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Evaluation of the HerpeSelect Express Rapid Test in the Detection of Herpes Simplex Virus Type 2 Antibodies in Patients With Genital Ulcer Disease

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Background: A rapid point-of-care test with high sensitivity and specificity is required in order to fulfill the need for early detection and screening of Herpes simplex virus type 2 (HSV-2) infection among patients with genital ulcer disease (GUD), for better management and control of virus transmission. *Methods:* The goal of this study is to evaluate the performance of the commercially available HerpeSelect Express rapid test in comparison with three ELISA assays: HerpeSelect ELISA, Kalon HSV-2 glycoprotein G2 assay, and monoclonal antibody blocking enzyme immunoassay, which was used as the gold standard for the detection of HSV-2 antibodies. *Results:* This study showed high sensitivity (ranging from 82.6 to 100%) and specificity (100%) of the HerpeSelect Express rapid test when compared to the three ELISA assays. The agreement between the HerpeSelect Express rapid test with the three ELISAs ranged from 93.3 to 100%. *Conclusion:* The HerpeSelect Express rapid test has adequate sensitivity and specificity for confirming HSV-2 infection in patients with GUD, indicating its suitability for epidemiological studies. J. Clin. Lab. Anal. 29:43–46, 2015. © 2014 Wiley Periodicals, Inc.

Key words: genital ulcer; herpes simplex virus 2; HerpeSelect ELISA; HSV-2 antibodies; monoclonal antibody blocking EIA

INTRODUCTION

Herpes simplex virus type 2 (HSV-2) is the main cause of genital ulcers and is one of the most prevalent sexually transmitted diseases worldwide (1). Infection with HSV-2 has also been shown to increase the risk of acquisition or transmission of HIV infection (2, 3). Antiviral therapy reduces subclinical shedding of HSV, thus significantly reducing transmission (4). Identification of individuals infected with HSV-2 is critical for the management of genital herpes as well as for epidemiological studies and clinical trials (5). Hence, there is a need for a rapid point-of-care test with high sensitivity and specificity.

Direct tests for virus isolation and antigen detection aim to demonstrate the presence of HSV-2 in a suspicious lesion or in genital secretions. The sample should be taken from a vesicular lesion that has been present for fewer than 24 h because once the lesion has begun to crust, the test sensitivity will decline. In addition, the test sensitivity is lower in patients with recurrent lesions than in those with first episodes (6). It is even possible that some recent HSV-2 infections may have been accompanied by a lack of HSV-2 DNA detection, since the delay between the onset of symptoms and presentation is quite long, allowing clearance of the virus (7).

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Seroepidemiological studies have been hampered by the lack of accurate and easy-to-use tests for the detection of antibodies against HSV-2 in different populations. Western blotting (WB), a time-consuming and expensive assay, has long been used as a "gold standard" for the detection of anti-HSV type specific antibodies (8). Another reference standard is the monoclonal antibody blocking enzyme immunoassay (MAb-EIA) adapted from the MAb radioimmunoassay produced at the Health Protection Agency (HPA), London, United Kingdom (9).

More recently, two HSV type specific antibody assays directed against glycoprotein G2 (gG2) have been developed and have become commercially available for the diagnosis of HSV-2 infections, including HerpeSelect ELISA and Kalon gG2 ELISA (5, 10). The FDA-approved and widely available HerpeSelect ELISA has high sensitivity and specificity, ranging from 96 to 100%, against the reference WB assay for testing of sera from North American or Western European populations (5, 11–13). Kalon gG2 specific ELISA was found to be as sensitive as and more specific than HerpeSelect, with WB as the reference method (13). Type-specific tests are useful diagnostic tools that allow differentiation of HSV-1 and HSV-2 infections (14, 15).

A rapid point-of-care test with high sensitivity and specificity is required in order to fulfill the need for early detection and screening of HSV-2 infection for better management and control of virus transmission. It is to be noted that the HSV-2 antibody assay result will not answer the question about the genital ulcer disease (GUD) in many patients, as there are other causes such as HSV-1. The goal of this study is to evaluate the performance of a commercially available HerpeSelect Express rapid test in comparison with three ELISA assays: HerpeSelect ELISA, Kalon HSV-2 gG2 assay, and MAb-EIA, which was used as the gold standard.

MATERIALS AND METHODS

A total of 60 patients were enrolled in the study (35 males and 25 females), the mean age of the patients was 37 ± 13.6 years. The patients were diagnosed with GUD by the dermatology consultant at the Dermatology Department, Qassim University polyclinic and King Fahad Specialist Hospital, Kingdom of Saudi Arabia, from April 2011 to November 2012. The study was approved by the local ethical committee and performed according to the ethical procedures. Written informed consent was obtained from each participant.

Blood samples were drawn from the patients and collected in pyrogen-free tubes under aseptic conditions, they were allowed to be clotted and centrifuged. The sera were stored frozen in aliquots at −20°C until used for analysis. The collected serum samples were thawed before starting the assays procedures. The aliquots were tested by the HerpeSelect Express rapid test, a qualitative immunochromatographic assay that utilizes nitrocellulose membrane lateral flow to detect HSV-2 IgG (Focus Technologies, Inc., Cypress, CA). The sera were also tested by HerpeSelect 2 ELISA IgG (Focus Technologies, Inc.) and by Kalon HSV-2 IgG ELISA (Kalon Biologicals Ltd., Guildford, United Kingdom). The commercial assays were performed at the Department of Microbiology of Qassim University according to the manufacturers' instructions. Samples with optical density index values >1.1 were recorded as positive, those with index values <0.9 were recorded as negative, and those with index values 0.9–1.1 were recorded as equivocal. The sera were also assayed by the reference serological test MAb-EIA. The MAb-EIA was performed as follows: recombinant HSV-2 gG-2 antigen had been precoated onto microtiter plate wells, serum samples were pipetted into the wells, an enzyme-linked monoclonal antibody specific for HSV-2 gG-2 was added to the wells. Following a wash to remove any unbound antibody enzyme reagent, a substrate solution was added. The color development was stopped and the intensity of the color was measured at 450 nm (16, 17).

STATISTICAL ANALYSIS

Data were analyzed using SPSS version 16. Values were expressed as mean ± SD. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the HerpeSelect Express rapid test compared to the three ELISA assays were calculated. The statistical significance of the differences in sensitivities and specificities was assessed by the Pearson Chi-square test. Phi coefficient test was used to assess the correlation between the results of the HerpeSelect Express rapid test with the three ELISA assays results. Results were considered statistically significant at P-value ≤0.05.

RESULTS

Considering MAb-EIA test as the gold standard for the diagnosis of HSV-2 infection in patients with a clinical picture suggestive of herpetic genital ulcer, the seropositivity of HSV-2 antibodies in our study was 38.3%. As shown in Table 1, the sensitivity and specificity of the HerpeSelect Express rapid test were 95 and 100%, respectively, when compared to HerpeSelect ELISA (Phi = 0.963); PPV and NPV were 100 and 97.56%, respectively. When it was compared to Kalon ELISA, the HerpeSelect Express rapid test had sensitivity and specificity of 100% (Phi = 1). Both PPV and NPV were 100%. When it was compared to MAb-EIA, it had sensitivity and specificity of 82.6 and 100%, respectively (Phi = 0.863); PPV and

TABLE 1. Performance of the HerpeSelect Express Rapid Test in Comparison to HerpeSelect ELISA, Kalon Assay, and MAb-EIA

	HerpeSelect Express								
	Positive *N* (%)	Negative *N* (%)	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*P*-value	Phi
HerpeSelect ELISA									
Positive	19 (95)	1 (5)	20	95	100	100	97.6	<0.001	0.963[a]
Negative	0 (0)	40 (100)	40						
Total	19	41	60						
Kalon									
Positive	19 (100)	0 (0)	19	100	100	100	100	<0.001	1[a]
Negative	0 (0)	41 (100)	41						
Total	19	41	60						
MAb-EIA									
Positive	19 (82.6)	4 (17.4)	23	82.6	100	100	90.2	<0.001	0.863[a]
Negative	0 (0)	37 (100)	37						
Total	19	41	60						

[a]The high values of Phi indicate good correlation between HerpeSelect Express rapid test and each of the three ELISA assays.

TABLE 2. Patients With Equivocal Results of HerpeSelect or Kalon ELISA

Patient no.	HerpeSelect ELISA	Kalon ELISA	HerpeSelect Express
2	1.09	0.98	Negative
22	0.9	1.07	Negative
32	0.98	0.92	Negative
51	0.93	1.04	Negative
59	0.9	0.86	Negative

NPV were 100 and 90.24%, respectively. The agreement between the HerpeSelect Express rapid test with the three ELISA assays was 98.3, 100, and 93.3% for the HerpeSelect ELISA, Kalon, and MAb EIA, respectively.

In this study, we recorded five patients with equivocal results by HerpeSelect and/or Kalon ELISA. Interestingly, all of the five patients were negative for the HerpeSelect express rapid test (Table 2).

DISCUSSION

The estimated seropositivity of HSV-2 antibodies in our study (38.3%) was in concordance with that obtained by Sen et al. in North India (33.5%) (18) but higher than that obtained by Ghazi et al. in Saudi Arabia, who estimated seropositivity of 27.1% (19). This could be due to regional and sociodemographic differences among the studies' groups.

This study showed high sensitivity (ranging from 82.6 to 100%) and specificity (100%) of the HerpeSelect Express rapid test when compared to the three ELISA assays. The agreement between the HerpeSelect Express rapid test and the three ELISAs ranged from 93.3 to 100%.

It was noticed that the five patients with equivocal results for HerpeSelect and/or Kalon ELISA assays were negative for the HerpeSelect rapid test. A previous study revealed that samples with equivocal results by the HerpeSelect ELISA were shown to be negative by WB. Thus, the categorization of samples with equivocal results as "negative" seems to be the most logical decision (20). However, it cannot be ruled out that a number of equivocal samples perhaps associated with seroconversion, as the HerpeSelect assay has been shown to detect seroconversion earlier than other tests (12, 21). The choice of HSV-2 serological assays needs to be dictated by circumstances, whether for an epidemiological study examining risk factors for HSV-2 or a clinical trial seeking to enroll HSV-2-seropositive patients, for which highly specific assays would be more desirable, or for the diagnosis and management of patients with possible early HSV-2 infection, for which a sensitive assay would be required to offer appropriate advice on management and counseling about the risk of HIV acquisition (7, 22).

Data from this study suggested a good correlation between the HerpeSelect Express rapid test and HerpeSelect ELISA and Kalon ELISA, and adequate correlation with MAb-EIA in Qassim region's HSV-2-infected patients. In conclusion, the HerpeSelect Express rapid test has adequate sensitivity and specificity for confirming HSV-2 infection in patients with GUD. This test can also be a useful screening test for HSV-2 infection in high-risk individuals to achieve early and better management and prevention of transmission. Further studies are required to assess the performance of the HerpeSelect Express rapid

test in large series of patients and to assess it as a screening test in epidemiological studies for HSV-2 infection in asymptomatic patients.

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CONFLICT OF INTEREST

There was not any potential conflict of interest in this paper.

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