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, planeagg, 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 objective 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, Planeagg, Germany]) using serum samples (n = 303) submitted to our
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.

Eumast immuneassay. All serum samples were tested according to the manufacturer’s instructions using the following EIA’s: Trep-ID, Trep-Check, 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 (TpM). 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-Check 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-Check 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 nontoxic T. pallidum (Nichols strain) as the substrate capture antigen for the detection of total antibodies against T. pallidum.

Multiplex flow immuneassay. 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 dried 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 arbitrary antibody concentration 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 Seirdia 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/TAT) × 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), κ = 0.96; TP-PA, 97.0% (94.4 to 98.5), κ = 0.93; Trep-Check EIA, 97.7% (95.2 to 99.0), κ = 0.95; Trep-Sure EIA, 95.4% (92.3 to 97.3), κ = 0.90; Trep-ID EIA, 98.4% (96.1 to 99.4), κ = 0.96; and ViraBlot IgG, 97.0% (94.4 to 98.5), κ = 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 treponema 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), κ = 0.98; FTA, 99.0 (97.0 to 99.8), κ = 0.98; TP-PA, 98.0% (95.6 to 99.2), κ = 0.95; Trep-Check EIA, 98.7% (96.5 to 99.6), κ = 0.97; Trep-Sure EIA, 95.7% (92.7 to 97.5), κ = 0.90; Trep-ID EIA, 99.3% (97.5 to 99.9), κ = 0.99; and ViraBlot IgG, 98.0% (95.6 to 99.2), κ = 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-Check 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
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 \( \times 100 \)). 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-negative FTA 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-negative 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 1. Comparison of six treponemal assays to the FTA assay using serum specimens

<table>
<thead>
<tr>
<th>Assay and result</th>
<th>FTA result (no. of samples)</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% Agreement (95% CI)</th>
<th>κ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>BioPlex syphilis IgG</td>
<td>94</td>
<td>3</td>
<td>96.9 (90.9, 99.3)</td>
<td>98.5 (95.6, 99.7)</td>
<td>98.0 (95.6, 99.2)</td>
</tr>
<tr>
<td>TP-PA</td>
<td>93</td>
<td>5</td>
<td>95.9 (89.5, 98.7)</td>
<td>97.6 (94.3, 99.1)</td>
<td>97.0 (94.4, 98.5)</td>
</tr>
<tr>
<td>Trep-Chek IgG</td>
<td>93</td>
<td>1</td>
<td>95.9 (89.5, 98.7)</td>
<td>98.5 (95.6, 99.7)</td>
<td>97.7 (95.2, 99.0)</td>
</tr>
<tr>
<td>Trep-Sure</td>
<td>94</td>
<td>4</td>
<td>96.9 (90.9, 99.3)</td>
<td>94.7 (90.6, 97.1)</td>
<td>95.4 (92.3, 97.3)</td>
</tr>
<tr>
<td>Trep-ID</td>
<td>94</td>
<td>2</td>
<td>96.9 (90.9, 99.3)</td>
<td>99.0 (96.3, 100)</td>
<td>98.4 (96.1, 99.4)</td>
</tr>
<tr>
<td>ViraBlot IgG</td>
<td>91</td>
<td>2</td>
<td>93.8 (86.9, 97.4)</td>
<td>98.5 (95.6, 99.7)</td>
<td>97.0 (94.4, 98.5)</td>
</tr>
</tbody>
</table>

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.
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/H11005 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 with recent infection due to positive results by the 6 other treponemal assays, as well as positive IgM and RPR results (titer/H11005 4), and therefore, was interpreted as a probable true-negative Trep-Sure result (Table 2).

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

<table>
<thead>
<tr>
<th>Assay and result</th>
<th>Consensus of panel (no. of samples)</th>
<th>Sensitivity (%) (95% CI)</th>
<th>Specificity (%) (95% CI)</th>
<th>Agreement (%) (95% CI)</th>
<th>( \kappa ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (94)</td>
<td>100 (95.3, 100)</td>
<td>98.6 (95.7, 99.7)</td>
<td>99.0 (97.0, 99.8)</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Negative (3)</td>
<td>0 (95)</td>
<td>98.6 (95.7, 99.7)</td>
<td>99.0 (97.0, 99.8)</td>
<td>0.98</td>
</tr>
<tr>
<td>FTA</td>
<td>Positive (94)</td>
<td>100 (95.3, 100)</td>
<td>98.6 (95.7, 99.7)</td>
<td>99.0 (97.0, 99.8)</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Negative (3)</td>
<td>0 (95)</td>
<td>98.6 (95.7, 99.7)</td>
<td>99.0 (97.0, 99.8)</td>
<td>0.98</td>
</tr>
<tr>
<td>Trep-Chek IgG</td>
<td>Positive (93)</td>
<td>98.9 (93.6, 99.9)</td>
<td>98.6 (95.7, 99.7)</td>
<td>98.7 (96.5, 99.6)</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Negative (1)</td>
<td>98.6 (95.7, 99.7)</td>
<td>98.7 (96.5, 99.6)</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Equivocal (0)</td>
<td>0 (95)</td>
<td>98.7 (96.5, 99.6)</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>TP-PA</td>
<td>Positive (93)</td>
<td>98.9 (93.6, 99.9)</td>
<td>97.6 (94.4, 99.1)</td>
<td>98.0 (95.6, 99.2)</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Negative (5)</td>
<td>98.9 (93.6, 99.9)</td>
<td>97.6 (94.4, 99.1)</td>
<td>98.0 (95.6, 99.2)</td>
<td>0.95</td>
</tr>
<tr>
<td>Trep-Sure</td>
<td>Positive (93)</td>
<td>98.9 (93.6, 99.9)</td>
<td>94.3 (90.1, 96.8)</td>
<td>95.7 (92.7, 97.5)</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Negative (1)</td>
<td>94.3 (90.1, 96.8)</td>
<td>95.7 (92.7, 97.5)</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Equivocal (0)</td>
<td>0 (95)</td>
<td>95.7 (92.7, 97.5)</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Trep-ID</td>
<td>Positive (94)</td>
<td>100 (95.3, 100)</td>
<td>99.0 (96.4, 99.9)</td>
<td>99.3 (97.5, 99.9)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Negative (2)</td>
<td>99.0 (96.4, 99.9)</td>
<td>99.3 (97.5, 99.9)</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>ViraBlot IgG</td>
<td>Positive (91)</td>
<td>96.8 (90.6, 99.3)</td>
<td>98.6 (95.7, 99.7)</td>
<td>98.0 (95.6, 99.2)</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Negative (2)</td>
<td>96.8 (90.6, 99.3)</td>
<td>98.0 (95.6, 99.2)</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Equivocal (1)</td>
<td>1 (3.6)</td>
<td>98.0 (95.6, 99.2)</td>
<td>0.95</td>
<td></td>
</tr>
</tbody>
</table>

* 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.

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

<table>
<thead>
<tr>
<th>Potentially cross-reactive analyte or condition (no. of sera tested)</th>
<th>BioPlex syphilis IgG</th>
<th>FTA</th>
<th>Trep-Chek IgG</th>
<th>TP-PA</th>
<th>Trep-Sure</th>
<th>Trep-ID</th>
<th>ViraBlot IgG</th>
<th>RPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV IgG (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>HSV IgM (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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</tr>
<tr>
<td>EBV IgG (5)</td>
<td>0 (0)</td>
<td>1 (20)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>EBV IgM (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<td>Rheumatoid factor (3)</td>
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<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Heterophile antibodies (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pregnancy (28)</td>
<td>0 (0)</td>
<td>1 (3.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (3.6)</td>
<td>1 (3.6)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* The cross-reactivity test panel comprised 53 sera.

b HSV, herpes simplex virus.

c This result was equivocal by the ViraBlot IgG assay.
were positive by FTA assay versus 94 (31.0%) by the panel and
strategies (8, 9), and further studies are needed. Interestingly,
Past reports have suggested advantages and limitations to both
screening with a treponema-specific assay is clinically or eco-
Third, the results from this study do not address whether
determining the significance of reactive treponemal screening
rates 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
treatment compared to screening with a non-treponemal
test (2).

In summary, our findings demonstrate comparable perfor-
ance among the 7 treponema-specific assays evaluated. How-
ever, our data suggest that each method has limitations, in-
cluding 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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