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