

Evaluation of the Bio-Rad BioPlex 2200 Syphilis Multiplex Flow Immunoassay for the Detection of IgM- and IgG-Class Antitreponemal Antibodies[∇]

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The laboratory diagnosis of syphilis is based primarily upon serologic findings. Historically, serologic testing for syphilis has relied on assays such as rapid plasma reagin, fluorescent treponemal antibody absorption, *Treponema pallidum* particle agglutination (TP-PA), and more recently, enzyme immunoassay (EIA). In this study, we evaluated the performance of a novel multiplex flow immunoassay (BioPlex 2200 Syphilis; Bio-Rad Laboratories, Hercules, CA) for the detection of antitreponemal IgG- and IgM-class antibodies. Serum specimens ($n = 1,008$) submitted for routine treponema-specific antibody testing by syphilis IgM and IgG EIA (Trep-Chek; Phoenix-Biotech, Mississauga, Ontario, Canada) were also analyzed by the BioPlex Syphilis multiplex assay. Specimens showing discordant results were repeat tested, with further discrepancies being arbitrated by TP-PA. Compared directly to the results of EIA, the BioPlex IgG assay demonstrated 98.7% (77/78) sensitivity and 99.4% (916/930) specificity. Compared to the Trep-Chek IgM EIA, the BioPlex IgM assay showed 80% (4/5) sensitivity and 97.9% (652/666) specificity. These results indicate that the BioPlex Syphilis multiplex assay shows similar serological agreement with EIA while allowing for a fully automated random-access platform that provides faster (1.7 h for 100 samples versus 4.5 h by EIA) and higher-throughput (800 samples per 9 h versus 200 samples by EIA) analysis of the syphilis serologic response.

Syphilis is a sexually transmitted infection caused by the spirochete *Treponema pallidum* and is diagnosed primarily by serology. *T. pallidum* infection induces an immunologic response in the host characterized by the production of nontreponemal and treponema-specific antibodies. Nontreponemal antibodies are targeted against a lipoidal antigen (e.g., cardiolipin) that is generated following infection and can be detected by the Venereal Disease Research Laboratories and rapid plasma reagin (RPR) tests. Nontreponemal tests are inexpensive but are labor-intensive and subjective (9). In contrast, treponema-specific tests such as the *T. pallidum* particle agglutination (TP-PA) and fluorescent treponemal antibody absorption (FTA-ABS) tests detect specific antibodies that react with treponemal antigens. These tests are more specific than nontreponemal assays but are also labor-intensive and subjective and require trained personnel (9).

Historically, serum samples have been tested initially by a nontreponemal test (e.g., RPR), with screen-positive samples being confirmed by a treponema-specific assay (e.g., FTA-ABS). However, in recent years, many clinical laboratories have adopted a reverse algorithm in which sera are first tested by a treponema-specific assay (e.g., enzyme immunoassay [EIA]), with positive samples being tested further by RPR to assess the patient's disease and treatment status (4). This approach may yield increased specificity over screening by RPR (3) and allows clinical laboratories to meet growing test volumes due to the ability to automate EIAs. Although testing for

treponema-specific IgG-class antibodies is most common, the detection of IgM-class antibodies may also be useful when evaluating patients with suspected early disease or congenital syphilis (10, 11, 13, 14, 16, 18, 20).

While a treponema-specific EIA offers a sensitive and specific approach (5), the detection and differentiation of IgM- and IgG-class antibodies by this method require separate assays to be performed. This potentially increases the sample volume required, as well as the turnaround time and cost associated with testing. In this study, we evaluated the performance of the BioPlex 2200 Syphilis multiplex assays (Bio-Rad Laboratories, Hercules, CA) for the detection of IgM- and IgG-class antibodies to *T. pallidum*. Results were compared to those obtained by routine testing using EIA, with discordant results being arbitrated by TP-PA.

MATERIALS AND METHODS

Serum specimens and study design. The use of biological specimens in this study was approved by an Institutional Review Board at the Mayo Clinic. From 2006 to 2008, prospective serum specimens ($n = 1,008$) submitted to our reference laboratory for serologic testing for syphilis by EIA (Trep-Chek; Phoenix-Biotech, Mississauga, Ontario, Canada) were also tested by the BioPlex Syphilis IgM and IgG assays. Syphilis IgG testing was performed on all 1,008 specimens, while IgM testing was performed on 671 specimens. Discordant results were resolved by repeat testing, with further discordant samples being tested by Serodia *Treponema pallidum* particle agglutination (TP-PA; Fujirebio Diagnostics, Malvern, PA). In addition, samples showing discordant IgM results were also tested by RPR (Becton Dickinson, Sparks, MD).

EIA. All serum specimens were tested and interpreted by the Trep-Chek IgM EIA and the Trep-Chek IgG EIA according to the manufacturer's instructions. These EIAs use recombinant proteins as the capture antigen to detect and differentiate IgM- and IgG-class antibodies. Testing by EIA was completed on the Triturus automated EIA analyzer (Grifols S.A., Barcelona, Spain).

Multiplex flow immunoassay (MFI). In addition to testing by EIA, samples were tested according to the manufacturer's instructions using the BioPlex 2200 Syphilis IgM and IgG kits on the BioPlex 2200 analyzer (Bio-Rad Laboratories).

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TABLE 1. Comparison of the Bio-Rad BioPlex Syphilis IgG assay and the Trep-Chek IgG EIA using 1,008 prospective serum samples

| Bio-Rad BioPlex Syphilis IgG assay result | No. of Trep-Chek IgG EIA results | | |
|---|----------------------------------|-----------------|-------|
| | Positive | Negative | Total |
| Positive | 77 | 14 ^a | 91 |
| Negative | 1 ^b | 916 | 917 |
| Total | 78 | 930 | 1,008 |

^a Eight out of these 14 samples were positive by TP-PA.

^b This sample became negative by EIA upon repeat testing.

The principle of MFI technology has been reviewed previously (12, 19). The BioPlex Syphilis IgG kit uses three different populations of microspheres coated with recombinant proteins from *T. pallidum* (15 kDa, 17 kDa, and 47 kDa). The syphilis IgM kit uses two different bead sets individually coated with recombinant proteins associated with *T. pallidum* (17 kDa and 47 kDa). Briefly, the patient specimen is added to a reaction vessel containing bead reagent and sample diluent. The sample is incubated at 37°C and washed, and a phycoerythrin-conjugated reporter antibody is then added. After a second incubation and washing step, the beads are read by a flow-based detector which quantitates each analyte and compares it to a pre-established calibration curve. In addition, up to three quality control bead sets are employed that check for detector fluctuations, sample integrity, and nonspecific binding. The data are initially calculated as relative fluorescence intensity and then converted to a fluorescence ratio (FR) using an internal standard bead. The FR is compared to an assay-specific calibration curve to determine the analyte concentration in antibody index (AI) units. For this study, the results were classified according to their AI values as negative (<1.0) or positive (≥1.0) based on the manufacturer’s recommendations. The BioPlex Syphilis IgG kit has been FDA approved, while the syphilis IgM kit was a prototype assay at the time of this evaluation.

Statistics. Statistical analyses were performed using JMP software, version 7 (SAS Institute, Inc., Cary, NC). In addition to percent agreement, kappa coefficients were calculated as a secondary measure of agreement. Result agreement by kappa values is categorized as nearly 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).

RESULTS

Among 1,008 serum samples tested for IgG-class antibodies to *T. pallidum*, the BioPlex IgG assay demonstrated 98.5% (993/1,008; 95% confidence interval [CI], 97.5 to 99.1) agreement with EIA ($\kappa = 0.903$) (Table 1). There was one EIA-positive, BioPlex-negative sample identified following initial testing. However, this sample was borderline positive by EIA and became negative upon repeat testing. In addition, there were 14 specimens that were EIA negative and BioPlex positive following initial testing. These samples were subsequently tested by TP-PA, and 8/14 (57.1%) were found to be positive. Of the 14 BioPlex IgG-positive and EIA-negative specimens, 12 had concomitant requests for IgM testing. Among these 12 samples, 11 (91.7%) were negative for IgM by both BioPlex and EIA, while 1 (8.3%) was positive for IgM by the BioPlex and negative by EIA. Compared directly to the Trep-Chek IgG EIA, the sensitivity and specificity of the BioPlex IgG assay were 98.7% (77/78; 95% CI, 92.1 to 99.9) and 98.5% (916/930; 95% CI, 97.5 to 99.1), respectively. After resolution of the discrepancies, the BioPlex IgG assay demonstrated a sensitivity of 100% (85/85) and a specificity of 99.3% (917/923) (Table 1).

Of the 1,008 serum samples submitted for syphilis IgG, 671 (66.6%) also had requests for syphilis IgM testing. Of the 671 samples tested for IgM, the BioPlex IgM assay demonstrated an overall agreement of 97.8% (656/671; 95% CI, 96.3 to 98.7)

TABLE 2. Comparison of the Bio-Rad BioPlex Syphilis prototype IgM assay and the Trep-Chek IgM EIA using 671 prospective serum samples

| Bio-Rad BioPlex Syphilis IgM assay result | No. of Trep-Chek IgM EIA results | | |
|---|----------------------------------|-----------------|-------|
| | Positive | Negative | Total |
| Positive | 4 | 14 ^a | 18 |
| Negative | 1 ^b | 652 | 653 |
| Total | 5 | 666 | 671 |

^a Four of these 14 samples were positive by TP-PA and RPR.

^b This sample became negative by EIA upon repeat testing.

with EIA ($\kappa = 0.340$) (Table 2). Following initial testing, there was one EIA-positive, BioPlex-negative sample. However, this sample became negative by EIA upon repeat testing. In addition, 14 specimens were positive by BioPlex and negative by EIA. These samples were subsequently tested by TP-PA and RPR, and 4/14 (28.6%) specimens were reactive by TP-PA and RPR. Among the 14 BioPlex IgM-positive and EIA-negative samples, 13 also had request for IgG testing by Trep-Chek EIA. Of these 13 samples, 8 (61.5%) were negative for IgG by both BioPlex and EIA, 4 (30.7%) were positive for IgG by both BioPlex and EIA, and 1 (7.7%) was positive for IgG by BioPlex and negative by EIA. Compared directly to EIA, the BioPlex IgM assay showed a sensitivity of 80% (4/5; 95% CI, 35.9 to 97.9) and a specificity of 97.9% (652/666; 95% CI, 96.5 to 98.8) (Table 2). After follow-up testing of discordant samples, the adjusted sensitivity and specificity of the BioPlex IgM assay were 100% (8/8) and 98.5% (653/663), respectively.

DISCUSSION

Due to the decreasing incidence of syphilis in the 1990s in the United States (6), the increased specificity of treponema-specific tests, and the potential to automate EIAs and MFI assays, many clinical laboratories are now using treponema-specific tests (e.g., EIAs) to screen specimens submitted for serologic testing for syphilis. The BioPlex Syphilis MFI assay offers a novel, unique method to analyze the IgM- and IgG-class antibody responses to *T. pallidum*. In this study, the BioPlex IgG assay showed excellent performance, with a sensitivity of 98.7% (77/78) and a specificity of 98.5% (916/930) compared to the Trep-Chek IgG EIA. Among the 14 serum samples that were BioPlex IgG positive and EIA negative, 8/14 (57.1%) were also positive by TP-PA. These findings suggest that the BioPlex IgG assay may be more sensitive than the Trep-Chek EIA.

The utility of IgM in the diagnosis of syphilis is not well defined. Although there have been multiple studies addressing the use of IgM (3, 15, 17), the results have conflicted somewhat and further studies are needed. Pedersen et al. reported that performing an IgM EIA increased the sensitivity and specificity of diagnosis compared to testing by IgG EIA alone (15). It has also been suggested that detection of syphilis IgM may correlate with active disease and assist in differentiating active from past, successfully treated syphilis (3). In our evaluation, the BioPlex IgM assay demonstrated a sensitivity of 80% (4/5) and a specificity of 97.9% (652/666) compared to the Trep-Chek IgM EIA. The BioPlex-negative, EIA-positive specimen was

noted to be borderline positive upon initial testing and yielded a negative result upon repeat testing by EIA. Furthermore, among the 14 BioPlex IgM-positive, EIA-negative specimens, 4 (28.6%) were positive by TP-PA and RPR. In addition, 8 of these 14 samples were found to be negative for IgG by both BioPlex and Trep-Chek EIA. Whether this indicates that the BioPlex IgM assay increases the clinical sensitivity for detecting early cases of syphilis in comparison to testing for IgG alone or represents potential false-positive results remains to be determined. To address this, further studies using higher numbers of specimens from patients with active disease are needed to better characterize the performance of the BioPlex IgM assay.

There are several limitations of this study. First, the results of the BioPlex IgM and IgG assays were compared to those of treponema-specific EIAs, using TP-PA and RPR to arbitrate discordant results. It should be emphasized that reactivity by TP-PA is likely, but not unequivocal, evidence that the BioPlex-positive, EIA-negative sera were from patients with syphilitic infection. Samples tested in this study were submitted to our reference laboratory without corresponding clinical information, so correlation of the test results with disease status was not possible. Therefore, the determination of clinical sensitivity and specificity was limited. Second, there were a limited number of samples positive for IgM, so further studies are needed to accurately define the performance of the BioPlex IgM test. Third, the clinical relevance of the IgM antibody tests was not assessed, and recommendations regarding the applicability of IgM testing for syphilis require further investigation. Finally, this study did not directly compare the conventional (e.g., screen RPR followed by FTA or EIA) and contemporary (e.g., screening EIA or MFI followed by RPR) algorithms for syphilis serologic testing, and therefore, clinical laboratories should establish which approach yields optimal performance based on the assays used and the prevalence of disease in their patient population.

Multiplex immunoassays offer the capacity to detect multiple analytes in a single assay, thereby reducing hands-on time, specimen volume requirements, and turnaround time. These advantages have been demonstrated in several recent studies evaluating MFI assays for the detection of autoantibodies in autoimmune disorders, as well as in the evaluation of Epstein-Barr and herpes simplex virus infections (1, 2, 7, 8). In this study, we found that the BioPlex Syphilis multiplex assay demonstrated a more rapid (1.7 h for 100 samples versus 4.5 h by EIA) and higher-throughput (800 samples per 9 h versus 200 samples by EIA) analysis of the syphilis antibody response while allowing for random-access capabilities. The BioPlex multiplex assay may prove beneficial to clinical laboratories experiencing increasing test volumes for syphilis serologic testing.

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REFERENCES

- Binnicker, M. J., D. J. Jespersen, and J. A. Harring. 2010. Evaluation of three multiplex flow immunoassays compared to an enzyme immunoassay for the detection and differentiation of IgG class antibodies to herpes simplex virus types 1 and 2. *Clin. Vaccine Immunol.* **17**:253–257.
- Binnicker, M. J., D. J. Jespersen, J. A. Harring, L. O. Rollins, and E. M. Beito. 2008. Evaluation of a multiplex flow immunoassay for detection of Epstein-Barr virus-specific antibodies. *Clin. Vaccine Immunol.* **15**:1410–1413.
- Castro, R., E. S. Prieto, I. Santo, J. Azevedo, and F. D. Exposto. 2003. Evaluation of an enzyme immunoassay technique for detection of antibodies against *Treponema pallidum*. *J. Clin. Microbiol.* **41**:250–253.
- Centers for Disease Control and Prevention. 2008. Syphilis testing algorithms using treponemal tests for initial screening—four laboratories, New York City, 2005–2006. Centers for Disease Control and Prevention, Atlanta, GA.
- Cole, M. J., K. R. Perry, and J. V. Parry. 2007. Comparative evaluation of 15 serological assays for the detection of syphilis infection. *Eur. J. Clin. Microbiol. Infect. Dis.* **26**:705–713.
- Department of Health and Human Services. 2009. Sexually transmitted diseases surveillance 2007 supplement: syphilis surveillance report. Department of Health and Human Services, Washington, DC.
- Hanly, J. G., K. Thompson, G. McCurdy, L. Fougere, C. Theriault, and K. Wilton. 2010. Measurement of autoantibodies using multiplex methodology in patients with systemic lupus erythematosus. *J. Immunol. Methods* **352**: 147–152.
- Kaul, R., K. Johnson, H. Scholz, and G. Marr. 2009. Performance of the BioPlex 2200 Autoimmune Vasculitis kit. *Autoimmun Rev.* **8**:224–227.
- Larsen, S. A., B. M. Steiner, and A. H. Rudolph. 1995. Laboratory diagnosis and interpretation of tests for syphilis. *Clin. Microbiol. Rev.* **8**:1–21.
- Lefevre, J. C., M. A. Bertrand, and R. Bauriaud. 1990. Evaluation of the Captia enzyme immunoassays for detection of immunoglobulins G and M to *Treponema pallidum* in syphilis. *J. Clin. Microbiol.* **28**:1704–1707.
- McMillan, A., and H. Young. 2008. Reactivity in the Venereal Diseases Research Laboratory test and the Mercia IgM enzyme immunoassay after treatment of early syphilis. *Int. J. STD AIDS* **19**:689–693.
- Morgan, E., R. Varro, H. Sepulveda, J. A. Ember, J. Apgar, J. Wilson, L. Lowe, R. Chen, L. Shivraj, A. Agadir, R. Campos, D. Ernst, and A. Gaur. 2004. Cytometric bead array: a multiplexed assay platform with applications in various areas of biology. *Clin. Immunol.* **110**:252–266.
- Müller, F., and M. Moskophidis. 1984. Evaluation of an enzyme immunoassay for IgM antibodies to *Treponema pallidum* in syphilis in man. *Br. J. Vener. Dis.* **60**:288–292.
- O'Neill, P., and C. S. Nicol. 1972. IgM class antitreponemal antibody in treated and untreated syphilis. *Br. J. Vener. Dis.* **48**:460–463.
- Pedersen, N. S., C. S. Petersen, and N. H. Axelsen. 1982. Enzyme-linked immunosorbent assay for detection of immunoglobulin M antibody against the Reiter treponeme flagellum in syphilis. *J. Clin. Microbiol.* **16**:608–614.
- Rawstron, S. A., S. Mehta, and K. Bromberg. 2004. Evaluation of a *Treponema pallidum*-specific IgM enzyme immunoassay and *Treponema pallidum* Western blot antibody detection in the diagnosis of maternal and congenital syphilis. *Sex Transm. Dis.* **31**:123–126.
- Schmidt, B. L., M. Edjlalipour, and A. Luger. 2000. Comparative evaluation of nine different enzyme-linked immunosorbent assays for determination of antibodies against *Treponema pallidum* in patients with primary syphilis. *J. Clin. Microbiol.* **38**:1279–1282.
- Shannon, R., and S. D. Booth. 1977. The pattern of immunological responses at various stages of syphilis. *Br. J. Vener. Dis.* **53**:281–286.
- Varro, R., R. Chen, H. Sepulveda, and J. Apgar. 2007. Bead-based multi-analyte flow immunoassays: the cytometric bead array system. *Methods Mol. Biol.* **378**:125–152.
- Young, H., J. Pryde, L. Duncan, and J. Dave. 2009. The Architect Syphilis assay for antibodies to *Treponema pallidum*: an automated screening assay with high sensitivity in primary syphilis. *Sex. Transm. Infect.* **85**:19–23.