Journal of Clinical Virology 111 (2019) 33–38

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Performance of four diagnostic assays for detecting herpes simplex virus type 2 antibodies in the Middle East and North Africa

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ARTICLE INFO

Keywords:
HSV-2
Serology
Middle East and North Africa
Cohen's kappa statistic
Sensitivity
Specificity
Concordance measures

ABSTRACT

Background: Assessments of commercial assays in detecting herpes simplex virus type 2 (HSV-2) antibodies have shown variable sensitivity and specificity, and variation in performance by global population.
Objective: To evaluate performance of four assays in detecting HSV-2 antibodies in a composite Middle Eastern and North African (MENA) population. The assays are two ELISA kits: HerpeSelect® 2 ELISA IgG and Euroimmun Anti-HSV-2 (gG2) ELISA (IgG), and two immunoblot (IB)/Western blot (WB) assays: HerpeSelect® 1 and 2 Immunoblot IgG and Euroimmun Anti-HSV-1/HSV-2 gG2 Euroline-WB (IgG/IgM).
Study design: Blood specimens were drawn from blood donors between 2013–2016 in Doha, Qatar. Twenty specimens from ten nationalities (Egypt, Iran, Jordan, Lebanon, Pakistan, Palestine, Qatar, Sudan, Syria, and Yemen; total = 200) were randomly selected and tested for HSV-2 antibodies.
Results: In the six possible assay comparisons, Cohen's kappa statistics indicated fair to good agreement, ranging between 0.57 (95% CI 0.28-0.86) and 0.69 (95% CI 0.44-0.95). Meanwhile, positive percent agreement ranged between 50.0 (95% CI 18.7–81.3%) and 63.6% (95% CI 30.8–89.1%); negative percent agreement ranged between 97.8% (95% CI 94.4–99.4%) and 99.5% (95% CI 97.0–100.0%); and overall percent agreement ranged between 95.8% (95% CI 91.9–97.9%) and 97.5% (95% CI 94.2–98.9%). The two ELISA kits demonstrated comparable sensitivities and specificities ≥50% and > 98%, respectively, with respect to the IB/WB assays.
Conclusion: The study provided, for the first time, primary data on performance of these assays in diagnosing HSV-2 infection in MENA populations. Findings support comparable performance and utility of these assays, and demonstrate challenges in establishing seropositivity (versus seronegativity).

1. Background

With nearly 20 million new infections every year and an estimated 400 million persons infected worldwide [1]{Looker, 2015 #1;Dargham, 2018 #33}, herpes simplex virus type 2 (HSV-2) continues to be one of the most common sexually transmitted infections and a global public health concern [2,3]. HSV-2 infection is mostly asymptomatic and non-curable once acquired [4,5], and is a main cause of genital ulcer disease worldwide [2,3,6,7]. The infection has been also implicated in enhancing HIV transmission, particularly in sub-Saharan Africa [8,9]. Despite the global interest in this infection including the ongoing development of prophylactic and therapeutic HSV-2 vaccines [10,11], the availability of low-cost, accessible, and reliable diagnostic assays for HSV-2 antibody detection is still a pressing need.

Different commercial assays have been developed to detect antibodies against HSV-2, and more precisely, antibodies to the type-2 specific glycoprotein, G-2 (gG-2) [12]. Assessments of these assays have shown a range of sensitivity and specificity outcomes [13–16], when compared to gold standard tests offered in academic or reference laboratory settings, such as the University of Washington (UW) Western blot (WB), or the monoclonal antibody blocking assay at the Central Public Health Laboratory in London [12,17]. In addition, individual commercial tests have been shown to perform differently in populations from different geographic locations [13–16].

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https://doi.org/10.1016/j.jcv.2019.01.001
Received 13 September 2018; Received in revised form 22 November 2018; Accepted 7 January 2019

2. Objectives

We recently assessed the performance of commercial assays in detecting herpes simplex virus type 1 (HSV-1) antibodies in a composite population derived from different Middle Eastern and North African (MENA) nationalities [18]. Though several studies have used tests that are not well characterized to estimate HSV-2 prevalence in MENA populations [19], no study, to our knowledge, has investigated the diagnostic performance of commercial tests in detecting HSV-2 antibodies in this region. Our aim in this study was to use a similar methodology to that described previously for HSV-1 test evaluation [18], to compare the diagnostic performance of four widely available commercial tests in detecting HSV-2 antibodies. We further aimed to assess the diagnostic performance of two enzyme-linked immunosorbent assays (ELISA) kits against two HSV-2 immunoblot (IB) or IB/WB assays, with the latter assays treated here as reference assays, to consider their possible use in confirming ELISA results. Accordingly, we provide, to our knowledge for the first time, an assessment of well-characterized, widely available HSV-2 type-specific commercial tests in detecting HSV-2 antibodies within a MENA population.

3. Study design

Study methodology followed closely that developed recently to assess the performance evaluation of commercial tests in detecting HSV-1 antibodies, as described previously by Aldisi et al. 2018 [18].

3.1. Study population

The study sample set was selected from a total of 4525 blood specimens previously drawn for other studies [20–23], between June 2013 and June 2016. Specimens were anonymously drawn from voluntary blood donors at Hamad Medical Corporation, the national healthcare organization in Qatar, and serum aliquots stored, frozen. These donors were men, ≥18 years of age (median of 37.0 years and range of 19.0–63.0 years), and were Qatari citizens or expatriates from different countries of the MENA region, who were mostly recent residents in Qatar [24,25].

Twenty frozen, archived sera were randomly selected from donors from each of ten countries (Egypt, Iran, Jordan, Lebanon, Pakistan, Palestine, Qatar, Sudan, Syria, and Yemen), to provide a total of 200 specimens—a sample broadly representative of the overall MENA population. Informed by previous studies in the literature [16,26,27], this sample size was deemed feasible and reasonable to estimate comparison metrics with acceptable confidence interval width, in particular Cohen's Kappa statistic [28]. Of notice that HSV-2 seroprevalence is similar across these national populations [25]. The research protocol met the ethical standards and was approved by the research committees in Qatar University, Hamad Medical Corporation, and Weill Cornell Medicine-Qatar.

3.2. Antibody detection

Four HSV-2 type-specific antibody tests were used. Two of these were ELISAs: 1) HerpeSelect® 2 ELISA IgG (Ref No. EL0920 G, Focus Diagnostics, USA) [29], and 2) Euroimmun Anti-HSV-2 (gG2) ELISA IgG (Ref No. EI 2532-9601-2 G, Euroimmun, Germany) [30]. HerpeSelect® 2 ELISA provides qualitative measurements [29], while Euroimmune Anti-HSV-2 ELISA provides qualitative or semi-quantitative measurement [30], for HSV-2 IgG antibodies that are reacting with the HSV-2 purified recombinant gG2 antigen.

The remaining two HSV-2 diagnostic assays used either IB or combined IB/WB formats: 1) HerpeSelect® 1 and 2 Immunoblot IgG (Ref. No. IB0900 G, Focus Diagnostic, USA) [31,32], and 2) Euroimmun Anti-HSV-1/HSV-2 gG2 Euroline-WB (IgG/IgM) (Ref. No. DY 2531-1 G, Euroimmun, Germany) [33,34]. The HerpeSelect® IB assay strip contains purified type-specific proteins for HSV-2 gG2 and HSV-1 gG-1, as well as a common protein mixture [31,32]. The Euroline-WB assay included strips of HSV-1 proteins that had been denatured, electrophoretically separated and subsequently transferred to paper strips ("WB") [33]. Glycoprotein gG-2 was then applied to a separate area on the Western blots, for the HSV-2 IB portion of the test [33].

Laboratory testing and interpretation of results were conducted following the manufacturers' instructions. For both ELISA assays, the results were obtained by photometric measurements of the reaction at a wavelength of 450 nm. The change in the color intensity after adding the stop reagent was measured by its optical density using Epoch 2 microplate spectrophotometer (BioTek instruments, U.S.A). Then, the index values were calculated by dividing the optical densities of the controls or specimens by the average of the cut-off calibrator absorbance values. For HerpeSelect® 2 ELISA, sera with index values of < 0.90 were considered anti-HSV-2 negative, > 1.10 were considered anti-HSV-2 positive, and inclusive values between 0.90 and 1.10 were considered anti-HSV-2 equivocal [29]. For Euroimmun Anti-HSV-2 ELISA, sera with optical density index values < 0.80 were considered anti-HSV-2 negative, ≥1.10 were considered anti-HSV-2 positive, and between 0.80 and 1.10 were considered anti-HSV-2 equivocal [30].

For HerpeSelect® IB, the produced band intensity of the tested sera was evaluated visually against bands produced from the positive and negative controls [31]. Each resulted band on the immunoblot strips was compared relative to the provided reading control band, which is a gG-2 band on the cutoff/positive control strip. Bands darker than the reading control band were considered positive while lighter bands were considered negative. For Euroline-WB, results were assessed using a EurolineScan software scanner [33]. The software was used to scan and digitally evaluate the strips according to the presence and intensity of clear recognizable bands. Wet blot strips were placed on a green sheet of paper (for maximum resolution) and scanned using a scanner connected to the software. The EUROLineScan is able to measure band intensities, and according to the number of units each band produces, it is categorized into either positive, negative, or borderline. Negative results were ≤12 units, borderline 13–20 units, and positive results correlated with ≥20 units.

3.3. Statistical analysis

The four assays were compared to each other by cross tabulating the results to provide six combinations of contingency tables. Equivocal outcomes were not included in analysis. The following comparison metrics were calculated: positive, negative, and overall percent agreements and Cohen's kappa statistic. The latter metric provides a robust and standard statistical measure of the degree of agreement (beyond chance) between any two diagnostic methods [28]. With a range of values between 0 and 1, Cohen's kappa statistic ≥0.75 indicates excellent agreement, 0.40-0.75 indicates fair to good agreement, and < 0.40 indicates poor agreement [18,28].

Treating each of HerpeSelect® IB and Euroline-WB as a reference standard for assessing the performance of the two ELISA kits, the following additional comparison metrics were calculated: sensitivity, specificity, and positive and negative predictive values.

Statistical significance was assumed at 5%, and a 95% confidence interval (CI) was estimated for each metric. All calculations were performed using Microsoft Excel 2016.

4. Results

All 200 MENA blood specimens were tested for HSV-2 antibodies using HerpeSelect® 2 ELISA, Euroimmun Anti-HSV-2 ELISA, and Euroline-WB, while 197 sera were tested using HerpeSelect® IB, as three specimens had insufficient volume. HSV-2 seropositivity was estimated at 5.0% (10/200; 95% CI 2.4–9.0%) by HerpeSelect® 2 ELISA, 3.5% (7/

Table 1
Results of the four assays of HerpeSelect® 2 ELISA, Euroimmun Anti-HSV-2 ELISA, HerpeSelect® IB, and Euroline-WB, for all specimens for which at least one assay detected positive or equivocal HSV-2 antibodies.

Specimen	HerpeSelect® 2 ELISA	Euroimmun Anti-HSV-2 ELISA	HerpeSelect® IB	Euroline-WB
1	POS	EQUI	POS	POS
2	NEG	NEG	EQUI	NEG
3	NEG	NEG	EQUI	NEG
4	NEG	NEG	NEG	EQUI
5	NEG	NEG	EQUI	NEG
6	NEG	NEG	EQUI	NEG
7	NEG	EQUI	NEG	NEG
8	NEG	EQUI	NEG	NEG
9	NEG	NEG	EQUI	NEG
10	NEG	NEG	EQUI	NEG
11	POS	NEG	NEG	NEG
12	NEG	NEG	POS	NEG
13	NEG	NEG	NEG	POS
14	NEG	NEG	NEG	POS
15	NEG	POS	NEG	NEG
16	POS	NEG	NEG	NEG
17	NEG	NEG	POS	NEG
18	NEG	NEG	POS	NEG
19	POS	NEG	POS	NEG
20	NEG	POS	NEG	POS
21	NEG	NEG	POS	POS
22	POS	NEG	NEG	POS
23	POS	POS	POS	POS
24	POS	POS	POS	POS
25	POS	POS	POS	POS
26	POS	POS	POS	POS
27	POS	POS	POS	POS

POS – Positive, NEG – Negative, EQUI – Equivocal.

200; 95% CI 1.4–7.1%) by Euroimmun Anti-HSV-2 ELISA, 5.6% (11/197; 95% CI 2.8–9.8%) by HerpeSelect® IB, and 5.5% (11/200; 95% CI 2.8–9.6%) by Euroline-WB.

Testing identified 27 specimens with at least one of the four assays detecting positive or equivocal HSV-2 antibodies (Table 1). Testing identified a total of ten specimens with an equivocal outcome (three by Euroimmun Anti-HSV-2 ELISA, one by Euroline-WB, six by HerpeSelect® IB, and none by more than one test). A total of 187 specimens had no equivocal results on any of the diagnostic assays. Among these 187 sera, eight (4.3%) specimens were tested positive for HSV-2 antibodies by only one of the four assays, four (2.1%) specimens were positive in combinations of two assays, none were positive in combinations of three assays, and five (2.7%) were positive and 170 (90.9%) were negative in all four assays. Of note that one specimen was tested positive by three diagnostic assays, but the remaining fourth assay reported an equivocal result.

The six cross-tabulations performed comparing the four assays are presented in Fig. 1. Results of the concordance assessment between the four diagnostic assays are reported in Table 2. Cohen's kappa measure was within the range of 0.40-0.75 for all six comparisons, thus indicating fair to good agreement. The highest and lowest Cohen's kappa measures were reported for the comparisons between Euroimmun Anti-HSV-2 ELISA and Euroline-WB (kappa = 0.69, 95% CI 0.44-0.95), and between Euroimmun Anti-HSV-2 ELISA and HerpeSelect® IB (kappa = 0.57, 95% CI 0.28-0.86), respectively.

The highest and lowest overall percent agreements were estimated at 97.5% (95% CI 94.2–98.9%) for the Euroline-WB and Euroimmun Anti-HSV-2 ELISA comparison, and 95.8% (95% CI 91.9–97.9%) for the Euroline-WB and HerpeSelect® IB comparison, respectively. The highest and lowest negative percent agreements were estimated at 99.5% (95% CI 97.0–100.0%) for the Euroline-WB and Euroimmun Anti-HSV-2 ELISA comparison, and 97.8% (95% CI 94.4–99.4%) for the Euroline-WB and HerpeSelect® IB comparison, respectively. The highest and lowest positive percent agreements were estimated at 63.6% (95% CI 30.8–89.1%) for the HerpeSelect® 2 ELISA and HerpeSelect® IB, and also for the HerpeSelect® 2 ELISA and Euroline-WB and the HerpeSelect® IB and Euroline-WB comparisons; and 50.0% (95% CI 18.7–81.3%) for the Euroimmun Anti-HSV-2 ELISA and HerpeSelect® IB comparison, respectively.

Performance of the HerpeSelect® 2 ELISA and the Euroimmun Anti-HSV-2 ELISA kits in detecting HSV-2 antibodies was evaluated with respect to the IB/WB assays, treated as reference standards. Results of the four diagnostic assessments are reported in Table 3. Both overall percent agreement and specificity for all comparisons were estimated at > 96% and > 98%, respectively, while sensitivity ranged between 50.0% and 63.6%. Positive and negative predictive values were estimated at ≥70% and > 97%, respectively.

Against HerpeSelect® IB, HerpeSelect® 2 ELISA demonstrated a sensitivity of 63.6% (7/11; 95% CI 30.8–89.1%) and a specificity of 98.3% (177/180; 95% CI 95.2–99.7%), while Euroimmun Anti-HSV-2 ELISA demonstrated a sensitivity of 50.0% (5/10; 95% CI 18.7–81.3%) and a specificity of 98.9% (176/178; 95% CI 96.0–99.9%). Against Euroline-WB, HerpeSelect® 2 ELISA demonstrated a sensitivity of 63.6% (7/11; 95% CI 30.8–89.1%) and a specificity of 98.4% (185/188; 95% CI 95.4–99.7%), while Euroimmun Anti-HSV-2 ELISA demonstrated a sensitivity of 60.0% (6/10; 95% CI 26.2–87.8%) and a specificity of 99.5% (185/186; 95% CI 97.0–100%).

5. Discussion

Four widely available commercial ELISA and IB/WB assays were examined for the performance of detecting HSV-2 antibodies in a composite population from the MENA region. Each of these tests has been compared (in existing literature) with accepted gold standard tests for sensitivity and specificity; usually, but not exclusively, among populations with relatively high HSV-2 seroprevalence [12–16,26,32,34]. Compared to each other, the four tests demonstrated fair to good agreement with a Cohen's kappa statistic of about 0.6 across the six comparisons. While the overall and negative percent agreements were high (> 95%), the positive percent agreement was imperfect at about 60%.

Compared to the two IB/WB assays, the specificity of the two ELISA kits was high (> 98%), but the sensitivity was low at about 60%—contrary to what one would have desired for such less expensive and automated ELISA tests. While these results support the comparable performance and utility of these four assays, they also affirm known challenges with commercial assays in the reliable detection of HSV-2 antibodies, such as in terms of the positive predictive value [13,14,16,35–38], in both low HSV-2 prevalence areas like the MENA region [3,19,25,39], and high HSV-2 prevalence populations elsewhere [3,13–16,39].

The results further highlight that the context in which a study is done; for example a low versus high HSV-2 prevalence setting, can affect considerably the interpretation of the results of the serology testing. Indeed, for clinical diagnosis, the results indicate that the usual confirmatory algorithm of inexpensive fast high sensitivity/low specificity test to screen, followed by more expensive, more time intensive confirmatory test to rule out false positives [35,40], does not appear to work well in this studied low-seroprevalence MENA population.

Given existing evidence for variation in anti-HSV-2 assay performance by geographic location of the tested population [13–16], our results provide useful data, for the first time, for the performance of these assays in a MENA population. Given the finding of unexpected (apparently) low sensitivity for the two ELISA tests, our data demonstrate the need to develop a multiple test algorithms in identifying HSV-2 antibody positivity, particularly in such low HSV-2 seroprevalence populations, as suggested earlier [16,26,37]. The findings suggest also that caution should be used in interpreting any of the four tests results, in isolation, until more extensive testing against gold standards is done.

We recently evaluated the performance of the HSV-1 versions of

A)

Euroimmun Anti-HSV-2 ELISA	Herpeselect® 2 ELISA Pos	Neg	Total
Pos	5	2	7
Neg	4	186	190
EQUI	1	2	3
Total	10	190	200

B)

Euroimmun Anti-HSV-2 ELISA	HerpeSelect® IB Pos	Neg	EQUI	NS	Total
Pos	5	2	0	0	7
Neg	5	176	6	3	190
EQUI	1	2	0	0	3
Total	11	180	6	3	200

C)

Herpeselect® 2 ELISA	Euroline-WB Pos	Neg	EQUI	Total
Pos	7	3	0	10
Neg	4	185	1	190
Total	11	188	1	200

D)

Herpeselect® 2 ELISA	HerpeSelect® IB Pos	Neg	EQUI	NS	Total
Pos	7	3	0	0	10
Neg	4	177	6	3	190
Total	11	180	6	3	200

E)

Euroimmun Anti-HSV-2 ELISA	Euroline-WB Pos	Neg	EQUI	Total
Pos	6	1	0	7
Neg	4	185	1	190
EQUI	1	2	0	3
Total	11	188	1	200

F)

Euroline-WB	HerpeSelect® IB Pos	Neg	EQUI	NS	Total
Pos	7	4	0	0	11
Neg	4	175	6	3	188
EQUI	0	1	0	0	1
Total	11	180	6	3	200

Pos - Positive; Neg - Negative; EQUI - Equivocal; NS - Not enough blood sample

Fig. 1. Results of the six cross-tabulations performed to compare the four assays of HerpeSelect® 2 ELISA, Euroimmun Anti-HSV-2 ELISA, HerpeSelect® IB, and Euroline-WB, for detecting HSV-2 antibodies.

these commercial assays for detecting HSV-1 antibodies [18], an infection of very high seroprevalence in MENA [24,41], unlike that of HSV-2 [19,25]. Notably, the assays showed excellent agreement with one another and in all comparison metrics for detecting HSV-1 antibodies [18], unlike the case for detecting HSV-2 antibodies (Tables 1 and 2). This further highlights the need for cautious interpretation of HSV-2 serology measures in particular, and in all global populations.

This study has limitations. First, we treated HerpeSelect® IB and Euroline-WB as reference standards, because of their unique test formats, but could not use, for cost and logistical reasons, one of several widely recognized reference standards such as the UW-WB [12], the immunodot enzyme assay [42–44], or the monoclonal antibody inhibition test used in the United Kingdom [12]. Next, HSV-2 seroprevalence was low (about 5%), as it is low in MENA populations [19,25], but such low seroprevalence resulted in wide confidence intervals for several of the comparison metrics, as most metrics, such as Cohen's kappa statistic, depend on infection seroprevalence [45]. Furthermore, it is difficult to determine whether observed results are due to regionally-specific factors, the small number of positive samples, the differences in performance of the kits, or the reference standards selected. However, given existing evidence that shows significant variations in the sensitivities in HSV-2 serology testing across different diagnostics [13–16], we are inclined to believe that this could be the main cause of the differences between the assays. To clarify this better in future research, larger sample sizes should be used in consideration of the low HSV-2 seroprevalence.

Further, we used existing sera from other studies [20–23], which was collected from men, thus preventing us from assessing sex-based

Table 2
Results of the concordance assessment between the four diagnostic assays of HerpeSelect® 2 ELISA, Euroimmun Anti-HSV-2 ELISA, HerpeSelect® IB, and Euroline-WB, in detecting HSV-2 antibodies.

	Cohen's Kappa	Overall Percent Agreement	Positive Percent Agreement	Negative Percent Agreement
HerpeSelect® 2 ELISA & Euroimmun Anti-HSV-2 ELISA	0.61 (0.32-0.90)	97.0 (93.5-98.6)	55.56 (21.2-86.3)	98.9 (96.2-99.9)
HerpeSelect® 2 ELISA & HerpeSelect® IB	0.65 (0.40-0.89)	96.3 (92.6-98.2)	63.6 (30.8-89.1)	98.3 (95.2-99.7)
HerpeSelect® 2 ELISA & Euroline-WB	0.65 (0.40-0.89)	96.5 (92.9-98.3)	63.6 (30.8-89.1)	98.4 (95.4-99.7)
Euroimmun Anti-HSV-2 ELISA & HerpeSelect® IB	0.57 (0.28-0.86)	96.3 (92.5-98.2)	50.0 (18.7-81.3)	98.9 (96.0-99.9)
Euroimmun Anti-HSV-2 ELISA & Euroline-WB	0.69 (0.44-0.95)	97.5 (94.2-98.9)	60.0 (26.2-87.8)	99.5 (97.0-100)
HerpeSelect® IB & Euroline-WB	0.61 (0.37-0.86)	95.8 (91.9-97.9)	63.6 (30.8-89.1)	97.8 (94.4-99.4)

Table 3
Performance assessment of the HerpeSelect® 2 ELISA and the Euroimmun Anti-HSV-2 ELISA kits in detecting HSV-2 antibodies with respect to the HerpeSelect® IB and Euroline-WB assays, treated as reference standards.

Name of Reference Standard	Name of ELISA Kit	Overall Percent Agreement % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive Predictive Value % (95% CI)	Negative Predictive Value % (95% CI)
HerpeSelect® IB	**HerpeSelect® 2 ELISA**	96.4 (92.6-98.2)	63.6 (30.8-89.1)	98.3 (95.2-99.7)	70.0 (39.7-89.2)	97.8 (94.5-99.1)
	Euroimmun Anti-HSV-2 ELISA	96.3 (92.5-98.2)	50.0 (18.7-81.3)	98.9 (96.0-99.9)	71.4 (35.9-91.8)	97.2 (93.7-98.8)
Euroline-WB	**HerpeSelect® 2 ELISA**	96.5 (92.9-98.2)	63.6 (30.8-89.1)	98.4 (95.4-99.7)	70.0 (39.7-89.2)	97.9 (94.7-99.2)
	Euroimmun Anti-HSV-2 ELISA	97.5 (94.2-98.9)	60.0 (26.2-87.8)	99.5 (97.0-100)	85.7 (48.7-97.4)	97.9 (94.7-99.2)

differences in assay performance—of note that women have a higher seroprevalence than men, because of higher biological susceptibility [3,39]. Finally, though our sample included populations from ten different MENA nationalities to be broadly representative of MENA populations, we did not have specimens from all of the 23 different MENA nationalities.

In conclusion, performance of four common commercial assays in detecting HSV-2 antibodies was analyzed in a composite sample of ten MENA national populations, to inform design of epidemiological studies for HSV-2 infection. The four assays demonstrated excellent negative and overall concordance with each other. The two ELISA kits were also comparable in their diagnostic performance when compared to the IB/WB assays. However, positive concordance between the assays was imperfect, leading to a fair to good Cohen's kappa statistic. Development and use of multiple test algorithms may provide the most accurate test results for HSV-2 in MENA populations, to optimize sensitivity and specificity. Meanwhile, these findings suggest caution in future use of these assays, alone, for HSV-2 antibody detection, in both epidemiologic scientific studies and clinical settings.

Conflict of interest

All authors declare that we have no conflict of interest to disclose.

Disclose funding received for this work

The authors acknowledge funding received from the following resources 1. GKN: Student grant No. QUST-CHS-SPR-15/16-7 from Qatar University, Qatar and UREP18-001-3-001 from Qatar National Research Fund, a member of Qatar Foundation (QF), Qatar. 2. LJA: NPRP9-040-3-008 from Qatar National Research Fund, a member of Qatar Foundation (QF), Qatar.

Acknowledgements

The authors gratefully acknowledge Professor Emeritus Rhoda Ashley Morrow, from the University of Washington, for a critical review of this manuscript. The authors are also grateful for the administrative support of Adona Canlas and Enas S. Al-Absi, and the laboratory support of the following students: Rana S. Aldisi, Mariam Y. Nofal, Layla I. Mohammed, and Somaya Harche. The author roles in the project was as follow: Conceptualization: SRD and LJA, Data curation: GKN, Formal analysis: SRD, MSE, ASS, Funding acquisition: LJA and GKN, Methodology: MSE and ASS, and GKN. Project administration: GKN, Resources: GKN and LJA, Software: LJA, Supervision: GKN, Writing the original draft: SRD. Review and editing: all authors. The findings achieved herein are solely the responsibility of the authors.

References

[1] K.J. Looker, A.S. Magaret, M.T. May, K.M. Turner, P. Vickerman, S.L. Gottlieb, et al., Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012, PLoS One 10 (2015) e0140765.
[2] J. Smith, N. Robinson, Age-SPecific prevalence of infection with herpes simplex virus types 2 and 1: a global review, J. Infect. Dis. 186 (2002) S3–S28.
[3] H. Weiss, Epidemiology of herpes simplex virus type 2 infection in the developing world, Herpes 11 (Suppl 1) (2004) 24A–35A.
[4] K.E. Mark, A. Wald, A.S. Magaret, S. Selke, L. Olin, M.L. Huang, et al., Rapidly cleared episodes of herpes simplex virus reactivation in immunocompetent adults, J. Infect. Dis. 198 (2008) 1141–1149.
[5] R. Gupta, T. Warren, A. Wald, Genital herpes, Lancet. 370 (2007) 2127–2137.
[6] K.K. Holmes, Sexually Transmitted Diseases, 4th ed., McGraw-Hill Medical, New York, 2008.
[7] S.A. Morse, Etiology of genital ulcer disease and its relationship to HIV infection, Sex. Transm. Dis. 26 (1999) 63–65.
[8] L.J. Abu-Raddad, A.S. Magaret, C. Celum, A. Wald, I.M. Longini Jr., S.G. Self, et al., Genital herpes has played a more important role than any other sexually transmitted infection in driving HIV prevalence in Africa, PLoS One 3 (2008) e2230.
[9] K.J. Looker, J.A.R. Elmes, S.L. Gottlieb, J.T. Schiffer, P. Vickerman, K.M.E. Turner, et al., Effect of HSV-2 infection on subsequent HIV acquisition: an updated systematic review and meta-analysis, Lancet Infect. Dis. 17 (2017) 1303–1316.
[10] S.L. Gottlieb, C.D. Deal, B. Giersing, H. Rees, G. Bolan, C. Johnston, et al., The global roadmap for advancing development of vaccines against sexually transmitted infections: update and next steps, Vaccine 34 (2016) 2939–2947.
[11] S.L. Gottlieb, B. Giersing, M.C. Boily, H. Chesson, K.J. Looker, J. Schiffer, et al., Modelling efforts needed to advance herpes simplex virus (HSV) vaccine development: Key findings from the World Health Organization Consultation on HSV Vaccine Impact Modelling, Vaccine. (2017).
[12] R.L. Ashley, Sorting out the new HSV type specific antibody tests, Sex. Transm. Infect. 77 (2001) 232–237.
[13] R. Ashley-Morrow, J. Nollkamper, N.J. Robinson, N. Bishop, J. Smith, 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 (2004) 530–536.
[14] A. Mujugira, R.A. Morrow, C. Celum, J. Lingappa, S. Delany-Moretlwe, K.H. Fife, et al., Performance of the Focus HerpeSelect-2 enzyme immunoassay for the detection of herpes simplex virus type 2 antibodies in seven African countries, Sex. Transm. Infect. 87 (2011) 238–241.
[15] E. van Dyck, A. Buve, H.A. Weiss, J.R. Glynn, D.W. Brown, B. De Deken, et al., Performance of commercially available enzyme immunoassays for detection of antibodies against herpes simplex virus type 2 in African populations, J. Clin. Microbiol. 42 (2004) 2961–2965.
[16] S. Delany-Moretlwe, U. Jentsch, H. Weiss, J. Moyes, R. Ashley-Morrow, W. Stevens, et al., Comparison of focus HerpesSelect and Kalon HSV-2 gG2 ELISA serological assays to detect herpes simplex virus type 2 antibodies in a South African population, Sex. Transm. Infect. 86 (2010) 46–50.
[17] S. Biraro, P. Mayaud, R.A. Morrow, H. Grosskurth, H.A. Weiss, 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. Dis. 38 (2011) 140–147.
[18] R.S. Aldisi, M.S. Elsidiq, S.R. Dargham, A.S. Sahara, E.S. Al-Absi, M.Y. Nofal, et al., Performance evaluation of four type-specific commercial assays for detection of herpes simplex virus type 1 antibodies in a Middle East and North Africa population, J. Clin. Virol. 103 (2018) 1–7.
[19] L.J. Abu-Raddad, J.T. Schiffer, R. Ashley, G. Mumtaz, R.A. Alsallaq, F.A. Akala, et al., HSV-2 serology can be predictive of HIV epidemic potential and hidden sexual risk behavior in the Middle East and North Africa, Epidemics. 2 (2010) 173–182.
[20] R. AbuOdeh, N. Al-Mawlawi, A.A. Al-Qahtani, M.F. Bohol, M.N. Al-Ahdal, H.A. Hasan, et al., Detection and genotyping of torque teno virus (TTV) in healthy blood donors and patients infected with HBV or HCV in Qatar, J. Med. Virol. 87 (2015) 1184–1191.
[21] R.O. AbuOdeh, E. Al-Absi, N.H. Ali, M. Khalili, N. Al-Mawlawi, T.A. Hadwan, et al., Detection and phylogenetic analysis of human pegivirus (GBV-C) among blood donors and patients infected with hepatitis B virus (HBV) in Qatar, J. Med. Virol. 87 (2015) 2074–2081.
[22] A.A. Al-Qahtani, E.S. Alabsi, R. AbuOdeh, L. Thalib, M.E. El Zowalaty, G.K. Nasrallah, Prevalence of anelloviruses (TTV, TTMDV, and TTMV) in healthy blood donors and in patients infected with HBV or HCV in Qatar, Virol. J. 13 (2016) 208.
[23] G.K. Nasrallah, E.S. Al Absi, R. Ghandour, N.H. Ali, S. Taleb, L. Hedaya, et al.,

Seroprevalence of hepatitis E virus among blood donors in Qatar (2013-2016), Transfusion. 57 (2017) 1801–1807.
[24] G.K. Nasrallah, S.R. Dargham, L.I. Mohammed, L.J. Abu-Raddad, Estimating seroprevalence of herpes simplex virus type 1 among different Middle East and North African male populations residing in Qatar, J. Med. Virol. 90 (2018) 184–190.
[25] S.R. Dargham, G.K. Nasrallah, E.S. Al-Absi, L.I. Mohammed, R.S. Al-Disi, M.Y. Nofal, et al., Herpes simplex virus type 2 seroprevalence among different national populations of Middle East and north african men, Sex. Transm. Dis. 45 (2018) 482–487.
[26] B.R. Eing, L. Lippelt, E.U. Lorentzen, W. Hafezi, W. Schlumberger, K. Steinhagen, et al., Evaluation of confirmatory strategies for detection of type-specific antibodies against herpes simplex virus type 2, J. Clin. Microbiol. 40 (2002) 407–413.
[27] S.M. Reddy, P. Balakrishnan, S. Uma, S.P. Thyagarajan, S. Solomon, Performance of two commercial enzyme-linked immunosorbent assay kits using recombinant glycoprotein G2 antigen for detection of herpes simplex virus type 2 specific antibodies, Clin. Diagn. Lab. Immunol. 12 (2005) 359–360.
[28] J.L. Fleiss, B. Levin, M.C. Paik, The Measurement of Interrater Agreement. Statistical Methods for Rates and Proportions, Wiley, 2013, pp. 598–626.
[29] Focus Diagnostics, HerpeSelect® 2 ELISA IgG (English), (2011).
[30] EUROIMMUN, Anti-HSV-2 (gG2) ELISA (IgG, (2016).
[31] Focus Diagnostics, HerpeSelect® 1 and 2 Immunoblot IgG (English), (2011).
[32] A. Wald, R. Ashley-Morrow, Serological testing for herpes simplex virus (HSV)-1 and HSV-2 infection, Clin. Infect. Dis. 35 (2002) S173–82.
[33] EUROIMMUN, Anti-HSV-1/HSV-2-gG2 EUROLINE-WB (IgG/IgM), (2011).
[34] J.D. Neal, A.A. Tobian, O. Laeyendecker, T.D. Ngo, A.D. Redd, S.J. Reynolds, et al., Performance of the Euroline Western blot assay in the detection of herpes simplex virus type 2 antibody in Uganda, China and the USA, Int. J. STD AIDS 22 (2011) 342–344.
[35] M.R. Golden, R. Ashley-Morrow, P. Swenson, W.R. Hogrefe, H.H. Handsfield, A. Wald, Herpes simplex virus type 2 (HSV-2) Western blot confirmatory testing among men testing positive for HSV-2 using the focus enzyme-linked immunosorbent assay in a sexually transmitted disease clinic, Sex. Transm. Dis. 32 (2005) 771–777.
[36] J. Lingappa, E. Nakku-Joloba, A. Magaret, D. Friedrich, J. Dragavon, F. Kambugu, et al., Sensitivity and specificity of herpes simplex virus-2 serological assays among HIV-infected and uninfected urban Ugandans, Int. J. STD AIDS 21 (2010) 611–616.
[37] C. Feltner, C. Grodensky, C. Ebel, J.C. Middleton, R.P. Harris, M. Ashok, et al., Serologic screening for genital herpes: an updated evidence report and systematic review for the US preventive services task force, JAMA 316 (2016) 2531–2543.
[38] H.D. Mark, J.P. Nanda, J. Roberts, A. Rompalo, J.H. Melendez, J. Zenilman, Performance of focus ELISA tests for HSV-1 and HSV-2 antibodies among university students with no history of genital herpes, Sex. Transm. Dis. 34 (2007) 681–685.
[39] J.S. Smith, N.J. Robinson, Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review, J. Infect. Dis. 186 (Suppl 1) (2002) S3–28.
[40] R.A. Morrow, D. Friedrich, A. Meier, L. Corey, Use of "biokit HSV-2 Rapid Assay" to improve the positive predictive value of Focus HerpeSelect HSV-2 ELISA, BMC Infect. Dis. 5 (2005) 84.
[41] S. Chaabane, M. Harfouche, H. Chemaitelly, L. Abu-Raddad, The Epidemiology of Herpes Simplex Virus Type 1 Seroprevalence in the Middle-east and North Africa: a Systematic Review and Meta-analysis, Under review (2018).
[42] R.L. Ashley, J. Militoni, F. Lee, A. Nahmias, L. Corey, Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera, J. Clin. Microbiol. 26 (1988) 662–667.
[43] D.T. Fleming, G.M. McQuillan, R.E. Johnson, A.J. Nahmias, S.O. Aral, F.K. Lee, et al., Herpes simplex virus type 2 in the United States, 1976 to 1994, N. Engl. J. Med. 337 (1997) 1105–1111.
[44] F. Xu, M.R. Sternberg, B.J. Kottiri, G.M. McQuillan, F.K. Lee, A.J. Nahmias, et al., Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States, JAMA 296 (2006) 964–973.
[45] U.S. Department of Health and Human Services, Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests, Accessed on Sept 2017). 2007 Available at: https://www.fda.gov/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm071148.htm#top.