Use of Commercial Enzyme Immunoassays To Detect Antibodies to the Herpes Simplex Virus Type 2 Glycoprotein G in a Low-Risk Population in Hanoi, Vietnam

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Sera from 1,238 Vietnamese women in Hanoi were tested for herpes simplex virus type 2 (HSV-2). HSV-2 prevalence was 2.0%. The Kalon and Biokit assays showed significantly higher concordance to Western blotting data than did the Focus assay (P < 0.01). Screening by Focus and then retesting with Kalon/Biokit of positive samples can reduce falsely positive results significantly (P < 0.01).

Herpes simplex virus type 2 (HSV-2) infections are increasing worldwide (6, 7), and this virus is the main cause of genital ulcer disease (1, 5). HSV-2 prevalence is often higher in developing countries than in developed countries, with higher rates in women than men (11). Additionally, HSV-2 infection appears to be a risk factor for human immunodeficiency virus acquisition and transmission (7, 13). Few epidemiologic data on HSV-2 infections are available for developing countries (5). There are increasing concerns about the performance and accuracy of various commercially available serological assays for the detection of HSV-2 antibodies (4, 9).

Enzyme-linked immunosorbent assays (ELISA) are used for the detection of antibodies to the HSV-2 glycoprotein G-2 (gG-2) (3). Rapid membrane assays are used as point-of-care tests specific for HSV-2 antibodies to gG-2 (10). Western blotting (WB) and gG monoclonal antibody blocking assays are used as the “gold standard” for confirmation (12). It cannot be inferred that the performance of an ELISA and rapid test for the detection of HSV-2 infection in industrialized countries is equally accurate in other regions of the world (8, 12).

From November to December 2004, 1,238 married women not infected with human immunodeficiency virus were recruited from four communes in the periurban Soc Son District of Hanoi, Vietnam. This study was approved by collaborating institutions’ review boards in Vietnam and the United States. A total of 1,238 serum samples were initially screened for HSV-2 antibodies with the HerpeSelect HSV-2 ELISA (Focus Technologies, Cypress, CA). A subset of 174 selected sera, including all Focus seropositive (n = 108), equivocal (n = 19), and seronegative samples (n = 47), was retested by WB performed by the University of Washington’s Diagnostic Virology Laboratory (Seattle), by Kalon ELISA (Kalon Biological, Ltd., Surrey, United Kingdom), and with a Biokit rapid assay (SureVue; Lexington, MA). Sera of 83 Focus ELISA HSV-2-positive but WB-negative samples were further investigated using a Focus inhibition assay performed by Focus Technologies (Cypress, CA).

The Focus ELISA detected an 8.7% (n = 108; median index value, 2.39) HSV-2 seroprevalence among the 1,238 subjects. Of the 1,111 negative samples by Focus ELISA, the median index value was 0.27 (interquartile range, 0.16 to 0.50), with 72.5% of the samples having index values below 0.5. HSV-2 prevalence was 2% (n = 25) and was screened by Focus and then confirmed by WB (Table 1).

The 174 selected sera that were tested further included 108 positive, 19 equivocal, and 47 negative by the Focus ELISA. The remaining 1,064 sera were not tested subsequently. Of the 174 serum samples, 108 (62.1%) were positive by Focus, 47 (27.0%) by Kalon, and 43 (24.7%) by Biokit (Table 1). The positive proportion for HSV-2 by all three assays was 16.7%. Focus showed 91 (52.3%) concordant results to WB, while 143 (83.4%) were seen by Kalon and 146 (83.9%) by Biokit. Within the HSV-2 Focus-negative group, six were positive by Biokit and five by Kalon. Among the 19 equivocal samples by Focus,

Table 1. Comparison of HSV-2 results determined by Focus ELISA to those from Biokit, Kalon, and Western blotting

| Focus index | No. of specimens | No. of specimens with indicated Focus index that tested positive by: |
|-------------|-----------------|------------------------------------------------------------------|
|             | Biokit | Kalon | WB |
| <0.5        | 7      | 1     | 1   | 0  |
| 0.5–0.9     | 40     | 5     | 4   | 0  |
| 0.9–1.1     | 19     | 3     | 3   | 0  |
| >1.1–2.5    | 57     | 14    | 13  | 4  |
| 2.5–5.0     | 25     | 2     | 9   | 7  |
| >5.0        | 26     | 18    | 17  | 14 |
| Total       | 174    | 43    | 47  | 25 |

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3 were also equivocal by Kalon and 3 were positive by Biokit. From the 108 HSV-2-seropositive samples by Focus, 26 had index values higher than 5.0, 25 fell in the 2.5 to 5.0 range, and 57 were low positives (1.1 to 2.5) (Table 1). Among Focus HSV-2-seropositive samples, there were 34 HSV-2-positive samples by Biokit and 39 by Kalon (Table 1).

The Focus inhibition assay showed 94% concordance to Focus ELISA results based on analysis of the 83 Focus HSV-2 ELISA-positive samples. Of the 83 samples tested in the inhibition assay, 3 were false positive by Focus ELISA (<60% inhibition), 6 were equivocal, 14 were negative, and 60 were true positive (≥60% inhibition). Kalon and Biokit results were 30.6% and 45.8% concordant with the Focus inhibition assay, respectively.

The median percent inhibition was 94% for Kalon-positive samples (that were also Focus seropositive) and 91% for Biokit-positive samples (that were also Focus seropositive), with both assays showing two false positives according to the Focus inhibition assay results. The median percent inhibition between Kalon/Biokit negatives and Kalon/Biokit positives was significantly different (P < 0.01). Of the 69 samples that indicated HSV-2 inhibition reactivity, 5 samples showed inhibition values of <60%, while 64 samples showed ≥60% inhibition (Fig. 1).

The performance of the inhibition assay showed high concordance to the Focus ELISA, similar to the results found in the HSV study from four African countries (8). However, it was not a helpful tool to evaluate the discrepancies between the Focus ELISA, Biokit, and Kalon assays.

False-negative results occurred with both the Kalon and Biokit, with 3 (12%) and 5 (20%) WB-positive samples, respectively. The false positives with Kalon (n = 36) and Biokit (n = 14) compared to those detected by Focus (n = 83) likely underestimated the actual false-positive number, because Kalon and Biokit tests were only performed on the 174 selected samples rather than the total population of 1,238.

Focus ELISA presented low concordance to WB results and detected a high number of false positives, probably due to its cutoff index value of 1.1. There was a significant difference in median index values (P < 0.01) between WB-positive and Focus-seropositive versus WB-negative but Focus-seronegative results (5.3 versus 2.9). When raising the index cutoff value of Focus from >1.1 to ≥1.8, there was a 42.2% (83 to 48) reduction in falsely positive samples. Kalon and Biokit rapid assays showed a low number of false positives within the 174 selected samples. Initial screening by Focus ELISA followed by retesting by Kalon showed false-positive results declined from 83 (47.7%) to 25 (14.4%). Retesting by Biokit of Focus ELISA positives showed a reduction to 23 (13.2%) false positives. Therefore, retesting with the Kalon ELISA and/or Biokit rapid assay of Focus HSV-2-positive samples subsequent to raising the Focus cutoff index value to ≥1.8 would reduce falsely positive samples significantly (P < 0.01).

The variations in performance among various serological tests to detect antibodies to HSV-2 may depend on the population and cross-reactivity to HSV-1 (9, 12). Most often, these serologic assays are assessed in patients attending sexually transmitted disease clinics in developed countries (2), while our study focused on sera from a population-based study in Vietnam. The inconsistencies of these assays raise concerns about their performance for the detection of HSV-2 infection in clinical settings, especially where prevalence is not well-defined.

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