particle agglutination; TPHA = *T. pallidum* hemagglutination assay; EIA = enzyme immunoassay; RPR = rapid plasma reagin; IgG = immunoglobulin G; IgM = immunoglobulin M; N/A = not applicable

*Performance characteristics are stratified by syphilis stage if available. Otherwise, the performance characteristics are derived from data that did not specify the stage of syphilis.

†Unpublished data submitted to the FDA for PMA class III approval.

Supplementary Appendix 1. APHL meeting attendees, conflict of interest disclosures, and key questions

APHL Attendees: Laura Bachmann, MD, MPH, Wake Forest School of Medicine, Winston-Salem, North Carolina; William Becker, DO, MPH, Quest Diagnostics Laboratory, Lenexa, Kansas; Eric Blank, DrPH, APHL, Silver Spring, Maryland; Marc Couturier, PhD, D(ABMM), ARUP Laboratories/University of Utah, Salt Lake City, Utah; Marilyn Freeman, PhD, M(ASCP), Virginia Division of Consolidated Laboratory Services, Richmond, Virginia; Anne Gaynor, PhD, APHL, Silver Spring, Maryland; Laura Gillim-Ross, PhD, HCLD (ABB), LabCorp Englewood, Colorado; William A. Glover II, PhD, Washington Public Health Laboratories, Seattle, Washington; Edward Hook, MD, University of Alabama at Birmingham, Birmingham, Alabama; Jeffrey Klausner, MD, MPH, University of California Los Angeles, Los Angeles, California; Michael Loeffelholz, PhD, University of Texas Medical Branch, Galveston, Texas; Ruth Lynfield, MD, Minnesota Department of Health, St. Paul, Minnesota; William C. Miller, MD, PhD, The Ohio State University, Columbus, Ohio; Daniel Ortiz, PhD, University of Texas Medical Branch, Galveston, Texas; Susan Philip, MD, MPH, San Francisco Department of Public Health, San Francisco, California; Arlene C Seña, MD, MPH, University of North Carolina, Chapel Hill, North Carolina; Jeanne Sheffield, MD, Johns Hopkins University, Baltimore, Maryland; Marty Soehnlen, PhD, MPH, Michigan Public Health Laboratory, Lansing, Michigan; Elitza Theel, PhD, Mayo Clinic, Rochester, Minnesota; Anthony Tran, DrPH, MPH, District of Columbia Public Health Laboratory, Washington, DC; Susan Tuddenham, MD, MPH, Johns Hopkins University, Baltimore, Maryland; George Wendel, PhD, American Board of Obstetrics and Gynecology, Dallas, Texas; Kelly Wroblewski, MPH, APHL, Silver Spring, Maryland.

Meeting Facilitators: Joan Jarret and Paul Marquardt, PhD, AlignOrg Solutions, Shawnee, Kansas.

CDC Attendees: Sevgi Aral, PhD; Roxanne Barrow, MD, MPH; Gail Bolan, MD; Cheng Chen, PhD; Yetunde Fakile, PhD; Joseph Kang, PhD; Samantha Katz, PhD; Ellen Kersh, PhD; Sarah Kidd, MD; Jonathan Mermin, MD, MPH; S. Michele Owen, PhD; Ina Park, MD, MS; Lara Pereira, PhD; Tom Peterman, MD; Allan Pillay, PhD; Raul Romaguera, MPH, DMD; Mayur Shukla, PhD; Benedict Truman, MD; Kimberly Workowski, MD, National Center for HIV, Viral Hepatitis, STD, and TB Prevention, CDC.

Non-CDC Federal Employee Attendees: Carolyn Deal, PhD, National Institutes of Health, Rockville, Maryland; Tamara Feldblyum, MS, PhD, U.S. Food and Drug Administration, Silver Spring, Maryland; Delmyra Turpin, RN, MPH, National Institutes of Health, Rockville, Maryland.

Conflict of Interest Disclosures: Laura Bachmann, research funds awarded directly to Wake Forest University Health Sciences Medical School from Becton-Dickenson, Cepheid, Atlas, National Institutes of Health, CDC; William Becker, CLIA Lab Director, Columbus Public Health; Jeffrey Klausner, Laboratory Director at AIDS Healthcare Foundation, received donated test kits for research from Hologic and Cepheid; Michael Loeffelholz, member CDC Office of Infectious Diseases Board of Scientific Counselors, has previously received grant funding from Fujirebio Inc; Ruth Lynfield, Committee of Infectious Diseases for the American Academy of Pediatrics; Ina Park, Medical Consultant, CDC Division of STD Prevention (Intergovernmental Personnel Act contractor).

Supplementary Appendix 2. Key questions and workgroup reviewers.

Key Question: What are the performance characteristics of each direct detection test for *Treponema pallidum* and what are the optimal specimen types for each test (darkfield microscopy, direct fluorescent antibody, PCR and immunohistochemical, or silver staining of tissue)?

Key Question: What options are available for molecular epidemiology and what should be considered for specimen collection and preservation?

APHL Workgroup Reviewer: Elitza Theel

Literature Search Terms: (syphilis OR Treponema pallidum) AND (genital ulcer disease OR primary syphilis OR secondary syphilis OR tertiary syphilis OR congenital syphilis OR ocular syphilis) AND (diagnosis OR lesions OR polymerase chain reaction OR PCR OR nucleic acid amplification test OR NAAT OR multiplex test OR silver stain OR silver staining OR immunohistochemistry OR IHC OR rabbit infectivity testing OR RIT OR direct detection OR dark field microscopy OR darkfield microscopy OR dark-field microscopy OR direct fluorescent antibody OR DFA OR direct fluorescent antibody for *T. pallidum* OR DFA-TP OR direct fluorescent antibody tissue test for *T. pallidum* OR DFAT-TP). Solely-based international studies were excluded from the literature search.

Key Question: What are the performance characteristics, stratified by the stage of syphilis, for non-treponemal serologic tests?

APHL Work Group Reviewers: Khalil Ghanem, MD, PhD and Susan Tuddenham, MD, MPH

Literature Search Terms: (syphilis (mesh) OR syphilis (tiab) OR maternal syphilis (tiab) OR syphilis in pregnancy (tiab) OR neurosyphilis (tiab)) AND (syphilis serodiagnosis (mesh) OR serofast (tiab) OR nontreponemal (tiab) OR non-treponemal (tiab) OR VDRL (tiab) OR venereal disease research laboratory (tiab) OR RPR (tiab) OR rapid plasma reagin (tiab) OR Toluidine Red Unheated Serum Test" (tiab)) NOT (review (publication type)) AND (1960/01/01 (PDat): 3000/12/31(PDat)) AND (English (lang)). Solely-based international studies were excluded from the literature search.

Key Question: What are the performance characteristics, stratified by the stage of syphilis, for treponemal serologic tests? (*T. pallidum* particle agglutination, fluorescent treponemal antibody-absorption, enzyme immunoassay, chemiluminescence assay, multiplex bead-based immunoassay)

APHL Work Group Reviewers: Ina Park, MD, MS and Anthony Tran, DrPH, MPH

Literature Search Terms: ((Treponema pallidum OR neurosyphilis OR syphilis) AND (sero-diagnos* OR serodiagnos* OR (serolog* AND (test* OR exam* OR assay* OR screen* OR lab* OR diagnos* OR nontreponemal OR treponemal OR algorithm* OR antibody titer)) OR serofast) NOT exp animals/ not exp humans/. Solely-based international studies were excluded from the literature search.

Key Question: Do laboratory tests perform differently when applied to special populations such as HIV positive individuals or pregnant women? What tests should be used in cases of suspected congenital syphilis?

APHL Work Group Reviewers: Jeanne Sheffield, MD and Ahizechukwu Eke, MD

Literature Search Terms: ((Treponema pallidum OR neurosyphilis OR syphilis) AND (sero-diagnos* OR serodiagnos* OR (serolog* AND (test* OR exam* OR assay* OR screen* OR lab* OR diagnos* OR nontreponemal OR treponemal OR algorithm* OR antibody titer)) OR serofast OR trimester OR rapid test*) NOT exp animals/ not exp humans/. Solely-based international studies were excluded from the literature search.

Key Question: What considerations (i.e., diagnostics and cost-effective implications) should be taken into account when screening for syphilis using either the traditional and reverse algorithm?

APHL Work Group Reviewers: Daniel Ortiz, PhD and Michael Loeffelholz, PhD

Literature Search Terms: ((Treponema pallidum OR neurosyphilis OR syphilis) AND (sero-diagnos* OR serodiagnos* OR (serolog* AND (test* OR exam* OR assay* OR screen* OR lab* OR diagnos* OR nontreponemal OR treponemal OR algorithm* OR antibody titer)) OR serofast) NOT exp animals/ not exp humans/. Solely-based international studies were excluded from the literature search.

Key Question: What serologic-based point-of-care (POC) tests are available to support a syphilis diagnosis, including single syphilis POC tests and combination syphilis/HIV and nontreponemal/treponemal POC tests, and what are the performance characteristics?

APHL Work Group Reviewer: Anthony Tran, DrPH, MPH

Literature Search Terms: (syphilis OR Treponema pallidum) AND (Syphilis Health Check OR rapid test OR point-of-care test OR point of care test OR POC test OR rapid point-of-care test OR rapid point of care test OR RPOC test OR diagnostic test OR combination test OR dual test OR multiplex test OR ASSURED OR rapid syphilis test OR RST OR saliva test OR immunochromatographic test OR finger-stick test). Solely-based international studies were excluded from the literature search.

Supplementary Appendix 3. Peer Review Panel

Megan Crumpler, PhD, HCLD
Laboratory Director
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Sheila Lukehart, PhD
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University of Washington, Seattle, Washington

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Senior Scientific Director, Head R&D, Infectious Disease & Immunology
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Arlene C. Seña, MD, MPH
Professor of Medicine
Institute for Global Health and Infectious Diseases
Adjunct Professor of Epidemiology
Gillings School of Public Health
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Charge to Peer Reviewers: We request your review of the body of literature used to develop "Recommendations for Tests to Detect *Treponema pallidum*, the Causative Agent of Syphilis." As you review the Background, Methods, and Results sections, we would appreciate your thoughts as to whether any key studies have been left out or, in your opinion, misinterpreted as well as comments on the appropriateness of the conclusions. Above all, we are interested in your thoughts about the determinations regarding the quality of the evidence and the strength of the recommendations that were drawn. The questions below will serve as a template to collect and organize your responses. Once you complete your review, please send the review back to the CDC. After the Division of STD Prevention (DSTDP) reviews your comments, they will be posted without attribution along with our responses on the DSTDP.

Template of specific questions:

1. Are there omissions of information or key studies that are critical for the intended audience of clinical laboratory scientists, clinicians, and community health workers? If so, what should be included?

2. Have we included inappropriate information? If so, what should be removed?

3. Does the current scientific understanding of the biology of *T. pallidum* align with the terms "nontreponemal tests" and "treponemal tests" as discussed under the section Syphilis Serologic Laboratory Testing Terminology? Should new terms for nontreponemal tests and treponemal tests be adopted if scientifically appropriate? Would updating these terms add to confusion in the literature? Do you foresee any regulatory implications regarding product insert literature if new terms are proposed? Please explain.

4. Are the recommendations appropriately drawn from the evidence presented? Please explain.

5. Is this document clear and comprehensible? If not, which sections should be revised?

6. Are the recommendations practical and achievable? For example, are resources available for laboratories interested in establishing darkfield microscopy? If not, do you have any suggestions regarding capacity building to ensure the recommendations are practical and achievable.

7. Other comments you might have?

References

1. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K150358). 2015; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K150358.pdf.

2. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K173376). 2018; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K173376.pdf.

3. Creegan L, Bauer HM, Samuel MC, Klausner J, Liska S, Bolan G. An evaluation of the relative sensitivities of the venereal disease research laboratory test and the *Treponema pallidum* particle agglutination test among patients diagnosed with primary syphilis. Sex Transm Dis 2007;34:1016-8. (https://doi.org/10.1097/olq.0b013e3181124473)

4. Huber TW, Storms S, Young P, et al. Reactivity of microhemagglutination, fluorescent treponemal antibody absorption, Venereal Disease Research Laboratory, and rapid plasma reagin tests in primary syphilis. J Clin Microbiol 1983;17:405-9. (https://doi.org/10.1128/jcm.17.3.405-409.1983)

5. Bossak HN, Duncan WP, Harris A, Falcone VH. Assay of tests for syphilis on unheated serum. Public Health Rep 1960;75:196-8. (https://www.ncbi.nlm.nih.gov/pubmed/13803076)

6. Dyckman JD, Wende RD, Gantenbein D, Williams RP. Evaluation of reagin screen, a new serological test for syphilis. J Clin Microbiol 1976;4:145-50. (https://doi.org/10.1128/jcm.4.2.145-150.1976)

7. Dyckman JD, Gatenbein D, Wende RD, Williams RP. Clinical evaluation of a new screening test for syphilis. Am J Clin Pathol 1978;70:918-21. (https://doi.org/10.1093/ajcp/70.6.918)

8. Falcone VH, Stout GW, Moore MB, Jr. Evaluation of Rapid Plasma Reagin (Circle) Card Test. Public Health Rep 1964;79:491-5. (https://www.ncbi.nlm.nih.gov/pubmed/14155846)

9. Sischy A, da L'Exposto F, Dangor Y, et al. Syphilis serology in patients with primary syphilis and non-treponemal sexually transmitted diseases in southern Africa. Genitourin Med 1991;67:129-32. (https://doi.org/10.1136/sti.67.2.129)

10. Moore MB, Jr., Knox JM. Sensitivity and specificity in syphilis serology: Clinical implications. South Med J 1965;58:963-8. (https://www.ncbi.nlm.nih.gov/pubmed/14315433)

11. Castro R, Prieto ES, Santo I, Azevedo J, Exposto Fda L. Evaluation of an enzyme immunoassay technique for detection of antibodies against *Treponema pallidum*. J Clin Microbiol 2003;41:250-3. (https://doi.org/10.1128/jcm.41.1.250-253.2003) (https://www.ncbi.nlm.nih.gov/pubmed/12517856)

12. Glicksman J, Short D, Wende RD, Knox J. Instant syphilis screening; evaluation of the rapid plasma reagin teardrop card test. Tex Med 1967;63:46-8. (https://www.ncbi.nlm.nih.gov/pubmed/6039007)

13. Singh AE, Wong T, De P. Characteristics of primary and late latent syphilis cases which were initially non-reactive with the rapid plasma reagin as the screening test. Int J STD AIDS 2008;19:464-8. (https://doi.org/10.1258/ijsa.2007.007302) (https://www.ncbi.nlm.nih.gov/pubmed/18574118)

14. Castro R, Prieto ES, da Luz Martins Pereira F. Nontreponemal tests in the diagnosis of neurosyphilis: an evaluation of the Venereal Disease Research Laboratory (VDRL) and the Rapid Plasma Reagin (RPR) tests. J Clin Lab Anal 2008;22:257-61. (https://doi.org/10.1002/jcla.20254) (https://www.ncbi.nlm.nih.gov/pubmed/18623120)

15. Dyckman JD, Wende RD. Comparison of serum and plasma specimens for syphilis serology using the reagin screen test. J Clin Microbiol 1980;11:16-8. (https://doi.org/10.1128/jcm.11.1.16-18.1980)

16. Dyckman JD, Storms S, Huber TW. Reactivity of microhemagglutination, fluorescent treponemal antibody absorption, and venereal disease research laboratory tests in primary syphilis. J Clin Microbiol 1980;12:629-30. (https://doi.org/10.1128/jcm.12.4.629-630.1980)

17. Greaves AB. A comparative study of serologic tests in early syphilis. Arch Dermatol 1962;85:641-3. (https://doi.org/10.1001/archderm.1962.01590050071013)

18. Lassus A, Mustakallio KK, Aho K, Putkonen T. The order of appearance of reactivity to treponemal and lipoidal tests in early syphilis. Acta Pathol Microbiol Scand 1967;69:612-3. (https://doi.org/10.1111/j.1699-0463.1967.tb03770.x)

19. Wende RD, Mudd RL, Knox JM, Holder WR. The VDRL slide test in 322 cases of darkfield positive primary syphilis. South Med J 1971;64:633-4. (https://www.ncbi.nlm.nih.gov/pubmed/5573085)

20. Backhouse JL, Nesteroff SI. *Treponema pallidum* western blot: comparison with the FTA-ABS test as a confirmatory test for syphilis. Diagn Microbiol Infect Dis 2001;39:9-14. (https://doi.org/10.1016/s0732-8893(00)00213-3) (https://www.ncbi.nlm.nih.gov/pubmed/11173185)

21. de Lemos EA, Belem ZR, Santos A, Ferreira AW. Characterization of the Western blotting IgG reactivity patterns in the clinical phases of acquired syphilis. Diagn Microbiol Infect Dis 2007;58:177-83. (https://doi.org/10.1016/j.diagmicrobio.2006.12.024) (https://www.ncbi.nlm.nih.gov/pubmed/17350208)

22. Gibowski M, Zaba R, Machonko T. Detection of specific IgM-CLASS antitreponemal antibodies in blood serum of patients with syphilis with the use of CAPTIA Syphilis-M reaction and comparing it with VDRL, FTA-ABS and TPHA reactions. Med Sci Monit 1998;4:PI882-PI8. (https://www.medscimonit.com/download/index/idArt/502060)

23. McMillan A, Young H. Qualitative and quantitative aspects of the serological diagnosis of early syphilis. Int J STD AIDS 2008;19:620-4. (https://doi.org/10.1258/ijsa.2008.008103) (https://www.ncbi.nlm.nih.gov/pubmed/18725554)

24. Park IU, Fakile YF, Chow JM, et al. Performance of treponemal tests for the diagnosis of syphilis. Clin Infect Dis 2019;68:913-8. (https://doi.org/10.1093/cid/ciy558)

25. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K112343). 2012; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K112343.pdf.

26. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K153730). 2016; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K153730.pdf.

27. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K093837). . 2010; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K093837.pdf.

28. Young H, Moyes, A., de Ste Croix, I., McMillan, A. A new recombinant antigen latex agglutination test (Syphilis Fast) for the rapid serological diagnosis of syphilis. Int J STD AIDS 1998;9:196-200. (https://doi.org/10.1258/0956462981922034)

29. Young H, Moyes A, Seagar L, McMillan A. Novel recombinant-antigen enzyme immunoassay for serological diagnosis of syphilis. J Clin Microbiol 1998;36:913-7. (https://doi.org/10.1128/jcm.36.4.913-917.1998) (https://jcm.asm.org/content/jcm/36/4/913.full.pdf)

30. Lefevre JC, Bertrand MA, Bauriaud R. Evaluation of the Captia enzyme immunoassays for detection of immunoglobulins G and M to *Treponema pallidum* in syphilis. J Clin Microbiol 1990;28:1704-7. (https://doi.org/10.1128/jcm.28.8.1704-1707.1990) (https://jcm.asm.org/content/jcm/28/8/1704.full.pdf)

31. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K160910). 2016; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K160910.pdf.

32. Ijsselmuiden OE, Meinardi MMHM, van der Sluis JJ, Menke HE, Stolz E, van Eijk RVW. Enzyme-linked immunofiltration assay for rapid serodiagnosis of syphilis. European Journal of Clinical Microbiology 1987;6:281-5. (10.1007/BF02017613) (https://doi.org/10.1007/BF02017613)

33. Ijsselmuiden OE, Schouls LM, Stolz E, et al. Sensitivity and specificity of an enzyme-linked immunosorbent assay using the recombinant DNA-derived *Treponema pallidum* protein TmpA for serodiagnosis of syphilis and the potential use of TmpA for assessing the effect of antibiotic therapy. J Clin Microbiol 1989;27:152-7. (https://doi.org/10.1128/jcm.27.1.152-157.1989) (https://www.ncbi.nlm.nih.gov/pubmed/2643617)

34. Romanowski B FE, Prasad E, Lukehart S, Tam M, Hook EW 3rd. Detection of *Treponema pallidum* by a fluorescent monoclonal antibody test. Sex Transm Dis 1987;14:156-9. (https://doi.org/10.1097/00007435-198707000-00007) (https://journals.lww.com/stdjournal/Fulltext/1987/07000/Detection_of_Treponema_pallidum_by_a_Fluorescent.7.aspx)

35. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K091361). 2009; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K091361.pdf.

36. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K061247). 2006; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K061247.pdf.

37. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K153145). 2016; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K153145.pdf.

38. Augenbraun M, Rolfs R, Johnson R, et al. Treponemal specific tests for the serodiagnosis of syphilis. Sex Transm Dis 1998;25:549-52. (https://journals.lww.com/stdjournal/Fulltext/1998/11000/Treponemal_Specific_Tests_for_the_Serodiagnosis_of.10.aspx)

39. Larsen SA, Hambie EA, Pettit DE, Perryman MW, Kraus SJ. Specificity, sensitivity, and reproducibility among the fluorescent treponemal antibody-absorption test, the microhemagglutination assay for *Treponema pallidum* antibodies, and the hemagglutination treponemal test for syphilis. J Clin Microbiol 1981;14:441-5. (https://doi.org/10.1128/jcm.14.4.441-445.1981) (https://jcm.asm.org/content/jcm/14/4/441.full.pdf)

40. Pope V, Hunter EF, Feeley JC. Evaluation of the microenzyme-linked immunosorbent assay with *Treponema pallidum* antigen. J Clin Microbiol 1982;15:630-4. (https://doi.org/10.1128/jcm.15.4.630-634.1982) (https://jcm.asm.org/content/jcm/15/4/630.full.pdf)

41. Coffey EM, Bradford LL, Naritomi LS, Wood RM. Evaluation of the qualitative and automated quantitative microhemagglutination assay for antibodies to *Treponema pallidum*. Appl Microbiol 1972;24:26-30. (https://doi.org/10.1128/am.24.1.26-30.1972) (https://www.ncbi.nlm.nih.gov/pubmed/4560472)

42. Manavi K, Young, H. & McMillan, A. The sensitivity of syphilis assays in detecting different stages of early syphilis. Int J STD AIDS 2006;17:768-71. (https://doi.org/10.1258/095646206778691185)

43. Lam TK, Lau HY, Lee YP, Fung SM, Leung WL, Kam KM. Comparative evaluation of the Inno-Lia syphilis score and the MarDx *Treponema pallidum* immunoglobulin G Marblot test assays for the serological diagnosis of syphilis. Int J STD AIDS 2010;21:110-3. (https://doi.org/10.1258/ijsa.2009.009026)

44. Gratzer B, Pohl D, Hotton AL. Evaluation of diagnostic serological results in cases of suspected primary syphilis infection. Sex Transm Dis 2014;41:285-9. (https://doi.org/10.1097/olq.0000000000000126) (https://www.ncbi.nlm.nih.gov/pubmed/24722379)

45. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K053570). 2006; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K053570.pdf.

46. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K102283). 2011; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K102283.pdf.

47. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K170413). 2017; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K170413.pdf.

48. Zhu L, Gu X, Peng RR, et al. Comparison of the cerebrospinal fluid (CSF) toluidine red unheated serum test and the CSF rapid plasma reagin test with the CSF venereal disease research laboratory test for diagnosis of neurosyphilis among HIV-negative syphilis patients in China. J Clin Microbiol 2014;52:736-40. (https://doi.org/10.1128/jcm.02522-13) (https://www.ncbi.nlm.nih.gov/pubmed/24335955)

49. Marra CM, Tantalo LC, Maxwell CL, Ho EL, Sahi SK, Jones T. The rapid plasma reagin test cannot replace the venereal disease research laboratory test for neurosyphilis diagnosis. Sex Transm Dis 2012;39:453-7. (https://doi.org/10.1097/olq.0b013e31824b1cde) (https://www.ncbi.nlm.nih.gov/pubmed/22592831)

50. Marra CM, Maxwell CL, Dunaway SB, Sahi SK, Tantalo LC. Cerebrospinal fluid *Treponema pallidum* particle agglutination assay for neurosyphilis diagnosis. J Clin Microbiol 2017;55:1865-70. (https://doi.org/10.1128/jcm.00310-17) (https://www.ncbi.nlm.nih.gov/pubmed/28381602)

51. Buffet M, Grange PA, Gerhardt P, et al. Diagnosing *Treponema pallidum* in secondary syphilis by PCR and immunohistochemistry. Journal of Investigative Dermatology 2007;127:2345-50. (https://doi.org/10.1038/sj.jid.5700888) (http://www.sciencedirect.com/science/article/pii/S0022202X15331444)

52. Daniels KC FH. Specific direct fluorescent antibody detection of *Treponema pallidum*. Health Laboratory Science 1977;14:164-71. (https://pubmed.ncbi.nlm.nih.gov/326728/)

53. Grange PA, Gressier L, Dion PL, et al. Evaluation of a PCR test for detection of *Treponema pallidum* in swabs and blood. J Clin Microbiol 2012;50:546-52. (https://doi.org/10.1128/jcm.00702-11) (https://www.ncbi.nlm.nih.gov/pubmed/22219306)

54. Hook EW, 3rd, Roddy RE, Lukehart SA, Hom J, Holmes KK, Tam MR. Detection of *Treponema pallidum* in lesion exudate with a pathogen-specific monoclonal antibody. J Clin Microbiol 1985;22:241-4. (https://doi.org/10.1128/jcm.22.2.241-244.1985) (https://www.ncbi.nlm.nih.gov/pubmed/3897267)

55. Lee WS, Lee MG, Chung KY, Lee JB. Detection of *Treponema pallidum* in tissue: a comparative study of the avidin-biotin-peroxidase complex, indirect immunoperoxidase, FTA-ABS complement techniques and the darkfield method. Yonsei Med J 1991;32:335-41. (https://doi.org/10.3349/ymj.1991.32.4.335)

56. Grimprel E, Sanchez PJ, Wendel GD, et al. Use of polymerase chain reaction and rabbit infectivity testing to detect *Treponema pallidum* in amniotic fluid, fetal and neonatal sera, and cerebrospinal fluid. J Clin Microbiol 1991;29:1711-8. (https://doi.org/10.1128/jcm.29.8.1711-1718.1991) (https://www.ncbi.nlm.nih.gov/pubmed/1761693)

57. Hollier LM, Harstad TW, Sanchez PJ, Twickler DM, Wendel GD. Fetal syphilis: clinical and laboratory characteristics. Obstetrics & Gynecology 2001;97:947-53. (https://doi.org/10.1016/S0029-7844(01)01367-9) (http://www.sciencedirect.com/science/article/pii/S0029784401013679)

58. Behrhof W, Springer E, Bräuninger W, Kirkpatrick CJ, Weber A. PCR testing for *Treponema pallidum* in paraffin-embedded skin biopsy specimens: test design and impact on the diagnosis of syphilis. J Clin Pathol 2008;61:390-5. (https://doi.org/10.1136/jcp.2007.046714) (https://jcp.bmj.com/content/jclinpath/61/3/390.full.pdf)

59. Hoang MP, High WA, Molberg KH. Secondary syphilis: a histologic and immunohistochemical evaluation. J Cutan Pathol 2004;31:595-9. (https://doi.org/10.1111/j.0303-6987.2004.00236.x) (https://onlinelibrary.wiley.com/doi/abs/10.1111/j.0303-6987.2004.00236.x)

60. Cruz AR, Pillay A, Zuluaga AV, et al. Secondary syphilis in cali, Colombia: new concepts in disease pathogenesis. PLoS Negl Trop Dis 2010;4:e690-e. (https://doi.org/10.1371/journal.pntd.0000690) (https://www.ncbi.nlm.nih.gov/pubmed/20502522)

61. Zoechling N, Schluepen E, Soyer H, Kerl H, Volkenandt M. Molecular detection of *Treponema pallidum* in secondary and tertiary syphilis. Brit J Dermatol 1997;136:683-6. (https://doi.org/10.1046/j.1365-2133.1997.6561614.x) (https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2133.1997.6561614.x)

62. Heymans R, van der Helm JJ, de Vries HJC, Fennema HSA, Coutinho RA, Bruisten SM. Clinical value of *Treponema pallidum* real-time PCR for diagnosis of syphilis. J Clin Microbiol 2010;48:497-502. (https://doi.org/10.1128/jcm.00720-09) (https://www.ncbi.nlm.nih.gov/pubmed/20007388)

63. Gayet-Ageron A, Ninet B, Toutous-Trellu L, et al. Assessment of a real-time PCR test to diagnose syphilis from diverse biological samples. Sex Transm Infect 2009;85:264-9. (https://doi.org/10.1136/sti.2008.034314) (https://sti.bmj.com/content/sextrans/85/4/264.full.pdf)

64. Orle KA, Gates CA, Martin DH, Body BA, Weiss JB. Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. J Clin Microbiol 1996;34:49-54. (https://doi.org/10.1128/jcm.34.1.49-54.1996) (https://www.ncbi.nlm.nih.gov/pubmed/8748271)

65. Palmer HM, Higgins SP, Herring AJ, Kingston MA. Use of PCR in the diagnosis of early syphilis in the United Kingdom. Sex Transm Infect 2003;79:479-83. (https://doi.org/10.1136/sti.79.6.479) (https://www.ncbi.nlm.nih.gov/pubmed/14663125)

66. Martin IE, Tsang RSW, Sutherland K, et al. Molecular characterization of syphilis in patients in Canada: azithromycin resistance and detection of *Treponema pallidum* DNA in whole-blood samples versus ulcerative swabs. J Clin Microbiol 2009;47:1668-73. (https://doi.org/10.1128/jcm.02392-08) (https://www.ncbi.nlm.nih.gov/pubmed/19339468)

67. Yang CJ, Chang SY, Wu BR, et al. Unexpectedly high prevalence of *Treponema pallidum* infection in the oral cavity of human immunodeficiency virus-infected patients with early syphilis who had engaged in unprotected sex practices. Clin Microbiol Infect 2015;21:787.e1-

.e7. (https://doi.org/10.1016/j.cmi.2015.04.018) (http://www.sciencedirect.com/science/article/pii/S1198743X15004310)

68. Fakile YF, Brinson M, Mobley V, Park IU, Gaynor AM. Performance of the Syphilis Health Check in clinic and laboratory-based settings. Sex Transm Dis 2019;46:250-3. (https://doi.org/10.1097/olq.0000000000000974) (https://journals.lww.com/stdjournal/Fulltext/2019/04000/Performance_of_the_Syphilis_Health_Check_in_Clinic.7.aspx)

69. Matthias J DP, Totten Y, Blackmore C, Wilson C, Peterman TA. Notes from the field. Evaluation of the sensitivity and specificity of a commercially available rapid syphilis test — Escambia County, Florida, 2016. MMWR Morb Mortal Wkly Rep 2016;65:1174-5. (http://dx.doi.org/10.15585/mmwr.mm6542a5)

70. Obafemi OA, Wendel KA, Anderson TS, et al. Rapid syphilis testing for men who have sex with men in outreach settings: Evaluation of test performance and impact on time to treatment. Sex Transm Dis 2019;46:191-5. (https://doi.org/10.1097/olq.0000000000000932) (https://journals.lww.com/stdjournal/Fulltext/2019/03000/Rapid_Syphilis_Testing_for_Men_Who_Have_Sex_With.8.aspx)

71. Fakile YF, Markowitz N, Zhu W, et al. Evaluation of a rapid syphilis test in an emergency department setting in Detroit, Michigan. Sex Transm Dis 2019;46:429-33. (https://doi.org/10.1097/olq.0000000000000993) (https://journals.lww.com/stdjournal/Fulltext/2019/07000/Evaluation_of_a_Rapid_Syphilis_Test_in_an.2.aspx)

72. Pereira LE, McCormick J, Dorji T, et al. Laboratory evaluation of a commercially available rapid syphilis test. J Clin Microbiol 2018;56:e00832-18. (https://doi.org/10.1128/jcm.00832-18) (https://www.ncbi.nlm.nih.gov/pubmed/30021825)

73. United States Food and Drug Administration. 510k substantial equivalence determination decision summary (K102400). 2011; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K102400.pdf.

74. Humphries RM, Woo JS, Chung JH, Sokovic A, Bristow CC, Klausner JD. Laboratory evaluation of three rapid diagnostic tests for dual detection of HIV and *Treponema pallidum* antibodies. J Clin Microbiol 2014;52:4394-7. (https://doi.org/10.1128/jcm.02468-14) (https://pubmed.ncbi.nlm.nih.gov/25297332)

75. Leon SR, Ramos LB, Vargas SK, et al. Laboratory evaluation of a Dual-Path Platform Assay for rapid point-of-care HIV and syphilis testing. J Clin Microbiol 2016;54:492-4. (https://doi.org/10.1128/JCM.03152-15)

76. United States Food and Drug Administration. DPP HIV-Syphilis System (PMA: BP180191). 2020; Available from: https://www.fda.gov/vaccines-blood-biologics/blood-blood-products/dpp-hiv-syphilis-system.