Performance of the BioPlex 2200 Multiplexing Immunoassay Platform for the Detection of Herpes Simplex Virus Type 2 Specific Antibodies in African Settings

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The BioPlex platform was evaluated for the detection of herpes simplex virus 2 (HSV-2) antibodies in sub-Saharan Africa individuals in comparison to clinicovirological standards and compared to HerpeSelect. The sensitivities and specificities were, respectively, 88.9% and 93.5% for BioPlex and 89.9% and 92.7% for HerpeSelect. The agreement between both assays was 95.7%.

The identification of individuals infected with herpes simplex virus (HSV) is critical for the management of herpes-related conditions, such as genital herpes, as well as for epidemiological studies and clinical or intervention trials. The performance of commercially available enzyme-linked immunosorbent assays (ELISAs) in detecting HSV-2-specific glycoprotein G2 (gG2) antibodies varies significantly between different study populations (1, 8, 17). The BioPlex platform was evaluated for the detection of HSV-2 antibodies among African (4, 17) and Brazilian (15) populations, requiring that the ELISA cutoff value be increased for better specificity in the general population. However, a critical element in evaluating the performance of these assays is the clinical stage of HSV-2 infection (2, 11, 14). For example, the FDA-approved HerpeSelect gG2-specific ELISA (Focus Technologies, Cypress Hill, CA) had high sensitivity in predicting genital HSV-2 infection, particularly first episodes of HSV-2 ulcers, in patients with genital ulcer disease (GUD) from the Central African Republic and Ghana (11).

In the present study, we evaluated the performance of the new BioPlex 2200 immunoassay platform (3) (Bio-Rad Laboratories, Hercules, CA) in detecting HSV-1 and HSV-2 antibodies in populations living in sub-Saharan Africa, including patients with proven genital HSV-2 infection. We used stored sera obtained during cross-sectional studies from two distinct clinicovirological populations. First, sera were obtained between May and July 2009 from 200 HIV-seronegative children (age 0 to 17) seen at the Complexé Pédia-trique of Bangui, Central African Republic, and clinically asymptomatic for genital herpes. Informed consent was obtained from the parents or guardians of these children or from the older children themselves. Second, sera were collected from women presenting with genital ulcer disease (GUD) and cervicovaginal lavages using molecular tests, as described previously (10, 12). From the 226 women enrolled in the trial who had detectable genital HSV-2 DNA and who were either HSV-2 seropositive or seronegative, 208 serum samples were available for this study (12).

Sera were aliquoted, frozen at −20°C, and further tested for HSV-1 and HSV-2-specific antibodies using the BioPlex 2200 HSV-1 and HSV-2 IgG kit. The BioPlex 2200 platform is a fully automated instrument that combines flow cytometric technology with antigen-coated fluoromagnetic bead chemistry. The BioPlex 2200 HSV-1 and HSV-2 IgG kit detects and differentiates IgG antibodies to HSV-1 and HSV-2 by using beads coated with recombinant peptides encompassing the gG1 N-terminal region (amino acids 1 to 173) and the region between amino acids 205 to 240 of the gG2, respectively. For every sample processed, three internal quality control beads are employed that can check for detector fluctuations, sample integrity, and nonspecific binding. The results are reported according to their antibody index (AI), with values of <0.9 considered negative, 0.9 to 1.0 equivocal, and >1.0 positive.

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HSV-2 seroprevalence by age. The HSV-1 seroprevalence was.

Figure 1 shows clear differences in the patterns of HSV-1 and.

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presence of passive maternal antibodies) and under the age of.

samples from children over the age of 1 year (to avoid the.

samples from children with high posterior probability to be HSV-2.

were used as the clinicovirological standard to.

HSV-2 seronegative were used as the clinicovirological standard.

It is customary in this instance to use.

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the presence of passive maternal antibodies) and under the age of.

sexual debut (in practice, before the teenage years). We there-

fore selected samples from 139 children aged 1 to 10 years.

from the 200 asymptomatic children as a reference standard in.

this study.

Using the Bio-Rad BioPlex 2200 immunoassay kit, 158.

(79%) and 12 (6.0%) of the 200 asymptomatic children were.

found to be seropositive for HSV-1 and HSV-2, respectively.

Figure 1 shows clear differences in the patterns of HSV-1 and.

HSV-2 seroprevalence by age. The HSV-1 seroprevalence was.

already 50% among infants aged <1 year and steadily in-

creased to 100% in young people aged 16 to 17 years. With.

regard to HSV-2, 25% of the infants aged <1 year had detect-

able antibodies, likely of maternal origin. The prevalence at.

older ages was low (below 10%). These observations are con-

sistent with the natural history of HSV-1 and HSV-2 infections.

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of HSV-2 infection around the time of puberty and beginning.

of sexual activity (5, 16, 18).

Among the 208 samples from patients with proven genital.

HSV-2 infection (true positive cases) and available results for.

both assays, the sensitivities of serological testing were 88.9%.

(95% confidence interval [CI], 83.9% to 92.9%) and 89.9%.

(95% CI, 85.0% to 93.6%) for HSV-2 BioPlex (AI > 1.0) and.

HerpeSelect (AI > 1.1), respectively (Table 1). The concor-

dance between the two assays was high (200/208; agreement =

96.2%; 95% CI, 92.3% to 98.3%; κ = 0.80; P < 0.0001). The.

HIV serostatus was available for 207 individuals. For both.

assays, sensitivity was higher in the 125 HIV-1-seropositive.

individuals than in the 82 HIV-seronegative individuals. For.

BioPlex, the sensitivities were 98.4% (95% CI, 94.3% to.

99.8%) in HIV-1 seropositive and 74.4% (95% CI, 63.6% to.

83.4%) in HIV-seronegative individuals, respectively (P <

0.001); for HerpeSelect, the sensitivities were 96.8% (95% CI,

92.0% to 99.1%) in HIV-1-seropositive individuals and 79.3%.

(95% CI, 68.9% to 87.4%) in HIV-seronegative individuals.

(P < 0.001). In this cohort, a high proportion (84%) of first-

episode HSV-2 infections (n = 25) occurred in HIV-seronege-

nate women, of whom roughly 75% seroconverted at day 28.

(11). This explains why some true HSV-2 infections were.

HSV-2 seronegative at inclusion and, hence, the apparently.

Reduced sensitivity among HIV-uninfected women. The con-

cordance between assays was higher in the HIV-seronegative.

individuals than in the 82 HIV-seronegative individuals, despite.

lower agreement (HIV seronegative, agreement = 92.7% and.

κ = 0.80; HIV-1 seropositive, agreement = 98.4% and κ =

0.66). The slightly better concordance observed in HIV-sero-

negative individuals is due to a higher expected agreement in.

HIV positives because of the very high HSV-2 seroprevalence.

among HIV-positive individuals. Using the 3.5 cutoff for Her-

peSelect, the sensitivities were 82.4% (95% CI, 74.6% to.

88.6%) in HIV-1-seropositive and 63.4% (95% CI, 52.4% to.

73.8%) in HIV-seronegative individuals, respectively (P =

0.001).

Among the 139 samples from children aged 1 to 10 years.

(assumed to be true negative cases), the specificities of both.

assays were high and comparable: 93.5% (95% CI, 88.1% to.

97.0%) for BioPlex (AI = 1.0) and HerpeSelect (AI = 1.1).

Specificities of both assays were high and comparable: 93.5%.

(95% CI, 88.1% to 97.0%) for BioPlex, and 97.8% (95% CI,

92.8%–99.6%) for HerpeSelect (Table 1). The specificity.

increased to 97.8% (95% CI, 95.4% to 100%) for HerpeSelect.

TABLE 1. Performance of HSV-2 Bio-Rad BioPlex 2200 system and.

HSV-2-specific HerpesSelect ELISA and overall agreement between both assays

| Parameter | Result [% (95% CI)] at indicated cutoff value |
|-----------|---------------------------------------------|
| BioPlex sensitivity | 88.9 (83.9–92.9) | 82.2 (76.3–87.2) |
| HerpeSelect sensitivity | 89.9 (85.0–93.6) | 75.0 (68.5–80.7) |
| Bioplex specificity | 93.5 (88.1–97.0) | 97.1 (92.8–99.2) |
| HerpesSelect specificity | 92.7 (87.0–96.4) | 97.8 (93.8–99.6) |
| Agreement between both assays | 95.7 (92.9–97.5) | 91.9 (88.5–94.5) |

κ coefficient

0.91 0.84

* Sensitivity and specificity were determined in comparison with clinicoviro-

ological reference standards. Samples from molecularly documented HSV-2 gen-

ital infection were used as the reference to determine sensitivity. Samples from.

asymptomatic children aged 1 to 10 years were used as the reference to deter-

mine specificity. The manufacturer’s recommended positivity cutoff for BioPlex is

1.0 and for HerpeSelect ELISA is 1.1; the use of a higher index value of 3.5 for HerpeSelect

has been recommended to increase the specificity in African populations (4).

The concordance between the Bio-Rad BioPlex 2200 system and the Herpe-

Select ELISA is evaluated by the kappa coefficient.
when the AI was raised to 3.5. Interestingly, using a higher cutoff for BioPlex (3.5) as well gave slightly better specificity (97.1%; 95% CI, 92.8% to 99.2%). Concordance between the two assays at the lower cutoff was high (130/137; agreement = 94.9%; 95% CI, 89.8% to 97.9%; \(\kappa = 0.60\); \(P < 0.0001\)).

The overall concordance between the two assays was excellent at both the manufacturer’s and the 3.5 cutoff (\(\kappa = 0.84\), respectively; \(P < 0.0001\)) (Table 1).

Taken together, our observations demonstrate that the Bio-Rad BioPlex 2200 immunoassay has strikingly similar performance to the HerpeSelect gG2 ELISA, with the advantage of simultaneous detection of HSV-1 and HSV-2 antibodies, thus providing an additional useful tool for HSV type-specific serology which can be used in epidemiological, clinical, or intervention studies in African populations.

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REFERENCES

1. Ashley-Morrow, R., Nollkamper, N., J. Robinson, N. Bishop, and J. Smith. 2004. Performance of Focus ELISA tests for herpes simplex virus type 1 (HSV-1) and HSV-2 antibodies among women in ten diverse geographical locations. Clin. Microbiol. Infect. 10:530–536.
2. Ashley Morrow, R., E. Krantz, D. Friedrich, and A. Wald. 2006. Clinical correlates of index values in the focus HerpeSelect ELISA for antibodies to herpes simplex virus type 1. J. Clin. Microbiol. 44:5781–5787.
3. 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. J. Clin. Virol. 48:130–136.
4. Biraro, S., P. Mayaud, R. A. Morrow, H. Grosskurth, and H. A. Weiss. 2011. Performance of commercial herpes simplex virus type-2 antibody tests using serum samples from sub-Saharan Africa: a systematic review and meta-analysis. Sex. Transm. Infect. 87:286–290.
5. Cowan, F. M., et al. 2003. Seroepidemiological study of herpes simplex virus types 1 and 2 in Brazil, Estonia, India, Morocco, and Sri Lanka. Sex. Transm. Infect. 79:286–290.
6. Delany, S., et al. 2009. Impact of aciclovir on genital and plasma HIV-1 RNA in HSV-2/HIV-1 co-infected women: a randomized placebo-controlled trial in South Africa. AIDS 23:641–649.
7. Gamiel, J. L., et al. 2008. Improved performance of enzyme-linked immunoassay for herpesserobacter and effects of human immunodeficiency virus co-infection on the serologic detection of herpes simplex virus type 2 in Rakai, Uganda. Clin. Vaccine Immunol. 15:888–890.
8. Hogreve, W., X. Su, J. Song, R. Ashley, and L. Kong. 2002. Detection of herpes simplex virus type 2-specific immunoglobulin G antibodies in African sera by using recombinant gG2 Western blotting and gG2 inhibition. J. Clin. Microbiol. 40:3635–3640.
9. Laeyendecker, O., et al. 2004. Performance of a commercial, type-specific enzyme-linked immunosorbent assay for detection of herpes simplex virus type 2 antibodies. J. Clin. Microbiol. 42:1794–1796.
10. Legoff, J., et al. 2006. Real-time PCR quantification of genital shedding of herpes simplex virus (HSV) and human immunodeficiency virus (HIV) in women coinfected with HSV and HIV. J. Clin. Microbiol. 44:432–433.
11. LeGoff, J., et al. 2008. Performance of HerpeSelect and Kalon assays in detection of antibodies to herpes simplex virus type 2. J. Clin. Microbiol. 46:1919–1918.
12. LeGoff, J., et al. 2007. Cervicovaginal HSV-1 and herpes simplex virus type 2 shedding during genital disease episodes. AIDS 21:1569–1578.
13. Mayaud, P., et al. 2009. Impact of acyclovir on genital and plasma HIV-1 RNA, genital herpes simplex virus type 2 DNA, and ulcer healing among HIV-1-infected African women with herpes ulcers: a randomized placebo-controlled trial. J. Infect. Dis. 200:216–226.
14. Morrow, R. A., D. Friedrich, and E. Krantz. 2003. Performance of the Focus and Kalon enzyme-linked immunosorbent assays for antibodies to herpes simplex virus type 2 glycoprotein G in culture-documented cases of genital herpes. J. Clin. Microbiol. 41:5212–5214.
15. Nascimento, M. C., et al. 2007. Performance of the HerpeSelect (Focus) and Kalon enzyme-linked immunosorbent assays for detection of antibodies against herpes simplex virus type 2 by use of monoclonal antibody-blocking enzyme immunoassay and clinicovirological reference standards in Brazil. J. Clin. Microbiol. 45:2309–2311.
16. Obasi, A., et al. 1999. Antibody to herpes simplex virus type 2 as a marker of sexual risk behavior in rural Tanzania. J. Infect. Dis. 179:16–24.
17. van Dyck, E., et al. 2004. Performance of commercially available enzyme immunoassays for detection of antibodies against herpes simplex virus type 2 in African populations. J. Clin. Microbiol. 42:2961–2965.
18. Weiss, H. A., et al. 2001. The epidemiology of HSV-2 infection and its association with HIV infection in four urban African populations. AIDS 15(Suppl. 4):S70–S108.