Journal of Clinical Virology 103 (2018) 1–7

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

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

Rana S. Aldisi[a,b], Malaz S. Elsidiq[a], Soha R. Dargham[c], Afifah S. Sahara[a], Enas S. Al-Absi[b], Mariam Y. Nofal[a], Layla I. Mohammed[b], Laith J. Abu-Raddad[c,d], Gheyath K. Nasrallah[a,b,*]

[a] Department of Biomedical Science, College of Health Sciences, Qatar University, Doha, Qatar
[b] BioMedical Research Center, Qatar University, Doha, Qatar
[c] Infectious Disease Epidemiology Group, Weill Cornell Medicine-Qatar, Cornell University, Qatar Foundation – Education City, Doha, Qatar
[d] Department of Healthcare Policy and Research, Weill Cornell Medicine, Cornell University, New York, USA

ARTICLE INFO

Keywords:
HSV-1
Serology
Concordance measures
Cohen's kappa statistic
Sensitivity
Specificity

ABSTRACT

Background: The number of diagnostic assays for the detection of herpes simplex virus type 1 (HSV-1) antibodies has increased over the years. However, their performance characteristics could vary among global populations.
Objective: To investigate performance of two commercial ELISA kits, HerpeSelect® 1 ELISA and Euroimmun Anti-HSV-1 (gC1) ELISA (IgG); and two commercial immunoblot (IB)/Western blot (WB) assays, HerpeSelect® 1 and 2 Immunoblot IgG, and Euroimmun Anti-HSV-1/HSV-2 gG2 Euroline-WB (IgG/IgM); in detecting HSV-1 antibodies in a Middle East and North Africa (MENA) population.
Study design: Blood specimens were collected from blood donors in Doha, Qatar, June 2013–2016. Twenty specimens were randomly selected from 10 MENA nationalities (Egypt, Iran, Jordan, Lebanon, Pakistan, Palestine, Qatar, Sudan, Syria, and Yemen; total = 200), and tested for HSV-1 antibodies.
Results: Across all six comparisons between assays, positive percent agreement ranged between 95.7% (95% CI: 91.4–98.3%) and 100.0% (95% CI: 97.8–100.0%). Negative percent agreement ranged between 86.2% (95% CI: 68.3-96.1%) and 96.2% (95% CI: 80.4–99.9%). Overall percent agreement ranged between 95.7% (95% CI: 91.7–97.8%) and 99.4% (95% CI: 96.7–99.9%). Cohen's kappa statistic ranged between 0.84 (95% CI: 0.73–0.95) and 0.98 (95% CI: 0.93–1.00). Compared against IB/WB, HerpeSelect® and Euroimmun had sensitivities and specificities > 96% and > 86%, respectively. Positive and negative predictive values were > 97% and > 83%, respectively.
Conclusion: The assays showed excellent concordance with one another, and with a high kappa statistic. The ELISA kits demonstrated robust diagnostic performance compared to the IB/WB assays. These findings support the assays' utility in clinical diagnosis and research in MENA populations.

1. Background

Herpes simplex virus type 1 (HSV-1) is one of the most prevalent (mostly asymptomatic) infections worldwide [1,2]. Infections with HSV-1 are associated with oral, ocular, cutaneous, and central nervous system manifestations, and can result in mild to severe morbidities such as gingivostomatitis, neonatal herpes, corneal blindness, meningitis, and encephalitis [3,4]. Although HSV-1 infection is commonly associated with orolabial herpes, evidence indicates a growing role for HSV-1 as a sexually transmitted infection (STI) and as a cause of genital herpes, even surpassing the role of HSV-2 for incident genital herpes in some settings [5–7].

The World Health Organization and other global partners have embarked on multiple activities to accelerate the global roadmap for STI vaccine development with a focus on an HSV vaccine [8,9]. In particular, a business case for HSV vaccines is being developed factoring global public health need, vaccines' potential impact, pathways of HSV vaccine implementation, anticipated cost-effectiveness, and return on investment [8]. To advance this global effort, it is essential to quantify infection levels for both HSV-1 and HSV-2 infections. Therefore, there is a need to have valid, reliable, and affordable diagnostic assays for the detection of HSV-1 antibodies among different global

* Corresponding author at: Department of Biomedical Science, College of Health Sciences, Qatar University, P.O. Box 2713, Doha, Qatar.
E-mail address: gheyath.nasrallah@qu.edu.qa (G.K. Nasrallah).

https://doi.org/10.1016/j.jcv.2018.03.011
Received 24 October 2017; Received in revised form 2 March 2018; Accepted 21 March 2018

populations.

The biologic and antigenic distinction between HSV-1 and HSV-2 was first described in the 1960s [10,11]. Although the viruses share 83% of their genome and more than 85% of their protein profile, they have a prominent antigenic difference in their envelope glycoprotein G expressed on the surface of the virion, glycoprotein G-1 (gG-1) and G-2 (gG-2), respectively [12]. Epitope mapping studies have shown that despite amino acid sequence similarities between gG from HSV-1 and HSV-2, functional antibodies against HSV-1 epitopes do not recognize gG from HSV-2 [13]. It has been also suggested that glycoprotein gC from HSV-1 may also be antigenically distinct from the gC of HSV-2 [14].

The number of type-specific commercial enzyme-linked immunosorbent assays (ELISA) and immunoblot (IB)/Western blot (WB) diagnostic assays for the detection of HSV-1 antibodies has increased over the years [15–19]. While these assays are a mainstay of clinical diagnosis and scientific research, their performance characteristics can vary among different global populations [20,21]. To our knowledge, no study has investigated the performance of such commercially-available diagnostic assays in the detection of HSV-1 antibodies in Middle East and North Africa (MENA) populations.

2. Objectives

With a large proportion of the population coming from other MENA countries, Qatar provides an opportune setting for comparing and evaluating different type-specific HSV-1 antibody diagnostic assays among MENA populations. Our main objective was to compare the performance of four commonly-used and commercially-available assays in detecting HSV-1 antibodies in a composite population derived from MENA countries. Specifically, we investigated and compared the concordance of HerpeSelect® 1 ELISA, Euroimmun Anti-HSV-1 (gC1) ELISA (IgG), HerpeSelect® 1 and 2 Immunoblot IgG, and Euroimmun Anti-HSV-1/HSV-2 gG2 Euroline-WB (IgG/IgM). Informed by prior studies [17,18,22], our secondary objective was to assess the diagnostic characteristics of the two ELISA kits with respect to the two IB/WB assays, treated here as reference assays due to their different format for HSV-1 antibody detection.

3. Study design

3.1. Study population

Blood specimens were collected from volunteer men blood donors attending Hamad Medical Corporation, the main healthcare provider in Doha, Qatar, between June 2013 and 2016. The blood specimens were originally collected for other studies [23–28]. A total of 4525 blood specimens were eligible for this study. The sample was comprised of Qataris and MENA expatriates aged ≥18 years old.

Informed by prior work [17,29], a sample size of 200 was estimated to be necessary to ensure narrow confidence interval for the Cohen's kappa statistic. Twenty specimens were randomly selected from each of 10 MENA populations resulting in a total sample of 200. These 10 MENA populations comprised subjects from Egypt, Iran, Jordan, Lebanon, Pakistan, Palestine, Qatar, Sudan, Syria, and Yemen. The research work was approved by the ethics boards and research committees at Hamad Medical Corporation, Qatar University, and Weill Cornell Medicine-Qatar.

3.2. Detection of anti-HSV-1 IgG

3.2.1. ELISA

Sera were tested for the presence of anti-HSV-1 antibodies using two commercial ELISA kits: HerpeSelect® 1 ELISA (Cat. No. EL0910G-5, Focus Diagnostics, USA) and Euroimmun Anti-HSV-1 (gC1) ELISA (IgG) kit (Cat. No. EI 2531–2 G, Euroimmun, Germany). The HerpeSelect® 1 ELISA kit offered qualitative measurements for HSV-1 IgG antibodies using purified recombinant gG1 antigen [30]. The Euroimmun ELISA kit was a semi-quantitative assay that used affinity chromatography purified-gC1 antigen to detect the presence of HSV-1 antibodies [31].

Both tests were carried out manually according to the manufacturers' instructions, except for the washing step, which was done automatically. The color intensity was measured using a spectrophotometer to read the optical density (OD) at 450 *nm*; an index value was then obtained by dividing the OD by the mean absorbance of the kit control sera. For HerpeSelect® 1 ELISA, sera with OD index values < 0.90 were considered anti-HSV-1 negative, values > 1.10 were considered anti-HSV-1 positive, and values ranging between 0.90 and 1.10 were considered anti-HSV-1 equivocal [30]. For Euroimmun Anti-HSV-1 (gC1) ELISA, sera with OD index values < 0.80 were considered anti-HSV-1 negative, values ≥1.10 were considered anti-HSV-1 positive, and values ranging between 0.80 and 1.10 were considered anti-HSV-1 equivocal [30–32].

3.2.2. IB/WB

ELISA tests may have cutoffs that maximize sensitivity at the possible expense of specificity. We compared the ELISA kits against two assays that utilize a different format: 1) HerpeSelect® 1 and 2 Immunoblot IgG (Cat. No. IB0900G, Focus Diagnostic, USA) and 2) Euroimmun Anti-HSV-1/HSV-2 gG2 Euroline-WB (IgG/IgM) (Cat. No. DY 2531-1G, Euroimmun, Germany). HerpeSelect® IB test strips were striped with purified type-specific proteins: HSV-1 gG-1 and HSV-2 gG2, and a common protein mixture [33]. Euroline-WB test strips contained antigenic extracts of HSV-1 that were electrophoretically separated, then transferred to paper strips [34].

The two IB/WB assays were performed and interpreted according to the manufacturers' instructions. The HerpeSelect® IB is designed to detect anti-HSV-1 and anti-HSV-2 antibodies. The kit strips contain four antigen bands: an anti-human serum control band, a herpes common antigen band (a blend of HSV-1 and HSV-2 native virus antigens), and recombinant antigen bands for gG-1 (HSV-1) and gG-2 (HSV-2). Each assay run must, at a minimum, include one antigen strip reacted with a positive control serum, and one antigen strip reacted with the negative control serum (provided by the kit). The gG-1 and gG-2 bands on the positive control are at a low positive "cut-off" staining intensity to provide a reading comparison. For the test to be considered valid, the anti-human band must be clearly visualized. In addition, the positive control serum must react with all of the four bands on the strip, while the negative control must react with, and only with, the anti-human serum control band.

Reading of the strips that were valid by the above definition was done visually by comparing the intensity of the gG-1 and gG-2 bands relative to the gG-1 and gG-2 bands on the positive control strip. If the band is as dark or darker than the respective positive control band, then the band in question is reactive (positive). In contrast, if the band is lighter than the reading control band, then the band is unreactive (negative). The overall reactivity of bands is then used to interpret the results as per a table provided by the manufacturer [33]. For instance, to be considered positive for anti-HSV-1, the specimen must provide positive reactions with the anti-human serum, HSV common antigen, and gG-1 bands. A valid negative specimen for anti-HSV-2 will have anti-human and HSV type common bands but no band for gG-1. An equivocal test result is defined as reactive for anti-human serum and HSV common antigen bands, but negative for gG-1 and gG-2 bands.

On the other hand, results from Euroline-WB were interpreted qualitatively using a scanner with a EurolineScan software [34]. The EUROLineScan software is used to scan and digitally evaluate the strips according to the presence and intensity of recognizable bands on the blot strips. 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 equivocal. Negative results were ≤12 units, equivocal 13–20 units, and positive results correlated with ≥20

units.

3.3. Statistical analysis

Results were cross-tabulated for one-to-one comparisons between the four assays. Equivocal and borderline results were excluded from the analysis. Four concordance measures were estimated: positive percent agreement, negative percent agreement, overall percent agreement, and Cohen's kappa statistic. Using either HerpeSelect® IB or Euroline-WB, separately, as the reference standard, sensitivity, specificity, positive predictive value, and negative predictive value were calculated to assess the performance of the two ELISA kits. Level of significance was set at 5%, and a 95% confidence interval (CI) was reported for each measure. All measures were estimated using Microsoft Excel 2016.

The Cohen's kappa is a robust statistic that measures the level of agreement (beyond chance) between two tests [35]. It ranges between 0 and 1. Informed by the literature, Cohen's kappa values greater than 0.75 represent excellent agreement, values between 0.40 and 0.75 represent fair to good agreement, and values below 0.40 represent poor agreement [35].

4. Results

Fig. 1 shows the cross-tabulations for all six comparisons between the four assays. Of the 200 MENA blood specimens tested, 171 (85.5%, 95% CI: 79.8–90.1%) were positive for HSV-1 antibodies by HerpeSelect® 1 ELISA, 158 (79.0%; 95% CI: 72.7–84.4%) by Euroimmun ELISA, and 163 (81.5%; 95% CI: 75.4–86.6%) by Euroline-WB. Due to insufficient sera, three specimens were not tested using HerpeSelect® IB. As such, of the 197 specimens tested, 162 (82.2%; 95% CI: 76.2–87.3%) were positive for HSV-1 antibodies by HerpeSelect® IB. No specimen was equivocal using HerpeSelect® 1 ELISA, while seven specimens were equivocal using Euroimmun ELISA. Six specimens were equivocal using HerpeSelect® IB, while eight specimens were equivocal using Euroline-WB.

There were 177 specimens that had no equivocal results on any diagnostic test. Among these, 21 (11.9%) were negative and 147 (83.1%) were positive for HSV-1 antibodies in all diagnostic tests used. Only 4 (2.3%) specimens were positive for HSV-1 antibodies in only one of the four diagnostic tests while 5 (2.8%) specimens were positive for HSV-1 antibodies in different combinations of the diagnostic tests.

Fig. 2 reports the concordance assessment measures between the four diagnostic assays. Positive percent agreement ranged between 95.7% (95% CI: 91.4–98.3%) and 100.0% (95% CI: 97.8–100.0%).

A)

HerpeSelect® ELISA / Euroimmun ELISA	Pos	Neg	Total
Pos	157	1	158
Neg	7	28	35
EQUI	7	0	7
Total	171	29	200

B)

HerpeSelect® IB / Euroimmun ELISA	Pos	Neg	EQUI	NS	Total
Pos	150	3	3	2	158
Neg	5	26	3	1	35
EQUI	7	0	0	0	7
Total	162	29	6	3	200

C)

Euroline-WB / HerpeSelect® ELISA	Pos	Neg	EQUI	Total
Pos	162	3	6	171
Neg	1	26	2	29
Total	163	29	8	200

D)

HerpeSelect® IB / HerpeSelect® ELISA	Pos	Neg	EQUI	NS	Total
Pos	162	4	3	2	158
Neg	0	25	3	1	35
Total	162	29	6	3	200

E)

Euroline-WB / Euroimmun ELISA	Pos	Neg	EQUI	Total
Pos	152	2	4	158
Neg	5	27	3	35
EQUI	6	0	1	7
Total	163	29	8	200

F)

HerpeSelect® IB / Euroline-WB	Pos	Neg	EQUI	NS	Total
Pos	157	1	3	2	163
Neg	0	25	3	1	29
EQUI	5	3	0	0	8
Total	162	29	6	3	200

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

Fig. 1. Cross-tabulated results of 200 specimens tested for HSV-1 antibodies by HerpeSelect® ELISA, Euroimmun ELISA, HerpeSelect® IB, and Euroline-WB assays.

A) Positive percent agreement and negative percent agreement

Positive percent agreement % (95% CI) / Negative percent agreement % (95% CI)	HerpeSelect® ELISA	Euroimmun ELISA	HerpeSelect® IB	Euroline-WB
HerpeSelect® ELISA		95.7 (91.4-98.3)	100.0 (97.8-100.0)	99.4 (96.6-100.0)
Euroimmun ELISA	96.6 (82.2-99.9)		96.8 (92.6-98.9)	96.8 (92.7-99.0)
HerpeSelect® IB	86.2 (68.3-96.1)	89.7 (77.7-97.8)		100.0 (97.7-100.0)
Euroline-WB	89.7 (72.7-97.8)	93.1 (77.2-99.2)	96.2 (80.4-99.9)	

B) Cohen's kappa statistic and overall percent agreement

Cohen's kappa statistic (95% CI) / Overall percent agreement % (95% CI)	HerpeSelect® ELISA	Euroimmun ELISA	HerpeSelect® IB	Euroline-WB
HerpeSelect® ELISA		0.85 (0.75-0.95)	0.91 (0.83-1.00)	0.92 (0.84-1.00)
Euroimmun ELISA	95.9 (92.0-97.9)		0.84 (0.73-0.95)	0.86 (0.76-0.96)
HerpeSelect® IB	97.9 (94.8-99.20)	95.7 (91.7-97.8)		0.98 (0.93-1.00)
Euroline-WB	97.9 (94.8-99.2)	96.2 (92.4-98.2)	99.4 (97.0-99.9)	

Fig. 2. Concordance assessment between the results of 200 specimens tested for HSV-1 antibodies by HerpeSelect® ELISA, Euroimmun ELISA, HerpeSelect® IB, and Euroline-WB assays.

Negative percent agreement ranged between 86.2% (95% CI: 68.3–96.1%) and 96.6% (95% CI: 82.2–99.9%). Overall percent agreement ranged between 95.7% (95% CI: 91.7–97.8%) and 99.4% (95% CI: 97.0–99.9%).

Specifically, the two ELISA kits had a positive percent agreement of 95.7% (95% CI: 91.4–98.3%), a negative percent agreement of 96.6% (95% CI: 82.2–99.9%), and an overall percent agreement of 95.9% (95% CI: 92.0–97.9%). The two IB/WB assays had a positive percent agreement of 100.0% (95% CI: 97.7–100.0%), a negative percent agreement of 96.2% (95% CI: 80.4–99.9%), and an overall percent agreement of 99.4% (95% CI: 97.0–99.9%).

The Cohen's kappa statistic for all six comparisons was ≥ 0.75 (p-value < 0.001 in all comparisons), indicating excellent agreement [35]. It ranged between 0.84 (95% CI: 0.73–0.95) for the comparison between Euroimmun ELISA and HerpeSelect® IB and 0.98 (95% CI: 0.93–1.00) for the comparison between HerpeSelect® IB and Euroline-WB. The Cohen's kappa statistic for the comparison between the two ELISA kits was estimated at 0.85 (95% CI: 0.75–0.95).

Fig. 3 shows the four diagnostic and performance assessments for HerpeSelect® ELISA and Euroimmun ELISA kits in detecting HSV-1 antibodies with respect to the reference IB/WB assays. Overall concordance for all four assessments was estimated at > 95%. Sensitivities and specificities for all comparisons were estimated at > 96% and > 86%, respectively. Positive and negative predictive values were estimated at > 97% and > 83%, respectively.

Against HerpeSelect® IB, HerpeSelect® ELISA detected 162/162 anti-HSV-1 positive specimens, resulting in a sensitivity of 100.0% (95% CI: 97.8–100.0%). Specificity was 86.2% (95% CI: 68.3–96.1%), while the positive and negative predictive values were 97.6% (95% CI: 94.0–99.1%) and 100.0% (95% CI 86.7–100.0%), respectively. Euroimmun ELISA detected 150/155 anti-HSV-1 positive specimens, resulting in a sensitivity of 96.8% (95% CI: 92.6–98.9%). Specificity was 89.7% (95% CI: 77.7–97.8%), while the positive and negative predictive values were 98.0% (95% CI: 94.4–99.3%) and 83.9% (95% CI 67.4–92.9%), respectively.

Against Euroline-WB, HerpeSelect® ELISA detected 162/163 anti-HSV-1 positive specimens, resulting in a sensitivity of 99.4% (95% CI: 96.6–100.0%). Specificity was 89.7% (95% CI: 72.7–97.8%), while the positive and negative predictive values were 98.2% (95% CI: 94.8–99.4%) and 96.3% (95% CI: 81.7–99.3%), respectively. Euroimmun ELISA detected 152/157 anti-HSV-1 positive specimens, resulting in a sensitivity of 96.8% (95% CI: 92.7–99.0%). Specificity was 93.1% (95% CI: 77.2–99.2%), while the positive and negative predictive values were 98.7% (95% CI: 95.4–99.6%) and 84.4% (95% CI: 68.3–93.1%), respectively.

5. Discussion

To our knowledge, this is the first examination of the performance of two commercial ELISA and two commercial IB/WB assays in detecting HSV-1 antibodies in a MENA population. Serological testing among this population identified high prevalence of positive HSV-1 sera using HerpeSelect® 1 ELISA (86%), Euroimmun ELISA (79%), Euroline-WB (82%), and HerpeSelect® IB (82%). However, our results have also identified several sera as equivocal by Euroline-WB and HerpeSelect IB. These borderline results reflect the presence of antibodies to HSV common antigens while simultaneously the absence of glycoprotein gG-1. Without the visible type specific band, the assay in these cases is not

A) HerpeSelect® IB

	Overall Percent Agreement % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive Predictive Value % (95% CI)	Negative Predictive Value % (95% CI)
HerpeSelect® ELISA	97.9 (94.8-99.2)	100.0 (97.8-100.0)	86.2 (68.3-96.1)	97.6 (94.0-99.1)	100.0 (86.7-100.0)
Euroimmun ELISA	95.7 (91.7-97.8)	96.8 (92.6-98.9)	89.7 (77.7-97.8)	98.0 (94.4-99.3)	83.9 (67.4-92.9)

B) Euroline-WB

	Overall percent agreement % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
HerpeSelect® ELISA	97.9 (94.8-99.2)	99.4 (96.6-100.0)	89.7 (72.7-97.8)	98.2 (94.8-99.4)	96.3 (81.7-99.3)
Euroimmun ELISA	96.2 (92.4-98.2)	96.8 (92.7-99.0)	93.1 (77.2-99.2)	98.7 (95.4-99.6)	84.4 (68.3-93.1)

Fig. 3. Performance assessment in detecting HSV-1 antibodies for HerpeSelect® ELISA and Euroimmun ELISA assays with respect to each of the two reference standards of HerpeSelect® IB, and Euroline-WB assays.

able to distinguish the type of HSV [36]. These results may be due to early seroconversion in which type common antibodies may appear before those to type specific antigens, but this phenomenon has not been specifically proven for assays, only for Western blot [37].

All assays showed excellent positive, negative, and overall concordance with one another. In the six comparisons between these four assays, the positive percent agreement was > 95%, the negative percent agreement was > 86%, and the overall percent agreement was > 95%. The Cohen's kappa statistic was also high in all comparisons (> 0.84). These results support the comparable performance and utility of using any of these assays to clinically diagnose exposure to this infection, and to assess HSV-1 seroprevalence in MENA populations.

We further found that the two ELISA kits demonstrated robust diagnostic performance when compared to each of the IB/WB assays as the reference assays. In all four comparisons, the sensitivity was > 96% and the specificity was > 86%. The positive and negative predictive values were also high at > 97% and > 83%, respectively. The sensitivities, specificities, and positive predictive values were also similar for both ELISA kits, though a slight difference was identified for the negative predictive value. The latter was higher for HerpeSelect® ELISA compared to Euroimmun ELISA. These results further support the utility of these two ELISA kits in clinically diagnosing exposure to this virus and measuring HSV-1 seroprevalence, particularly in MENA populations.

There is evidence for variation in the sensitivity and specificity of HSV-1 assays by population [20,30,38]. Data from the United States of America suggests that HerpeSelect® ELISA has less than optimal sensitivity and may miss > 20% of seropositive cases compared to WB [39–42]. Our results here and our earlier HSV-1 seroprevalence study [27], however, suggests that this may not be the case for MENA populations—we measured very high HSV-1 seroprevalence in several MENA populations that was > 90%. Different levels of exposure and re-challenge to this infection and different immune activation levels, as well as global diversity within and between HSV-1 and HSV-2 glycoproteins [43], may explain some of the observed differences in sensitivity by population. Consequently, this provides an opportunity for further research to conduct similar comparison studies in other global populations, and to compare the similarities and variations in the results from one population to another.

The similar outcomes of these assays and their concordance suggest that any of them could be used for clinical diagnosis and scientific research. However, logistical factors may favor the use of one as opposed to another in a specific setting—the choice of assay may depend on the kit's availability, its costs, and laboratory infrastructure and demands for its use. For example, the total incubation duration for the Euroimmun ELISA kit is 75 min [31], in comparison to 100 min for the HerpeSelect® ELISA kit [30], which could be an important factor if a large number of specimens need to be tested in a specific laboratory. Meanwhile, Focus has automated platforms for their ELISA that can reduce the hands-on time in processing the samples.

There are limitations to this study. Our sample included blood specimens only from men, as we used existing sera that was collected from men. There were three specimens (two were positive by ELISA and confirmed positive by Euroline-WB) that were not tested using HerpeSelect® IB due to insufficient sera. Measures of agreement between assays, such as Cohen's kappa statistic, can depend on the true seroprevalence of infection [44], but our sample may not be representative for the true seroprevalence in the population. However, the observed seroprevalence in this sample was similar to that observed in MENA populations [27,45], and we employed a sampling strategy that enhances the representativeness of this sample of diverse MENA populations (20 random specimens selected per nationality for 10 nationalities). We treated HerpeSelect® IB and Euroline-WB as reference standards for the purpose of comparison, based on their IB/WB biological format and because we could not use (for logistical reasons) the University of Washington's WB, considered widely as the reference gold standard for HSV serology [15]. The use of HerpeSelect® IB and Euroline-WB for diagnosis and confirmation has been reported in the literature [15–17], but, ideally, larger studies will affirm their accuracy for HSV-1 detection.

In conclusion, we conducted a comparison study of four commonly-used commercial assays in detecting HSV-1 antibodies in a MENA population. All assays showed excellent positive, negative, and overall concordance with one another, and with a high Cohen's kappa statistic.

The two ELISA kits demonstrated robust and similar diagnostic characteristics when compared to each of the IB/WB assays. These findings support the utility of using these assays in clinical diagnosis and scientific research in MENA populations.

Conflict of interest

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

Disclose funding received for this work

Others.

Acknowledgements

The authors gratefully acknowledge Professor Emeritus Rhoda Ashley Morrow, from the University of Washington, for critically reviewing the manuscript. The authors are also grateful for the administrative support of Ms. Adona Canlas and the laboratory support of Ms. Somaya Harche. Testing kits were provided through pilot funding by the Biomedical Research Program at Weill Cornell Medicine-Qatar. GKN acknowledges support by Qatar University internal grant No. QUST-CHS-SPR-15/16-7. LJA and SRD acknowledge study conception and design support through NPRP grant number 9-040-3-008 from the Qatar National Research Fund (a member of Qatar Foundation), and GKN acknowledges support from the Qatar National Research Fund UREP grant number UREP18-001-3-001. Infrastructure support was provided by the Biostatistics, Epidemiology, and Biomathematics Research Core at Weill Cornell Medicine-Qatar. 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] R.C. Brady, D.I. Bernstein, Treatment of herpes simplex virus infections, Antiviral Res. 61 (2004) 73–81.
[4] M. Fatahzadeh, R.A. Schwartz, Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management, J. Am. Acad. Dermatol. 57 (2007) 737–763 (quiz 64-6).
[5] D.I. Bernstein, A.R. Bellamy, E.W. Hook Jr., M.J. Levin, A. Wald, M.G. Ewell, et al., Epidemiology, clinical presentation, and antibody response to primary infection with herpes simplex virus type 1 and type 2 in young women, Clin. Infect. Dis. 56 (2013) 344–351.
[6] J.W. Gnann Jr., R.J. Whitley, Genital herpes, N. Engl. J. Med. 375 (2016) 666–674.
[7] N. Ryder, F. Jin, A.M. McNulty, A.E. Grulich, B. Donovan, Increasing role of herpes simplex virus type 1 in first-episode anogenital herpes in heterosexual women and younger men who have sex with men, 1992–2006, Sex. Transm. Infect. 85 (2009) 416–419.
[8] 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), http://dx.doi.org/10.1016/j.vaccine.2017.03.074 in press https://www.sciencedirect.com/science/article/pii/S0264410X17304085?via%3Dihub.
[9] 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.
[10] W.R. Dowdle, A.J. Nahmias, R.W. Harwell, F.P. Pauls, Association of antigenic type of Herpesvirus hominis with site of viral recovery, J. Immunol. 99 (1967) 974–980.
[11] A.J. Nahmias, W.R. Dowdle, Antigenic and biologic differences in herpesvirus hominis, Prog. Med. Virol. 10 (1968) 110–159.
[12] A. Dolan, F.E. Jamieson, C. Cunningham, B.C. Barnett, D.J. McGeoch, The genome sequence of herpes simplex virus type 2, J. Virol. 72 (1998) 2010–2021.
[13] P. Tunback, J.A. Liljeqvist, G.B. Lowhagen, T. Bergstrom, Glycoprotein G of herpes simplex virus type 1: identification of type-specific epitopes by human antibodies, J. Gen. Virol. 81 (2000) 1033–1040.
[14] T. Scheper, S. Saschenbrecker, K. Steinhagen, A. Sauerbrei, W. Suer, W. Meyer, et al., The glycoproteins C and G are equivalent target antigens for the determination of herpes simplex virus type 1-specific antibodies, J. Virol. Methods 166 (2010) 42–47.
[15] T.B. Martins, R.J. Welch, H.R. Hill, C.M. Litwin, Comparison of a multiplexed herpes simplex virus type-specific immunoglobulin G serology assay to immunoblot, Western blot, and enzyme-linked immunosorbent assays, Clin. Vaccine Immunol. 16 (2009) 55–60.
[16] R.L. Ashley, Sorting out the new HSV type specific antibody tests, Sex. Transm. Infect. 77 (2001) 232–237.
[17] 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.
[18] A. Sauerbrei, P. Wutzler, Novel recombinant ELISA assays for determination of type-specific IgG antibodies against HSV-1 and HSV-2, J. Virol. Methods 144 (2007) 138–142.
[19] A. Wald, R. Ashley-Morrow, Serological testing for herpes simplex virus (HSV)-1 and HSV-2 infection, Clin. Infect. Dis. 35 (2002) S173–82.
[20] 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.
[21] R.L. Ashley, Performance and use of HSV type-specific serology test kits, Herpes 9 (2002) 38–45.
[22] M.J. Kasubi, A. Nilsen, H.S. Marsden, T. Bergstrom, N. Langeland, L. Haarr, Prevalence of antibodies against herpes simplex virus types 1 and 2 in children and young people in an urban region in Tanzania, J. Clin. Microbiol. 44 (2006) 2801–2807.
[23] 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.
[24] 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.
[25] 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.
[26] 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.
[27] 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.
[28] 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 males, Sex. Transm. Dis. (2018), http://dx.doi.org/10.1097/OLQ.0000000000000791 https://journals.lww.com/stdjournal/Abstract/publishahead/Herpes_Simplex_Virus_Type_2_Seroprevalence_among.98314.aspx.
[29] 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.
[30] Focus Diagnostics. HerpeSelect 1 ELISA IgG (English). 2011.
[31] Euroimmun. Anti-HSV-1 (gC1) ELISA (IgG). 2016.
[32] G. Robbins, S. Lammert, A. Rompalo, L. Riley, D. Daskalakis, R. Morrow, et al., Serologic assays for the diagnosis of herpes virus 1 (HSV-1) herpes virus 2 (HSV-2): test characteristics of FDA approved type-specific assays in an ethnically, racially, and economically diverse patient population, Open Forum Infectious Diseases, Oxford University Press, 2015.
[33] Diagnostics F. HerpeSelect® 1 and 2 Immunoblot IgG (English). 2011.
[34] Euroimmun. Anti-HSV-1/HSV-2 gG2 Euroline-WB (IgG/IgM) 2017.
[35] J.L. Fleiss, B. Levin, M.C. Paik, The Measurement of Interrater Agreement Statistical Methods for Rates and Proportions, Wiley, 2013, pp. 598–626.
[36] J. LeGoff, H. Pere, L. Belec, Diagnosis of genital herpes simplex virus infection in the clinical laboratory, Virol. J. 11 (2014) 83.
[37] R. Ashley, D. Koelle, Immune responses to genital herpes infection, in: T.C. Quinn (Ed.), Advances in Host Defense Mechanisms. Vol. 8 Sexually Transmitted Diseases, NY: Raven Press, New York, 1992, pp. 201–238.
[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] A. Elfriede, Performance of commercial enzyme-Linked immunosorbent assays for diagnosis of HSV-1 and HSV-2 infection in a clinical setting, 2016 STD Prevention Conference (Conference Abstract), (2016).
[40] Wald A. Personal communication regarding performance metrics of HerpeSelect® ELISA for HSV-1. 2017.
[41] G. Robbins, S. Lammert, A. Rompalo, L. Riley, D. Daskalakis, R. Morrow, et al., Serologic assays for the diagnosis of herpes virus 1 (HSV-1) herpes virus 2 (HSV-2): test characteristics of FDA approved type-specific assays in an ethnically, racially, and economically diverse patient population, Open Forum Infect. Dis. 2 (2015) 1561–1561.
[42] F. Xu, F.K. Lee, R.A. Morrow, M.R. Sternberg, K.E. Luther, G. Dubin, et al., Seroprevalence of herpes simplex virus type 1 in children in the United States, J. Pediatr. 151 (2007) 374–377.
[43] S.L. Lamers, R.M. Newman, O. Laeyendecker, A.A. Tobian, R.C. Colgrove, S.C. Ray, et al., Global diversity within and between human herpesvirus 1 and 2

glycoproteins, J. Virol. 89 (2015) 8206–8218.
[44] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health, Diagnostic Devices Branch, Division of Biostatistics, Office of Surveillance and Biometrics. Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests. Available at: https://www.fda.gov/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm071148.htm#top. (Accessed on Sept 2017). 2007.
[45] 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, (2018) (Under preparation).