

Treponema-Specific Tests for Serodiagnosis of Syphilis: Comparative Evaluation of Seven Assays[∇]

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The diagnosis of syphilis is challenging and often relies on serologic tests to detect treponemal or nontreponemal antibodies. Recently, the Centers for Disease Control and Prevention and the Association of Public Health Laboratories proposed an update to the syphilis serology testing algorithm, in which serum samples are first tested using a treponema-specific test and positive samples are analyzed with a nontreponemal assay. The goal of this study was to compare the performance of seven treponemal assays (BioPlex 2200 syphilis IgG [Bio-Rad, Hercules, CA], fluorescent treponemal antibody [FTA] assay [Zeus Scientific, Raritan, NJ], *Treponema pallidum* particle agglutination [TP-PA; Fujirebio Diagnostics, Malvern, PA], Trep-Sure enzyme immunoassay [EIA; Phoenix Biotech, Oakville, Ontario, Canada], Trep-Chek EIA [Phoenix Biotech], Trep-ID EIA [Phoenix Biotech], and Treponema ViraBlot IgG [Viramed Biotech AG, Planegg, Germany]) using serum samples ($n = 303$) submitted to our reference laboratory. In addition to testing with these 7 assays, all samples were tested by a rapid plasma reagin (RPR) assay and a treponemal IgM Western blot assay (Viramed ViraBlot). Compared to the FTA assay as the gold standard, the evaluated treponemal tests demonstrated comparable levels of performance, with percent agreement ranging from 95.4% (95% confidence interval, 92.3 to 97.3) for the Trep-Sure EIA to 98.4% (96.1 to 99.4) for the Trep-ID EIA. Compared to a “consensus of the test panel” (defined as at least 4 of 7 treponemal tests being in agreement), the percent agreement ranged from 95.7% (92.7 to 97.5) for Trep-Sure to 99.3% (97.5 to 99.9) for Trep-ID. These data may assist clinical laboratories that are considering implementing a treponemal test for screening or confirmatory purposes.

The diagnosis of syphilis is often based on the results of serology using assays designed to detect either nontreponemal (e.g., rapid plasma reagin [RPR]) or treponema-specific antibodies (e.g., fluorescent treponemal antibody [FTA]). Historically, serum samples have been screened using a nontreponemal test, with positive samples being confirmed by a treponemal assay (5). While this approach is cost effective and demonstrates reliable performance in areas of high disease prevalence, it has several limitations, including low test throughput and the subjective interpretation of nontreponemal screening results. Recently, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) released an updated algorithm for laboratory testing and result interpretation of samples from patients with suspected *Treponema pallidum* infection (1). This algorithm suggests that in areas of low disease prevalence (e.g., a rate of <2.2 per 100,000 population; <http://www.cdc.gov/std/stats09/figures/37.htm>), samples may be screened using a treponema-specific assay (e.g., enzyme immunoassay [EIA]), with positive samples being analyzed with a nontreponemal test to assess disease and treatment status.

Treponemal assays based on EIA, chemiluminescence immunoassay (CIA), or multiplex flow immunoassay (MFI) technology are often chosen for screening over conventional methods, such as FTA or *Treponema pallidum* particle agglutination (TP-PA) assay, due to higher testing throughput and the ob-

jective interpretation of results. However, the use of a treponemal test (whether it is a conventional or contemporary method) for screening purposes is not without limitations. With the increasing implementation of treponema-specific assays as first-line syphilis screening tests, health care providers are now faced with patients who are positive by a treponema-specific screening test yet are negative by nontreponemal tests (10). This discordance in test results is commonly observed in our laboratory and is the source of much confusion and anxiety among health care providers and patients. Although such result discordance may suggest a false-positive screening test, it may also occur in patients with past or recently treated syphilis and in patients with very early or late/latent disease (8). Given these variables in interpretation, health care providers must perform careful reviews of their patients' disease and treatment histories. If a false-positive screening test is suspected based on a low pretest probability of disease, a second treponema-specific test (e.g., FTA) is recommended before ruling out the diagnosis of syphilis. Similarly, if such result discordance is observed in a patient without a history of treatment, a second treponema-specific test should be performed to rule out early or late/latent disease (1).

The goal of this study was to compare the performance of seven commercially available treponema-specific assays (BioPlex 2200 syphilis IgG [Bio-Rad, Hercules, CA], FTA [Zeus Scientific, Raritan, NJ], Serodia *Treponema pallidum* particle agglutination [Fujirebio Diagnostics, Malvern, PA], Trep-Sure EIA [Phoenix Biotech, Oakville, Ontario, Canada], Trep-Chek EIA [Phoenix Biotech], Trep-ID EIA [Phoenix Biotech], and Treponema ViraBlot IgG [Viramed Biotech AG, Planegg, Germany]) using serum samples ($n = 303$) submitted to our

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reference laboratory. The study was designed to assess whether contemporary treponema-specific assays based on EIA, Western blot (WB), or multiplex flow immunoassay technology yield results comparable to those of conventional methods (e.g., FTA or TP-PA) that are commonly chosen for confirmatory purposes.

MATERIALS AND METHODS

Study design. Serum samples ($n = 303$) submitted to our reference laboratory were tested with the 7 treponema-specific assays described below. In addition, each sample was tested with an RPR and an IgM WB assay (Viramed Treponema ViraBlot) to assess potential recent infection. Among the 303 serum samples, 203 (67.0%) were submitted consecutively from hospitals and clinics throughout the United States, while the remaining 100 (33.0%) samples were selected based on the results of prior syphilis testing in our laboratory. Samples were collected and tested over the study period (~60 days), with technologists blinded to the results of other tests. Samples were stored at 4°C until all testing was complete so that analyses were performed in the same freeze-thaw cycle. The study protocol was reviewed by the institutional review board at our center.

Enzyme immunoassay. All serum samples were tested according to the manufacturer's instructions using the following EIAs: Trep-ID, Trep-Chek, and Trep-Sure (Phoenix Biotech). The Trep-ID EIA is designed for the qualitative detection of total (IgG and/or IgM) antibodies against *T. pallidum* (Tp) and utilizes the recombinant treponemal antigens Tp47, Tp17, Tp15, and Tp44 (TmpA). The results are calculated as index values (optical density of sample/cutoff value) and are then classified as negative (<1.0) or positive (≥ 1.0). The Trep-Chek EIA is a qualitative test designed to detect IgG class antibodies to *T. pallidum* and uses a cocktail of proprietary recombinant antigens. The results of the Trep-Chek EIA are calculated as index values and reported as negative (<0.9), equivocal (0.9 to 1.1), or positive (>1.1). The Trep-Sure EIA qualitatively measures total (IgG and/or IgM) antibodies using proprietary recombinant treponemal antigens. The results are reported as negative (<0.8), equivocal (0.8 to 1.2), or positive (>1.2). All testing by EIA was performed on a Triturus automated analyzer (Grifols, Inc., Barcelona, Spain).

Fluorescent treponemal antibody absorption. Testing using the FTA assay (Zeus Scientific) was performed according to the manufacturer's instructions. The Zeus FTA assay employs nonviable *T. pallidum* (Nichols strain) as the substrate capture antigen for the detection of total antibodies against *T. pallidum*.

Multiplex flow immunoassay. Testing by MFI was performed according to the manufacturer's instructions, using the BioPlex 2200 syphilis IgG kit on a BioPlex 2200 analyzer (3). The BioPlex syphilis IgG kit consists of three different populations of dyed beads that are coated with recombinant proteins derived from *T. pallidum* (Tp15, Tp17, and Tp47). Following flow cytometric analysis, the data are initially calculated in relative fluorescence intensity (RFI) and are then converted to a fluorescence ratio (FR) using an internal standard bead. The FR is compared to an assay-specific calibration curve to determine analyte concentration in antibody index (AI) units. The interpretive criteria were established by the manufacturer, and results are defined as negative (≤ 0.8 AI), equivocal (0.9 to 1.0 AI), or positive (≥ 1.1 AI).

Rapid plasma reagin assay. Testing by the RPR assay was performed according to the manufacturer's instructions using the BD Macro-Vue assay (Becton Dickinson, Franklin Lakes, NJ). Serum samples were tested undiluted, and in addition, a 2-fold dilution series was prepared using 0.9% sodium chloride diluent as outlined in the manufacturer's instructions.

Treponema pallidum particle agglutination. Samples were tested with the Serodia TP-PA assay (Fujirebio, Inc.) according to the manufacturer's instructions. This assay is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigen. Testing and result interpretation were performed in strict accordance with the recommendations outlined in the manufacturer's instructions.

Western blot analysis for IgG and IgM class antibodies. Testing by WB was performed according to the manufacturer's instructions using the Treponema ViraBlot IgG and IgM assays (Viramed Biotech AG). These assays utilize nitrocellulose strips with *T. pallidum*-specific antigens Tp47, Tp44.5, Tp17, and Tp15. Sample processing was performed using a BeeBlot (BeeRobotics, Gwynedd, United Kingdom). Test strips were then scanned and analyzed using the ViraScan interpretive software (Viramed Biotech AG), with the final interpretation of results being made by a laboratory technologist.

Assessment of analytical specificity. In order to assess the analytical specificity of the evaluated treponemal assays, sera known to be positive for potentially cross-reactive analytes (anti-herpes simplex virus IgM [$n = 2$] or IgG [$n = 5$],

anti-Epstein-Barr viral capsid antigen [VCA] IgM [$n = 5$] or IgG [$n = 5$], rheumatoid factor [$n = 3$], or heterophile antibodies [$n = 5$]) were tested with each treponema-specific assay and the RPR assay. In addition, sera collected from pregnant females ($n = 28$) for routine prenatal serology were tested.

Analysis of turnaround time, sample throughput, and cost. The approximate turnaround time (TAT) for testing and reporting of 100 serum samples for each treponema-specific assay was calculated using incubation and reaction times provided in the manufacturer's instructions for use. Estimations were made based on the use of a single instrument or performing technologist. The sample throughput of each assay was then calculated for a 9-h shift using the following equation: $(9/\text{TAT}) \times 100$. The cost-per-patient for each treponema-specific test was determined as the list price for reagents, as supplied by the manufacturer, and does not account for instrumentation or personnel cost associated with testing.

Statistics. Statistical analyses were performed using GraphPad software (GraphPad Software, Inc.; <http://graphpad.com/quickcalcs/index.cfm>). In addition to percent agreement, kappa coefficients were calculated as a secondary measure of agreement. The agreement of the results by kappa (κ) values is categorized as near perfect (0.81 to 1.0), substantial (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), slight (0 to 0.2), or poor (<0) (4).

RESULTS

Comparison of six treponemal assays to the FTA assay.

Following testing of 303 serum samples, the results of each treponema-specific assay were compared to those of the FTA assay, which was established as the gold standard method, similar to numerous prior studies (6, 11). The overall percent agreement and corresponding kappa values were as follows: BioPlex syphilis IgG, 98.0% (95% confidence interval [CI] = 95.6 to 99.2), $\kappa = 0.96$; TP-PA, 97.0% (94.4 to 98.5), $\kappa = 0.93$; Trep-Chek EIA, 97.7% (95.2 to 99.0), $\kappa = 0.95$; Trep-Sure EIA, 95.4% (92.3 to 97.3), $\kappa = 0.90$; Trep-ID EIA, 98.4% (96.1 to 99.4), $\kappa = 0.96$; and ViraBlot IgG, 97.0% (94.4 to 98.5), $\kappa = 0.93$ (Table 1).

Comparison of seven treponemal assays to a consensus of the test panel. Due to the limitations of the FTA assay as a gold standard (e.g., subjective interpretation resulting in inter- and intrareader variability), we also analyzed the data by comparing the results of each treponema-specific assay to a "consensus of the test panel," which was defined as at least 4 of the 7 treponemal test results being in agreement. The overall percent agreement and corresponding kappa values were as follows: BioPlex syphilis IgG, 99.0% (97.0 to 99.8), $\kappa = 0.98$; FTA, 99.0 (97.0 to 99.8), $\kappa = 0.98$; TP-PA, 98.0% (95.6 to 99.2), $\kappa = 0.95$; Trep-Chek EIA, 98.7% (96.5 to 99.6), $\kappa = 0.97$; Trep-Sure EIA, 95.7% (92.7 to 97.5), $\kappa = 0.90$; Trep-ID EIA, 99.3% (97.5 to 99.9), $\kappa = 0.99$; and ViraBlot IgG, 98.0% (95.6 to 99.2), $\kappa = 0.95$ (Table 2).

Assessment of analytical specificity. All members of the cross-reactivity panel, including 28 sera from pregnant females, were negative when tested with the BioPlex syphilis IgG, Trep-Chek IgG, TP-PA, and Trep-Sure assays. The FTA assay yielded negative results for all members of the cross-reactivity panel, with the exception of 1 of 5 (20%) samples known to be positive for anti-Epstein-Barr virus (EBV) VCA IgG and 1 of 28 (3.6%) sera collected from pregnant females. All cross-reactivity samples were negative with the ViraBlot IgG assay, with the exception of 1 of 28 (3.6%) sera collected from pregnant females, which resulted as equivocal with the ViraBlot IgG assay. The Trep-ID EIA yielded negative results for all members of the cross-reactivity panel, except for 1 of 28 (3.6%) samples from pregnant females, which was positive by this

TABLE 1. Comparison of six treponemal assays to the FTA assay using serum specimens^a

Assay and result	FTA result (no. of samples)		% Sensitivity (95% CI)	% Specificity (95% CI)	% Agreement (95% CI)	κ value
	Positive	Negative				
BioPlex syphilis IgG ^b						
Positive	94	3	96.9 (90.9, 99.3)	98.5 (95.6, 99.7)	98.0 (95.6, 99.2)	0.96
Negative	3	202				
TP-PA						
Positive	93	5	95.9 (89.5, 98.7)	97.6 (94.3, 99.1)	97.0 (94.4, 98.5)	0.93
Negative	4	201				
Trep-Chek IgG						
Positive	93	1	95.9 (89.5, 98.7)	98.5 (95.6, 99.7)	97.7 (95.2, 99.0)	0.95
Negative	4	203				
Equivocal	0	2				
Trep-Sure						
Positive	94	4	96.9 (90.9, 99.3)	94.7 (90.6, 97.1)	95.4 (92.3, 97.3)	0.90
Negative	3	195				
Equivocal	0	7				
Trep-ID						
Positive	94	2	96.9 (90.9, 99.3)	99.0 (96.3, 100)	98.4 (96.1, 99.4)	0.96
Negative	3	204				
ViraBlot IgG						
Positive	91	2	93.8 (86.9, 97.4)	98.5 (95.6, 99.7)	97.0 (94.4, 98.5)	0.93
Negative	5	203				
Equivocal	1	1				

^a A total of 303 samples were analyzed.

^b Only 302 samples were analyzed for the BioPlex, as one sample did not yield a result due to an instrument error code.

assay. Finally, all cross-reactivity samples were negative by the RPR assay, with the exception of 1 of 5 (20%) samples that was positive for anti-EBV VCA IgG (Table 3).

Turnaround time, sample throughput, and reagent cost.

The BioPlex syphilis IgG assay was estimated to yield the shortest TAT (1.75 h) for the analysis and reporting of 100 samples. In contrast, the Trep-ID assay had an estimated TAT of 5.7 h for 100 samples, using a single instrument and interpreting technologist. The BioPlex yielded the highest estimated sample throughput (514 samples) during a 9-h shift, while the Trep-ID assay was estimated to generate the lowest sample throughput (158 samples). The list price reagent cost (cost per patient) ranged from \$1.73 (TP-PA) to \$18.75 (Trep-ID); however, these values do not account for instrumentation or associated personnel cost (Table 4).

DISCUSSION

Recent updates to the syphilis testing algorithm propose the use of a treponema-specific assay (e.g., EIA) for screening purposes, with positive samples being analyzed by a nontreponemal test (1). This paradigm shift represents a reversal of a long-held practice and has generated substantial confusion among health care providers and patients, especially when results are positive by a treponemal screening assay but negative by nontreponemal tests. This discordance in test results is commonly observed in our laboratory and prompted us to evaluate and implement a second treponema-specific assay for supplemental/confirmatory purposes.

Despite our findings showing comparable performance of the 7 treponemal assays, there were samples with discordant results

that became a focus for further investigation. In order to potentially resolve these discrepancies, we reviewed the results of all other treponemal tests, as well as those of the RPR and IgM assays, to determine the likelihood of past or recent infection. Among the 3 samples that were BioPlex positive, consensus of the panel (“panel” hereinafter) negative, 1 sample showed results consistent with recent infection due to positive results by 2 other treponemal tests (Trep-Sure EIA and ViraScan IgG), as well as positive IgM and RPR results (titer = 16). The remaining 2 samples were negative by all other tests and were interpreted as probable false-positive BioPlex results (Table 2).

When we compared the FTA results to the consensus of the panel, we identified 3 discordant samples, with FTA-positive, panel-negative results. One of these 3 samples was also positive by the Trep-Sure assay but negative by all other tests. The remaining 2 samples were negative by all other tests (including the RPR and IgM assays) and were interpreted as probable false-positive FTA results (Table 2).

Among the 2 Trep-Chek discordant samples (Table 2), 1 sample was Trep-Chek positive, panel negative. This sample was also positive by the Trep-Sure assay but was negative by all other tests. The second discordant sample (Trep-Chek negative, panel positive) showed results consistent with infection due to positive results by the 6 other treponemal assays, as well as a positive RPR assay (titer = 2). We interpreted this sample as a probable false-negative Trep-Chek IgG result.

During our data analysis, we identified 6 TP-PA discordant samples. Among the 5 TP-PA-positive, panel-negative samples, 1 was also positive by the ViraBlot IgG assay but was negative by all

TABLE 2. Comparison of seven treponemal assays to the consensus of the test panel using serum specimens^a

Assay and result	Consensus of panel (no. of samples) ^b		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Agreement (%) (95% CI)	κ value
	Positive	Negative				
BioPlex syphilis IgG ^c						
Positive	94	3	100 (95.3, 100)	98.6 (95.7, 99.7)	99.0 (97.0, 99.8)	0.98
Negative	0	205				
FTA						
Positive	94	3	100 (95.3, 100)	98.6 (95.7, 99.7)	99.0 (97.0, 99.8)	0.98
Negative	0	206				
Trep-Chek IgG						
Positive	93	1	98.9 (93.6, 99.9)	98.6 (95.7, 99.7)	98.7 (96.5, 99.6)	0.97
Negative	1	206				
Equivocal	0	2				
TP-PA						
Positive	93	5	98.9 (93.6, 99.9)	97.6 (94.4, 99.1)	98.0 (95.6, 99.2)	0.95
Negative	1	204				
Trep-Sure						
Positive	93	5	98.9 (93.6, 99.9)	94.3 (90.1, 96.8)	95.7 (92.7, 97.5)	0.90
Negative	1	197				
Equivocal	0	7				
Trep-ID						
Positive	94	2	100 (95.3, 100)	99.0 (96.4, 99.9)	99.3 (97.5, 99.9)	0.99
Negative	0	207				
ViraBlot IgG						
Positive	91	2	96.8 (90.6, 99.3)	98.6 (95.7, 99.7)	98.0 (95.6, 99.2)	0.95
Negative	2	206				
Equivocal	1	1				

^a A total of 303 samples were analyzed.

^b Consensus of the panel was defined as at least 4 of 7 treponemal IgG or total antibody tests being in agreement.

^c Only 302 samples were analyzed for the BioPlex, as one sample did not yield a result due to an instrument error code.

other tests. The remaining 4 samples were negative by all other tests and were interpreted as probable false-positive TP-PA results. There was also 1 sample that was TP-PA negative, panel positive. This sample showed results consistent with recent infection due to positive results by the 6 other treponemal assays, as well as positive IgM and RPR results (titer = 4), and therefore, was interpreted as a probable false-negative TP-PA result (Table 2).

Similarly, there were 6 Trep-Sure discordant samples when the results were compared to those of the panel. Among the 5 Trep-Sure-positive, panel-negative samples (Table 2), 2 were

positive by 1 additional treponemal assay (FTA or Trep-Chek IgG) but negative by all other tests. One sample showed results consistent with recent infection due to positive results for 2 other treponemal assays (BioPlex IgG and ViraBlot IgG), as well as positive IgM and RPR results (titer = 16). This sample was interpreted as a probable true positive by the Trep-Sure assay. The remaining 2 Trep-Sure-positive, panel-negative samples were negative by all other tests and probably represented false-positive Trep-Sure results. The single Trep-Sure-negative, panel-positive sample showed results consistent

TABLE 3. Cross-reactivity serum panel^a tested by seven treponemal assays and the RPR assay

Potentially cross-reactive analyte or condition (no. of sera tested)	No. (%) of sera testing positive or equivocal by:							
	BioPlex syphilis IgG	FTA	Trep-Chek IgG	TP-PA	Trep-Sure	Trep-ID	ViraBlot IgG	RPR
HSV ^b IgG (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
HSV IgM (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
EBV IgG (5)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)
EBV IgM (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Rheumatoid factor (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Heterophile antibodies (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pregnancy (28)	0 (0)	1 (3.6)	0 (0)	0 (0)	0 (0)	1 (3.6)	1 (3.6) ^c	0 (0)

^a The cross-reactivity test panel comprised 53 sera.

^b HSV, herpes simplex virus.

^c This result was equivocal by the ViraBlot IgG assay.

TABLE 4. Comparison of turnaround time, sample throughput, and reagent cost among 7 treponema-specific assays

Assay	Turnaround time (h) ^a	Sample throughput (no. of samples) ^{a,b}	Reagent cost (\$) ^c
BioPlex syphilis IgG	1.75	514	9.00
FTA	3.3	272	3.45
TP-PA	4.0	225	1.73
Trep-Chek IgG	2.2	409	2.86
Trep-ID	5.7	158	18.75
Trep-Sure	2.3	391	3.07
ViraBlot IgG	5.5	163	15.00

^a Results were calculated for 100 patient samples tested by a single instrument or performing technologist.

^b Sample throughput was estimated for a 9-h shift using a single instrument or performing technologist.

^c List price (cost per patient) for reagents as supplied by the manufacturer. The amount does not include instrument or personnel cost associated with performing the test. The reagent fee for RPR was \$0.51 per patient.

with past, treated infection due to positive results by the 6 other treponemal tests but negative IgM and RPR results (Table 2).

Of the 2 Trep-ID discordant samples, both were Trep-ID positive, panel negative. These samples were negative by all other tests and were interpreted as probable false-positive Trep-ID results. Finally, our data analysis revealed 4 discordant samples when we compared the results of the ViraBlot IgG to those of the panel. Of the 2 ViraBlot-positive, panel-negative samples, one was also positive by the TP-PA assay but negative by all other tests, while the second sample showed results consistent with recent infection due to positive results by 2 other treponemal tests (BioPlex IgG and Trep-Sure) and positive IgM and RPR results (titer = 16). There were also 2 samples that were ViraBlot negative, panel positive. One of these 2 samples showed results consistent with past, treated infection due to positive results by the 6 other treponemal tests but negative IgM and RPR results. The second sample showed results consistent with recent infection due to positive results by the 6 other treponemal tests, as well as positive IgM and RPR results (titer = 4). Due to these findings, we interpreted both of these samples as probably false-negative ViraBlot IgG results.

This study has several limitations. First, the serum samples were submitted without corresponding clinical data, so we were unable to correlate results to the clinical presentation or treatment history. Despite this, each sample was analyzed by 7 treponemal assays, as well as IgM and RPR assays, and this allowed for a robust characterization of the serologic status of each sample. A second limitation of our study is that a subset of the serum samples was selected based on prior results, and therefore, we could not determine the positive and negative predictive values of each test. Our laboratory typically observes a reactive rate of ~5% for syphilis IgG, so samples were selected to increase the number of positives in our evaluation. Third, the results from this study do not address whether screening with a treponema-specific assay is clinically or economically advantageous compared to screening by RPR assay. Past reports have suggested advantages and limitations to both strategies (8, 9), and further studies are needed. Interestingly, among the 303 serum samples tested in our study, 97 (32.0%) were positive by FTA assay versus 94 (31.0%) by the panel and

only 66 (21.8%) by RPR assay. Among the samples that were positive by FTA assay ($n = 97$) or the panel ($n = 94$), the results of the RPR assay were also positive in 61 (62.9% and 64.9%, respectively). These data are consistent with the results of prior studies, which have shown increased percent-positive rates when screening with a treponemal assay in comparison to the percent-positive rates with the RPR assay (2, 8). This has important clinical implications, as treponema-specific assays may be positive in patients with either active syphilis or past, successfully treated disease. Therefore, it is often difficult to determine the significance of reactive treponemal screening results when nontreponemal tests are negative, especially in patients without a history of treatment for syphilis. This can complicate the interpretation of results and may lead to higher rates of treatment compared to screening with a nontreponemal test (2).

In summary, our findings demonstrate comparable performance among the 7 treponema-specific assays evaluated. However, our data suggest that each method has limitations, including the potential for false-positive and false-negative results. Therefore, serum samples testing positive by a first-line treponemal assay (e.g., MFI) but negative by RPR assay should be analyzed with a second treponemal test (e.g., FTA, EIA, or WB) (1, 7). In addition, it is important to underscore that health care providers must perform a thorough review of each patient’s clinical and treatment history when interpreting the results of syphilis serology.

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