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# Evaluation of the BioPlex 2200 Syphilis System as a First-Line Method of Reverse-Sequence Screening for Syphilis Diagnosis

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**Despite recent technological advances, the diagnosis of syphilis remains a challenging enterprise. Actually, most high-volume laboratories have adopted the "reverse algorithm" due several factors, including the potential to automate testing. Recently, immunoassays processed on random-access systems have been proposed as screening tests. The purpose of this study was to evaluate diagnostic performances of BioPlex 2200 Syphilis IgG and BioPlex 2200 Syphilis IgM, tests based on Multiplex Flow technology, in comparison with the performance of Architect Syphilis TP, a chemiluminescent immunoassay for the detection of IgG and/or IgM anti-*Treponema pallidum* antibodies. A retrospective study was performed with a panel of 100 blood donor sera, a panel of 350 clinical and laboratory-characterized syphilitic sera, and 170 samples obtained from subjects with potentially interfering conditions. Moreover, 200 unselected samples submitted to the Microbiology Laboratory of St. Orsola Hospital in Bologna for routine screening for syphilis were evaluated. As confirmatory tests, *T. pallidum* hemagglutination and Western blot assays were used. Considering the IgG Western blot (WB) assay to be the gold standard method, BioPlex 2200 Syphilis IgG specificity was far higher than Architect Syphilis TP specificity (89.7% versus 78.4%, respectively), whereas the sensitivity was 100% for both automated methods. Compared to the IgM WB assay, BioPlex 2200 Syphilis IgM performed with a specificity of 94.9%, whereas the sensitivity was 84.8%. Considering the excellent ease of use and automation, the high sample throughput and its valuable analytical performances, BioPlex Syphilis 2200 IgG could represent a suitable choice for high-volume laboratories. BioPlex Syphilis 2200 IgM could be considered a good addition to IgG testing for uncovering active infections.**

Syphilis is a sexually transmitted infection caused by the spirochete *Treponema pallidum* subsp. *pallidum*, which remains a public health problem worldwide (1). Developing countries show higher levels of prevalence and incidence, but recent outbreaks have also been reported in several cities in Europe and North America, in particular among men who have sex with men (MSM), in association with a rise in unsafe sexual behavior (2–4).

Diagnosis of syphilis is primarily based on serology, since the natural course of the infection is characterized by periods without clinical manifestations (5, 6). Serologic tests are divided into nontreponemal and treponemal categories. Nontreponemal tests, such as Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) tests, have low specificity but are necessary to monitor therapy. However, since positivity with respect to treponemal tests lasts a lifetime, they cannot be useful in follow-up. Treponemal tests include the serum fluorescent treponemal antibody absorption test (FTA-ABS), *T. pallidum* hemagglutination (TPHA) test, enzyme immunoassay (EIA), and Western blot (WB) assay; both EIA and WB tests can be based on either whole-cell lysate (7–11) or recombinant (12–16) treponemal antigens. Recently, chemiluminescent immunoassays set up with recombinant antigens have been evaluated (17, 18).

In developed countries, the merging of small- and medium-sized laboratories in high-volume centers has led to daily testing of huge numbers of samples. Therefore, most laboratories have adopted the "reverse algorithm" to diagnosis syphilis (19): in this approach, a reactive treponemal screening assay is followed by a quantitative nontreponemal assay to diagnose active disease and to monitor response to treatment. This algorithm also consists of a second and different treponemal assay.

Because syphilis is a sexually transmitted disease (STD), high sensitivity is the first characteristic required for tests, but specificity is also central, since false-positive results can lead to very unpleasant situations for those involved.

Simultaneous IgM and IgG detection has been reported to possibly increase sensitivity and specificity of diagnosis, compared to IgG testing only, suggesting in particular the utility of IgM for the diagnosis of very early infections (20, 21). Nevertheless, there are so far only a few data that support this hypothesis.

IgM tests remain basic for diagnosis of congenital syphilis (CS) because maternal IgM antibodies, in contrast to IgG ones, do not cross the placenta. It follows that a positive IgM result in a newborn's serum should be considered evidence of *T. pallidum* congenital infection (22, 23). Actually, IgM WB is considered the gold standard for diagnosis of CS, considering its high sensitivity and specificity (24, 25).

The purpose of this study was to evaluate diagnostic performances of BioPlex 2200 Syphilis IgG and BioPlex 2200 Syphilis IgM (Bio-Rad, Bio-Rad Laboratories, Hercules, CA), innovative tests (26) based on MFI (multiplex flow immunoassay) technology, in comparison with Architect Syphilis TP (Abbott Japan Co., Tokyo, Japan), a chemiluminescent microparticle immunoassay (CMIA).

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## MATERIALS AND METHODS

**Study groups.** For the retrospective study, all the samples have been selected basing on their clinical and diagnostic results and they have been coded to ensure full anonymity. The study protocol was reviewed by the institutional Ethics committee at our center. Sera were obtained from different subject groups (A to G), as follows: group A included 100 consecutive blood donor samples submitted to the Microbiology Laboratory of St. Orsola Hospital in Bologna for routine screening for syphilis.

Then, 350 sera were obtained from syphilis patients attending the STD Outpatients Clinic of Dermatology, St. Orsola Hospital, Bologna. In particular, 100 specimens were from patients with untreated early syphilis (group B) and 250 samples were from previously treated subjects (group C). The staging of the disease was done following the clinical and laboratory criteria proposed by Norris and Larsen (27).

Group D of samples consisted of 100 sera chosen because they were reactive by Architect testing but negative by TPHA, WB, and RPR testing. In particular, it is noteworthy that 37 of these samples were from healthy blood donors, already evaluated in a previous study (11), and 12 samples were from healthy pregnant women, whereas the remaining 51 were from elderly patients (over 75 years of age) hospitalized for different disorders. Taking into account that consecutive samples drawn from the same patients, in the absence of therapy, gave positive results only by CMIA and not by other tests, it follows that CMIA reactivity was consistent with two different hypotheses: false-positive results or syphilis infections in the remote past in previously treated elderly patients.

Panels E and F of sera were obtained from patients suffering from some of the most common biological conditions possibly resulting in false-positive reactivity in syphilis serology. In particular, 15 samples were drawn from patients with culture-confirmed Lyme disease (group E) (28) and 55 samples were obtained from patients with positive results by BioPlex 2200 using an ANA screen kit (29). These sera were reactive to at least one of the following antigens: double-stranded DNA (dsDNA), chromatin, SSA (52 kDa and 60 kDa), SSB, Sm, Sm/RNP, RNP-A, RNP (68 kDa), Scl70, centromere B, Jo-1, and P ribosomal proteins (group F).

Finally, 200 unselected consecutive samples submitted to the Microbiology Laboratory of St. Orsola Hospital in Bologna for routine screening for syphilis were evaluated (group G).

**BioPlex 2200 Syphilis system.** Specimens were tested on a BioPlex 2200 analyzer according to the manufacturer's instructions. All samples, frozen after being tested by CMIA, TPHA, WB, and RPR, were allowed to thaw at room temperature and subjected to a vortex porcedure before testing by the BioPlex 2200 Syphilis system. The BioPlex 2200 Syphilis IgG kit uses three different sets of beads coated with recombinant proteins from *T. pallidum* (15 kDa, 17 kDa, and 47 kDa), whereas the Syphilis IgM kit uses only two sets (17 kDa and 47 kDa).

The principle of MFI technology for syphilis diagnosis has been previously explained (26). Briefly, samples are added to reaction vessels containing bead reagent and sample diluent. After two incubations and the corresponding washing steps, the beads are read by a flow-based detector which quantifies each analyte and compares it to a pre-established calibration curve. Data are calculated as relative fluorescence intensities and 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 and classified as negative ($<0.9$), equivocal (0.9 to 1.1), or positive ($>1.1$).

**Architect Syphilis TP.** Specimens were tested according to the manufacturer's instructions on an Architect i2000SR analyzer. Architect Syphilis TP is a two-step immunoassay for qualitative detection of IgG and/or IgM to *T. pallidum* in human serum or plasma using chemiluminescent microparticle immunoassay (CMIA) technology. The results of the chemiluminescent reaction are measured as relative light units (RLUs), the level of which is directly related to the amount of anti-*T. pallidum* antibodies in the serum. Samples are considered reactive when the RLU value is $\geq 1.0$.

**Confirmatory tests.** Commercial tests were used for TPHA and RPR (Randox, United Kingdom). Titers $\geq$ 80 were considered positive for TPHA testing.

The WB test was performed according to Towbin et al. (30). An IgG WB test was considered positive when at least three of the four bands corresponding to TpN47, TmpA, TpN17, and TpN15 were clearly recognized. IgM WB tests were considered positive when at least two of the four diagnostic bands were visible, including at least one with low molecular mass (15, 31, 32).

**Statistics.** Statistical analyses were performed using Stata/SE version 12.1 (StataCorp LP, College Station, TX). Percent agreement, kappa coefficient, and sensitivity and specificity with 95% confidence intervals (CI) were calculated. 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 ($<0$) (26).

## RESULTS

**BioPlex 2200 Syphilis IgG assay.** Used to define the immune response to *T. pallidum* in sera from syphilis patients (groups B and C), both BioPlex 2200 Syphilis IgG and Architect Syphilis TP showed total agreement with WB and TPHA findings. No sample from group A was reactive when analyzed by all methods studied; moreover, two samples belonging to group G were found reactive by all methods (100% agreement). When sera with potential interfering situations (groups E and F) were analyzed, no sample was found reactive by Bioplex 2200 Syphilis IgG, whereas 1 sample from a Lyme disease's patient was scored positive by Architect Syphilis TP.

Analyzed by IgG WB, samples of group D gave only faint reactions and no sera recognized more than two bands. In particular, the most frequently recognized antigen was TpN47 (47 sera), as expected, followed by TmpA (29 sera). TpN17 and TpN15 were hardly recognized (16 cases and 5 cases, respectively), confirming that low-molecular-mass proteins are the most specific antigens among the diagnostic ones. Only 42 sera recognized two bands (in particular, 24 sera recognized both Tp47 and TmpA, 11 sera recognized both Tp47 and Tp17, 4 sera recognized both TmpA and Tp17, and 3 sera recognized both Tp47 and Tp15). All these 42 sera were drawn from elderly patients, whereas only a few single very faint reactions to TpN47 (25 cases) and to TmpA, TpN17, and TpN15 (2 cases for each protein) were observed in analyzing sera drawn from pregnant women or blood donors. As already observed (11), the most frequent source of false positivity of Architect Syphilis TP seems to be other than cross-reaction of antibodies to treponemal antigens, at least in healthy subjects, but at present the identity of the source of the false positivity is still unknown.

In contrast with Architect Syphilis TP, BioPlex 2200 Syphilis IgG scored as positive 48/100 specimens of this group. In particular, all these 48 sera were obtained from elderly patients, and all the specimens from healthy blood donors or pregnant women were negative by this method. In detail, 42 sera were reactive to two antigens and 6 sera recognized TpN47 and TpN17 as single antigens (5 cases and 1 case, respectively). None of the 48 elderly patients were aware of their serologic syphilis status before hospitalization, and none of them recalled any specific treatment in the past. Anyway, the possibility of a syphilis infection having occurred in the very remote past cannot be fully excluded.

Results obtained by BioPlex Syphilis 2200 IgG, Architect Syphilis TP, and TPHA compared to IgG WB are summarized in Table 1.

Considering IgG WB as the gold standard method, BioPlex

**TABLE 1** Comparison of BioPlex 2200 Syphilis IgG, ARCHITECT Syphilis TP, and TPHA to IgG WB assay

| Assay and result | IgG WB result (no. of samples) | | % sensitivity (95% CI) | % specificity (95% CI) | % agreement (95% CI) | κ value |
|---|---|---|---|---|---|---|
| | Positive | Negative | | | | |
| BioPlex 2200 Syphilis IgG | | | | | | |
| Positive | 352 | 48 | 100 (99–100) | 89.7 (86.6–92.3) | 94.2 (92.5–95.8) | 0.88 |
| Negative | 0 | 420 | | | | |
| ARCHITECT Syphilis TP | | | | | | |
| Positive | 352 | 101 | 100 (99–100) | 78.4 (74.4–82.1) | 87.7 (85.4–89.9) | 0.76 |
| Negative | 0 | 367 | | | | |
| TPHA | | | | | | |
| Positive | 351 | 1 | 99.7 (98.5–99.9) | 99.8 (98.8–100) | 99.8 (99.4–99.9) | 0.98 |
| Negative | 1 | 467 | | | | |

2200 Syphilis IgG specificity was far higher than Architect Syphilis TP specificity at 89.7% (95% CI, 86.6 to 92.3) versus 78.4% (95% CI, 74.4 to 82.1), respectively, whereas the sensitivity was 100% (95% CI, 99.0 to 100.0) for both automated methods. The overall percent agreement and corresponding kappa values were as follows: for BioPlex 2200 Syphilis IgG, 94.2% agreement and $\kappa = 0.88$; for Architect Syphilis TP, 87.7% agreement and $\kappa = 0.76$; and for TPHA, 99.8% agreement and $\kappa = 0.98$.

**BioPlex 2200 Syphilis IgM assay.** As expected, most of the positive samples found by BioPlex 2200 Syphilis IgM belonged to untreated early-syphilis patients (group B; 34/100) followed by samples of group C (23/250). In particular, BioPlex 2200 Syphilis IgM performed well when sera from untreated primary and secondary syphilis patients were studied, since 58.3% and 41.4% of studied samples, respectively, were IgM reactive. All these sera showed RPR titers higher than 1:8 (titers ranging from 1:8 to 1:128). Among sera of group B, those scored negative by BioPlex 2200 Syphilis IgM had RPR titers ranging between 1:2 and 1:8. Regarding false reactivities, 2 sera of group A and 1 of group G were reactive only when tested by BioPlex 2200 Syphilis IgM, being negative when tested by all other methods (TpN47 was hardly recognized by both IgG and IgM WB). Moreover, 5 samples of group D, as well as 2 ANA-positive sera (group F) and 1 sample from a Lyme disease patient (group E), were scored positive by BioPlex 2200 Syphilis IgM. Again, the only diagnostic antigen faintly recognized by both IgG and IgM WB was TpN47. Considering IgM WB to be the gold standard method, BioPlex 2200 Syphilis IgM specificity was 94.9% (95% CI, 93.1 to 96.3), whereas the sensitivity was 84.8% (95% CI, 68.1 to 94.9). The overall percent agreement and corresponding kappa values for BioPlex 2200 Syphilis IgM were 94.5% agreement and $\kappa = 0.53$. Interestingly, if only sera of untreated syphilis patients are considered (group B), the kappa value is raised ($\kappa = 0.75$), and the agreement with IgM WB is 89.0% (Table 2).

## DISCUSSION

Serologic testing is a crucial element of syphilis surveillance campaigns, and in addition to traditional methods, a great variety of new commercial kits are nowadays proposed as first-line tests of the "reverse algorithm." Many studies are available comparing performances of different methods for syphilis diagnosis in routine use, taking into account sensitivity and specificity, as well as cost, ease of use, and suitability of automation (9–12, 33, 34). Regarding new MFI technology, Gomez et al. tested the BioPlex 2200 Syphilis system (26), suggesting that it may prove beneficial to clinical laboratories experiencing increasing test volumes for syphilis serologic testing. Nevertheless, in that study, as stressed by the authors themselves, the lack of clinical information to arbitrate discordant results between different assays was a major limit for the further interpretation of the results. Moreover, the clinical relevance of the IgM antibody testing was not assessed, leaving unsolved the issue of whether IgM testing could increase sensitivity in the early stages of syphilis or whether it would rather be a source of problems due to false-positive results.

The present study, based on a great number of clinical and

**TABLE 2** Comparison of BioPlex 2200 Syphilis IgM to IgM WB

| Assay and result | IgM WB result (no. of samples) | | % sensitivity (95% CI) | % specificity (95% CI) | % agreement (95% CI) | κ value |
|---|---|---|---|---|---|---|
| | Positive | Negative | | | | |
| BioPlex 2200 Syphilis IgM[a] | | | | | | |
| Positive | 28 | 5 | 84.8 (68.1–94.9) | 94.9 (93.1–96.3) | 94.5 (92.9–96.1) | 0.53 |
| Negative | 40 | 747 | | | | |
| BioPlex 2200 Syphilis IgM[b] | | | | | | |
| Positive | 28 | 5 | 84.8 (68.1–94.9) | 91.0 (81.5–96.6) | 89.0 (86.6–91.2) | 0.75 |
| Negative | 6 | 61 | | | | |

[a] Data represent total results, considering all the 820 sera.
[b] Data represent results obtained when sera obtained from untreated syphilis patients were studied.

laboratory records, can be an aid in shedding light on these controversial issues.

When BioPlex 2200 Syphilis IgG results were analyzed, the most reactive antigen was found to be the 17-kDa protein, followed by the 15-kDa protein, whereas the 47-kDa protein was the least recognized. This result depends on the particular mix of antigens used for coating the beads, and the greater amount of 17-kDa protein, compared to the 47-kDa protein, was probably chosen by the manufacturer to improve the IgG assay's specificity. In the present study, indeed, BioPlex 2200 Syphilis IgG specificity was far higher than Architect Syphilis TP specificity (89.7% versus 78.4%, respectively), considering WB to be the gold standard method.

IgM assays are often characterized by a larger number of false-positive reactions than IgG assays, in particular in patients with autoimmune disorders, pregnant women, or patients with acute viral infections (35, 36). In the present study, Architect Syphilis TP, a method for detecting total IgG/IgM anti-*T. pallidum* antibodies, showed lower specificity than BioPlex 2200 IgG, a method for detecting IgG only. The choice to set up two different kits to be run on the BioPlex 2200 system seems to have increased the specificity of IgG detection.

Anyway, the good specificity shown by BioPlex 2200 Syphilis IgG did not affect sensitivity, since it was as impressive as Architect Syphilis TP sensitivity (100%).

These findings are partially discordant with those of a study in which BioPlex 2200 Syphilis IgG showed lower sensitivity than another automated assay run on a random-access system (37). It is important to underline that in that previous work, commercially obtained sera were used, in contrast with the clinically and laboratory-characterized samples used in the present study. Moreover, when a panel of well-preserved specimens was used (33), BioPlex 2200 Syphilis IgG showed the highest agreement among the various methods studied.

The results obtained in the present study showed that the routine use of an IgG assay, such as BioPlex 2200 Syphilis IgG, instead of a combined assay, such as Architect Syphilis TP, did not affect sensitivity but strongly increased specificity.

BioPlex 2200 Syphilis IgM performed with much higher sensitivity in the group of untreated early syphilis patients than in the group of previously treated subjects, thus suggesting that IgM detection could be a valuable resource to distinguish acute infections. Nevertheless, it is worth underlining that IgM findings alone are not sufficient for a correct diagnosis, considering the lower specificity of the IgM assay compared to that of the IgG one. This could be explained by considering that, in contrast to the IgG assay, the most reactive antigen recognized by IgM assay was 47 kDa, far less specific than the 17-kDa antigen (15). It can be summarized that IgM testing should never be considered an alternative to IgG testing but should rather be considered an addition to IgG testing.

IgM testing could be performed as a second step when an IgG-positive result is obtained in order to add useful information for the clinicians.

The routine use of automated IgM tests should always be very careful evaluated when IgM testing is essential for a correct diagnosis, namely, for congenital syphilis (22, 23, 38). As a preliminary study, we tested by BioPlex 2200 Syphilis IgM 10 sera obtained from babies born to mothers who were found infected during their pregnancies or at delivery. Among these specimens, BioPlex Syphilis 2200 IgM scored as positive one sample, drawn from the only congenital syphilis case. Anyway, since in the present study the relative sensitivity of the MFI method compared to IgM WB is 84.8%, we suggest that only IgM WB, since it has the highest sensitivity combined with the highest specificity, should be used to rule out congenital syphilis. In contrast, we do not suggest the use of IgM WB for the diagnosis of acute infections in adult patients, because even if IgM WB is very sensitive and specific, its use is strongly limited by being a manual and expensive method; therefore, its use should be restricted to the diagnosis of probable congenital syphilis cases.

In conclusion, considering the excellent ease of use and automation, the high sample throughput, and its good sensitivity and specificity, BioPlex 2200 Syphilis IgG could represent an outstanding choice for a screening test for high-volume laboratories. In this context, BioPlex 2200 Syphilis IgM could be an aid for the correct diagnosis of acute infections, even if IgM results should be carefully evaluated in association with other laboratory and clinical findings.

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