Comparison of Three Commercial Immunoassays for Detection of Herpes Simplex Virus Type 2 Antibodies in Commercial Sex Workers in Yunnan Province, China

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Five hundred commercial sex workers in China were tested for herpes simplex virus type 2 by three immunoassays and Western blotting. Sensitivities for the Focus, Kalon, and Biokit assays were 86.7%, 82.3%, and 34.9%, respectively, and specificities were 91.8%, 94.2%, and 60.1%, respectively. The Focus assay performed optimally at an index of 1.5 (95.2% sensitivity and 93.4% specificity), and the Kalon assay performed optimally at an index of 1.2 (93.3% sensitivity and 95.2% specificity).

Herpes simplex virus type 2 (HSV-2) infections increase the likelihood of the transmission and acquisition of human immunodeficiency virus (HIV) (8, 16, 19). HSV-2 antibody status can provide an objective measure of the extent of polypartnerism, early age of sexual debut, and acquisition rates of genital herpes (14). These relationships serve as the impetus for the accurate diagnosis and control of HSV-2 infection, especially among high-risk groups.

Three tests are commonly used for the diagnosis of HSV-2 infection. HerpeSelect and Kalon enzyme-linked immunosorbent assays (ELISAs) have shown high sensitivities (95% to 100%) and specificities (95% to 100%) in comparison to various “gold standards” (1, 12, 18). A rapid membrane assay, the Biokit assay, was developed as a point-of-care test specific for HSV-2 antibodies and showed premarket evaluations of 96% sensitivity and 98% specificity (3).

These assays were developed for clinical use in sexually transmitted disease (STD) clinics in industrialized countries. The scope of their performance among different populations in China is limited. The HerpeSelect assay has been introduced in China, and it has been used in STD clinics and for epidemiological studies (20). This study evaluated the performance of three immunoassays in comparison to Western blotting (WB) for the detection of HSV-2 infections among commercial sex workers (CSWs) in Kunming, Yunnan Province, China.

Sera from 500 CSWs were tested for HSV-2 antibodies by HerpeSelect HSV-2 ELISA (Focus Technologies, Cypress, CA), Kalon HSV-2 ELISA (Kalon Biological, Ltd., Surrey, United Kingdom), and Biokit rapid assay (Sure-Vue, Lexington, MA). The HerpeSelect ELISA is referred as the Focus assay. All seropositive samples (n = 275) detected by the Biokit, Focus, and Kalon assays were confirmed by WB for HSV-1 and HSV-2, performed by the University of Washington (Seattle) as previously described (2). The Committees on Human Research at the Johns Hopkins University, Baltimore, Maryland, the National Institutes of Health, Bethesda, Maryland, and Yunnan University, Yunnan, China, approved the study protocol. Since only seropositive results were confirmed by WB, biased estimates of sensitivity and specificity would have resulted if calculations were made based on the entire population (5). Taking into account this confirmatory strategy, appropriate mathematical adjustments were considered according to the method of Kosinski and Barnhart in order to avoid verification bias (9).

HSV-2 seroprevalence was 36.8% (95% confidence interval [95% CI], 32.6 to 41.0%) by Focus assay, 33.8% (95% CI, 29.3 to 37.6%) by Kalon assay, and 46.6% (95% CI, 42.2 to 51.0%) by Biokit assay. After all seropositive results by the three immunoassays were confirmed by WB, HSV-2 seroprevalence was 33.0% (95% CI, 28.9 to 37.1%). Estimated sensitivities with adjustment for verification bias for the Focus, Kalon, and Biokit assays were 86.7%, 82.3%, and 34.9%, respectively. Estimated specificities were 91.8%, 94.2%, and 60.1%, respectively, compared to that of WB. The Focus, Kalon, and Biokit assay results were 88.0%, 91.2%, and 55.6% concordant to WB results, respectively. These calculations were based on the manufacturers’ recommended index cutoff value of 1.1.

The receiver operating characteristic (ROC) curve showed that the Focus assay performed optimally with an index cutoff value of 1.5, with 95.2% sensitivity, 93.4% specificity, and 94% concordance. The optimal index cutoff for the Kalon assay was

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1.2, with 93.3% sensitivity, 95.2% specificity, and 94.6% concordance (Fig. 1).

With the 275 seropositive samples that were confirmed by WB, the Biokit assay had 34.5% (n = 95) false-positive results, with 9.5% (n = 26) false-positive results by Focus assay and 5.8% (n = 16) false-positive results by Kalon assay. Within the subset of 95 sera that were only HSV-1 positive by WB, the Biokit assay detected 88.4% (n = 84) as positive for HSV-2. The median index values among Biokit assay-positive samples were 0.22 by Focus assay and 0.29 by Kalon assay, which were significantly lower than the index values of 5.7 by Focus assay and 4.7 by Kalon assay for Biokit assay/WB HSV-2-positive samples (n = 138) (P < 0.01).

Among Focus assay HSV-2-seronegative samples (n = 91) (index values of <1.1), the Biokit assay detected 83 (91.2%) samples as HSV-2 positive, while only 7 (7.7%) were positive by WB (P < 0.001). The difference in the proportions of positive sera between the Biokit assay and WB became narrower as Focus assay index values increased. The proportion of Focus assay/WB-positive samples also increased, from 61.1% at index values of 1.1 to 2.0 to 77.3% at index values of 2.0 to 3.5 and, finally, to 95.1% at index values of >3.5.

Of the Kalon assay-seronegative sera (n = 106) (index values of <1.1), the Biokit assay identified 94 (88.7%) HSV-2-positive samples, whereas 12 (11.3%) samples were positive by WB (P < 0.001). The proportions of positive samples between Biokit assay and WB became more similar as Kalon assay index values increased to >1.1. Additionally, the proportion of positive sera by Kalon assay/WB increased from 73.0% at index values of 1.1 to 2.0 to 92.3% at index values of 2.0 to 3.5 and, finally, to 96.2% at index values of >3.5 (Fig. 2).

The Kalon assay performed with the highest concordance to WB and had the smallest number of false-positive results, followed by the Focus assay and then the Biokit assay. Previous studies suggested raising the index cutoff value of 1.1 for optimal performance among the ELISAs (7, 10, 13, 15). A study on the effect of HIV coinfection on the performance of HSV-2 immunoassays in Uganda recommended raising the cutoff value to 3.2 for the Focus assay and to 1.5 for the Kalon assay for optimal performance (6). Another study among Chinese STD patients showed that the Focus assay performed optimally with a cutoff value of 0.9 (93.1% sensitivity and 93.6% specificity) (20). ROC curve analyses in this study showed that the Focus assay performed optimally at an index of 1.5 (95.2% sensitivity and 95.4% specificity) and the Kalon assay performed optimally at an index of 1.2 (93.3% sensitivity and 95.2% specificity).

Among men who have sex with men in the United States, the Biokit assay was an effective confirmatory method for the Focus assay by reducing falsely positive results (4). In our study, sequential testing of Focus and Kalon assay-seropositive results by the Biokit assay showed 50% and 43% reductions, respectively, in the numbers of falsely positive samples (P < 0.01). Since the Biokit assay showed a stronger correlation with WB at Focus and Kalon assay index values of >1.1 than at
index values of <1.1, it may be useful as a confirmatory test of Focus and Kalon assay-seropositive results.

This study provided greater insight into the performance characteristics of three immunoassays among CSWs in China. Not all 500 samples were confirmed by WB. This may have an influence on our calculations, even with appropriate mathematical adjustments. Variations in test characteristics among these assays and various settings may depend on the population study, seroconversion factors, and the cross-reactivity with HSV-1 (4, 11, 17).

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