Performance of Focus, Kalon, and Biokit for the Detection of Herpes Simplex Virus Type 2- Antibodies among Commercial Sex Workers in Yunnan Province, China

Running Title: Detection of HSV-2 among CSWs in China

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Abstract

500 CSWs in China were tested for HSV-2 by three immunoassays and Western blot.

Sensitivities for Focus, Kalon, Biokit were 86.7%, 82.3%, 34.9%, and specificities were 91.8%, 94.2%, 60.1%, respectively. Focus performed optimal at index 1.5 (95.2% sensitivity; 93.4% specificity), and Kalon at 1.2 (93.3% sensitivity; 95.2% specificity).

Keywords: Herpes simplex virus type 2 (HSV-2), commercial sex workers (CSWs), China, Focus, Kalon, Biokit
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 (ELISA) have shown high sensitivities (93% to 100%) and specificities (95% to 100%) when evaluated against various “gold standards” (1, 12, 18). The rapid membrane assay, Biokit, was developed as a point-of-care test specific for HSV-2 antibodies showed pre-market evaluations of 96% sensitivity and 98% specificity (3).

These assays were developed for clinical use in 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 STDs clinics and among epidemiological studies (20). This study evaluated the performance of three immunoassays to Western blotting (WB) in the detection of HSV-2 infections among commercial sex workers (CSWs) in Kunming, Yunnan Province of China.

Sera from 500 CSWs were tested for HSV-2 antibodies by HerpeSelect HSV-2 ELISA (Focus Technologies; Cypress, CA, USA), Kalon HSV-2 ELISA (Kalon Biological, Ltd.; Surrey, UK), and Biokit rapid assay (Sure-Vue; Lexington, MA, USA). The HerpeSelect ELISA is referred as Focus. All seropositive samples (N=275) by Biokit, Focus and Kalon were further confirmed by WB for HSV-1 and HSV-2 performed by University of Washington (Seattle, Washington, USA) previously described (2). The Committees on Human Research at the Johns
Hopkins University, Maryland, the National Institutes of Health, Bethesda, Maryland, and at the 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 Kosinski and Barnhart in order to avoid verification bias (9).

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

The receiver operating characteristic (ROC) curve showed that Focus performed optimally at index cutoff value of 1.5 with 95.2% sensitivity, 93.4% specificity, and 94% concordance. The optimal index cutoff for Kalon was 1.2 with 93.3% sensitivity, 95.2% specificity, and 94.6% concordance. (Figure 1)

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

Among Focus HSV-2 seronegative samples (n=91) (index value <1.1), Biokit 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 proportion of positive sera between Biokit and WB became narrower as Focus index values increased. The proportion of Focus/WB positive samples also increased from 61.1% at index values 1.1-2.0, to 77.3% at index values 2.0-3.5, and finally to 95.1% at index values >3.5.

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

Kalon performed with the highest concordance to WB, and it had the least number of false positive results, followed by Focus, then Biokit. 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 co-infection on the performance of HSV-2 immunoassays in Uganda recommended raising the cutoff values to 3.2 for Focus and 1.5 for Kalon for optimal performance (6). Another study among Chinese STD patients showed that Focus performed optimally at cutoff value 0.9 (93.1% sensitivity, 93.6% specificity) (20). ROC curve analyses in this study showed that Focus performed optimally at index 1.5 (95.2% sensitivity, 93.4% specificity) and 1.2 for Kalon (93.3 % sensitivity, 95.2 % specificity).
Among men who have sex with men in the United States, Biokit was an effective confirmatory method for Focus by reducing falsely positive results (4). In our study, sequential testing of Focus and Kalon seropositive results by Biokit showed 50% and 43% reduction in falsely positive samples \( (p<0.01) \), respectively. Since Biokit showed stronger correlation to WB at Focus and Kalon index values >1.1 compared to <1.1, it may be useful as a confirmatory test of Focus and Kalon seropositive results.

This study provided greater insight to 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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FIGURE 1. ROC Curve for Focus and Kalon ELISAs Using WB as the “Gold Standard”
Mathematical adjustments were considered to avoid verification bias based on Kosinski and Barnhart (9).
FIGURE 2. Western Blot and Biokit Results Sorted by Focus and Kalon HSV-2 ELISA Index Values. Proportion (%) positive determined from the 275 samples tested by Western blotting (WB). Number of sera (N) contributing to each subset is given below designated index value ranges.