Validating the BioPlex 2200 Total IgG/IgM Multiplex Flow Immunoassay for detection of syphilis antibodies

Authors: D. Riner, K. Smith, K. Hunsanger, H. McCracken, D. Huntzinger and M. Soehnlen

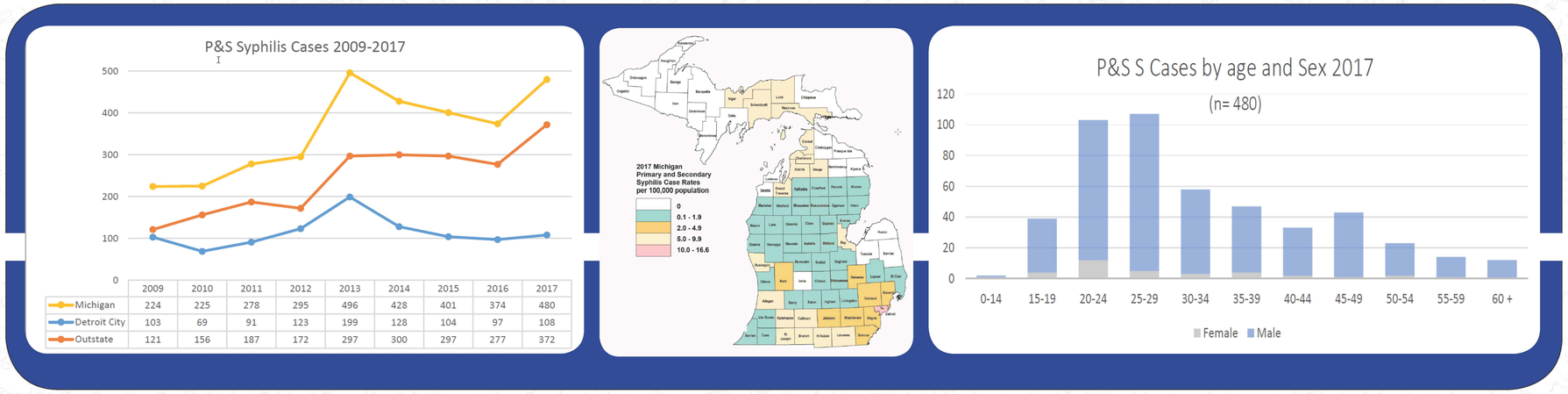
Contact Information

E-Mail: rinerd@michigan.gov
Michigan Department of Health & Human Services
Bureau of Laboratories
3350 N. Martin Luther King Jr., Blvd.
Lansing, Michigan 48906

Introduction

Sexually transmitted diseases (STD's) are responsible for excessive morbidity, mortality and healthcare costs particularly for women, adolescents, newborns and other vulnerable populations including men who have sex with men (MSM). The Michigan Department of Health and Human Services (MDHHS) is committed to decreasing STD rates in Michigan citizens through increased screening, treatment, and partner referrals. Michigan saw an increase in syphilis cases in 2017. A total of 1,305 cases were reported in 2017 with 480 reported as primary or secondary syphilis, which is an increase of 28% from the previous year (Figure 1).

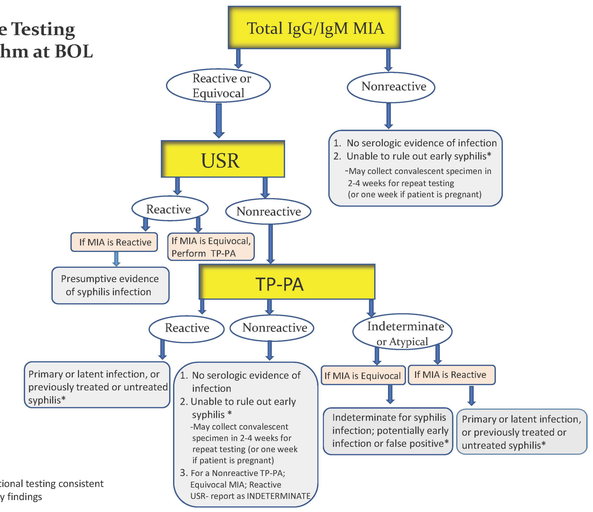
In May 2018, the Michigan Bureau of Laboratories (MI-BOL) switched from the forward to reverse testing algorithm (Figure 2). To support the algorithm change, MI-BOL validated Bio-Rad's Total IgM/IgG Multiflow Immunoassay (MIA) on the BioPlex 2200 instrument. A validation was performed to ensure that the MIA was a suitable method for testing the over 32,000 syphilis test specimens received annually. After instituting the reverse algorithm, BOL initially saw a few negative MIA results with patients showing past positive *Treponema pallidum* particle agglutination (TP-PA) results and nonreactive MIAs with clinician reports of positive treponemal screens. Due to this observation, a post validation study was performed to ensure the BioPlex 2200 was performing as expected.



	2009	2010	2011	2012	2013	2014	2015	2016	2017
Michigan	224	225	278	295	496	428	401	374	480
Detroit City	103	69	91	123	199	128	104	97	108
Outstate	121	156	187	172	297	300	297	277	372

Figure 1. Syphilis cases, rates and geographic spread in Michigan (A.) Syphilis cases in Michigan from 2009 to 2017 showing the increase of reported cases in 2017. (B) Case rates per 100,000 of syphilis by county for 2017. (C.) Age distribution of syphilis cases for Michigan in 2017.

Reverse Testing Algorithm at BOL



*Recommend additional testing consistent with clinical history findings

Figure 2. Diagram of reverse algorithm testing for Syphilis at the Michigan Bureau of Laboratories

Methods

The BioPlex 2200 Syphilis Total & RPR (Rapid Plasma Reagin) kit employs *Treponema pallidum* fusion protein (rTP47/rTP17) and cardiolipin antigen-coated fluoromagnetic beads with unique fluorescent signatures to identify the presence of IgG and IgM antibodies to *Treponema pallidum* and nontreponemal reagin antibodies in a two-step assay format. BOL chose to run only the Syphilis Total antibody as a standalone assay. The results are expressed in antibody index (AI). The Syphilis Total assay results are reported as nonreactive ($\leq$ 0.8 AI), equivocal (0.9, 1.0 AI) or reactive ($\geq$ 1.1 AI).

The initial validation study was performed using 109 specimens (21 negative and 88 positive) previously tested specimens formerly using the forward algorithm. Assay accuracy and precision/reproducibility were determined. To pass validation, test accuracy, precision, and reproducibility needed to exceed 90%. Post validation studies were done during May 2018 flagging MIA negative results in the range of 0.2 to 0.8 (22/606), July 2018 and November 2018 – January 2019 flagging MIA negative results in the range of 0.3 to 0.8 (112/4090), September-October 2018 flagging all MIA negative results in the range of 0.4 to 0.8 (29/1326). These specimens were all re-tested by the *Treponema pallidum* particle agglutination (TP-PA) assay.

Results and Discussion

During the initial validation, BOL determined a 98% sensitivity and 100% specificity comparing the MIA with the TP-PA (Table 1). The total accuracy of the MIA test was 98.1%. The intra-assay precision was 100%.

During the 4 observation periods, a total of 6022 specimens were run. During the observation periods, no specimens with an AI of 0.2 were TP-PA reactive and only one specimen with an AI of 0.3 was TP-PA reactive. Therefore, the AI range of 0.4 to 0.8 was then designated as "high negatives", and all specimens in that range during the 4 observation periods were combined. A total of 113 specimens were in the AI range of 0.4 to 0.8, in which 28 of these were reactive by the TP-PA assay (Table 2). A trend was observed that with increasing AI values in that "high negative" range and an increase in the percentage of TP-PA reactive specimens was seen. (Table 2). The collection of demographic and clinical information from individuals with discordant results is ongoing in collaboration with the Michigan Bureau of Epidemiology STD and HIV Prevention Section.

The median age for the TP-PA reactive/MIA nonreactive group was 55 years (range 25-76 years) and was significantly higher than seen for the TP-PA non-reactive group which was 29 years (range 15-77 years) ($p < 0.0001$ Mann-Whitney U test). Clinical information was provided for 9 of the 25 TP-PA reactive/MIA nonreactive individuals. Of those individuals, 6 had previously confirmed cases of syphilis (Table 3). Considering these results, the Michigan Bureau of Laboratories will continue to reflex all AI results in the "high negative" range for TP-PA testing for a 9-month period and will use that data to guide testing changes if warranted

BioPlex 2200 Total IgG/IgM Multiplex Flow Immunoassay Sensitivity and Specificity

	TP-PA Reactive	TP-PA Nonreactive
Total MIA Reactive	86	0
Total MIA Nonreactive	1	21
Total MIA Indeterminate	1	

Total MIA Sensitivity: 86/(86+2) X 100=98%

Total MIA Specificity: 21/(21+0) = 100%

Table 1. Sensitivity and Specificity of the BioPlex 2200 total IgG/IgM multiplex flow immunoassay (MIA).

Percentage of MIA Negative/TP-PA positive test results during the observation periods

MIA Result	Number (N)	TP-PA Reactive (N)	% TP-PA reactive
0.4	35	4	11.4%
0.5	24	4	16.7%
0.6**	24	12	50.0%
0.7	16	4	25.0%
0.8	14	4	28.6%
Total*	113	28	

*Out of 6022 MIA tests run during the observation periods

** 3 individuals had repeat testing with specimens collected between 4 to 7 months apart

Table 2. Percentage of "high negative" results that were *Treponema pallidum* particle agglutination (TP-PA) reactive.

Discordant MIA TP-PA results

Specimen #	MIA Result	TPPA Result	Syphilis Case status
1	0.4	1+	Under investigation
2	0.4	2/3+	Under investigation
3	0.4	4+	Unconfirmed
4	0.4	4+	Unconfirmed
5	0.5	1/2+	Under investigation
6	0.5	1+	Under investigation
7	0.5	2/3+	Under investigation
8	0.5	2+	Under investigation
9	0.6	1+	Under investigation
10	0.6	2/3+	Under investigation
11	0.6	2+	Confirmed Case 2017
12	0.6	2+	Confirmed Case 1992
13	0.6	2+	Under investigation
14-1*	0.6	3/4+	Under investigation
14-2	0.6	3+	
15	0.6	4+	Confirmed Case 1996
16-1*	0.6	4+	Confirmed Case 2018
16-2	0.6	4+	
17-1*	0.6	4+	Under investigation
17-2	0.6	4+	
18	0.7	4+	Under investigation
19	0.7	4+	Confirmed Case 2000
20	0.7	4+	Confirmed Case 2003
21	0.7	4+	Under investigation
22	0.8	2/3+	Under investigation
23	0.8	2+	Under investigation
24	0.8	2+	Under investigation
25	0.8	3/4+	Unconfirmed

*Same individual with testing repeated between 3 to 7 months apart

Table 3. Table of discordant BioPlex 2200 total IgG/IgM MIA TP-PA results and clinical information if known.

Conclusion

With the sensitivity, specificity, precision/reproducibility, and the accuracy of the test being over 90%, the BioPlex 2200 was found to be suitable for syphilis testing purposes. Investigations are ongoing to try to resolve discordant MIA and TP-PA results.