Diagnostic Microbiology and Infectious Disease 95 (2019) 114864

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Herpes simplex virus type 2 (HSV-2) IgG index values in two immunoassays in relation to HSV-2 IgG inhibition assay results☆

Harry E. Prince *, Hollis J. Batterman, Dale A. Schwab

Quest Diagnostics Infectious Disease, Inc., San Juan, Capistrano, CA, USA

ARTICLE INFO

Article history:
Received 4 June 2019
Accepted 3 July 2019
Available online 13 July 2019

Keywords:
Herpes simplex virus type 2
IgG Inhibition assay
Confirmatory testing

ABSTRACT

CDC guidelines recommend confirmatory testing of sera with low-positive indices (1.10–3.50) in the HerpeSelect® (HSLT) HSV-2 IgG screening assay. To determine if this recommendation is adequate for our patient population, we reviewed HSLT HSV-2 IgG screening indices for 262 screen-positive sera (index >1.10) tested in our confirmatory assay, which assesses inhibition of binding to recombinant gG2 by HSV-1- and HSV-2-infected cell lysates. To determine how the recommendation affects other screening assays, we tested these samples in the Liaison® HSV-2 IgG assay. Of 124 false-positive sera, 20% and 39% had an index >3.50 in the HSLT and Liaison screening assays, respectively. In both assays, 51% of 63 indeterminate sera (inhibition by HSV-1 lysate) had indices >3.50. Similarly, ≥75% of 75 true-positive samples exhibited indices >3.50 in both assays. Thus, confirmatory testing only of sera with low-positive HSV-2 IgG indices misses some false-positive and indeterminate samples, leading to misdiagnosis of HSV-2 infection.

1. Introduction

Herpes simplex virus 2 (HSV-2) type-specific IgG testing has emerged as a major laboratory tool for diagnosing HSV-2-associated genital herpes (Ashley 2002; Ho et al. 1992). Given the clinical and psychosocial impact of this diagnosis, it is important that HSV-2 IgG assay results exhibit the highest accuracy possible. The most commonly-used HSV-2 IgG screening test, the HerpeSelect® (HSLT) HSV-2 IgG enzyme immunoassay (EIA), may yield false positive (FP) results compared to the gold standard Western blot (WB) assay; FP results are most common among samples with a low-positive HSLT EIA screen index value, defined as 1.10–3.50 (Ashley Morrow et al. 2005; Ashley-Morrow et al. 2004; Delaney et al. 2010; Smith et al. 2009). Based on these findings, the Centers for Disease Control and Prevention (CDC) (2015) recommends that serum samples with positive HSLT HSV-2 IgG screening indices of 1.10–3.50 be tested with a confirmatory assay that identifies true-positive (TP) reactivity.

The HSV-2 IgG confirmatory assay employed at our laboratory is a modification of the HSLT HSV-2 IgG EIA; it assesses the differential inhibitory effects of HSV-1-infected and HSV-2-infected cell lysates on IgG binding to recombinant gG2 protein (Hogrefe et al. 2002). As shown in several studies, agreement of results between this inhibition assay and WB is >95%; the few discordant samples (TP by the inhibition assay but WB negative) most likely represented earlier detection of seroconversion by HSLT versus WB (Ashley-Morrow et al. 2003; Golden et al. 2005; Hogrefe et al. 2002; Ngo et al. 2008; Smith et al. 2009).

Our HSV-2 IgG inhibition assay was launched in 2002, well before the CDC recommendation was issued in 2015, and is thus offered for any HSV-2 IgG-positive sample (index ≥1.10) rather than samples with a low-positive index value of 1.10–3.50 only (CDC, 2015). We capitalized on this difference in reflex testing protocols to assess what proportion of samples with an inhibition result other than TP (i.e., FP or indeterminate [IND]) would be misinterpreted as TP if the CDC recommendation to test only low-positive samples is followed. In addition, we also tested the study samples using an HSV-2 IgG chemiluminescent screening assay (Liaison®, Diasorin) to determine if the same relationships observed between HSLT screening indices and inhibition assay results also characterize another screening assay.

2. Materials and methods

2.1. Study design

The study included 187 consecutive residual sera that were positive in the HSLT HSV-2 IgG screening EIA and had a FP (n = 124) or IND (n = 63) result in the HSV-2 IgG inhibition assay. Also included in the study were 75 samples with a TP result in the inhibition assay; these samples were randomly selected during the collection period without

Abbreviations: HSLT, HerpeSelect®; FP, false positive; IND, indeterminate; TP, true positive.

☆ Declaration of interest: All authors are employees of Quest Diagnostics Infectious Disease, Inc.

* Corresponding author. Tel.: +1-949-728-1855; fax: +1-949-728-4334.
E-mail addresses: harry.e.prince@questdiagnostics.com (H.E. Prince), hollis.j.batterman@questdiagnostics.com (H.J. Batterman), dale.a.schwab@questdiagnostics.com (D.A. Schwab).

https://doi.org/10.1016/j.diagmicrobio.2019.07.002
0732-8893/

knowledge of the HSLT screening index. After recording the HSLT HSV-2 IgG screening assay index of the 262 samples, they were tested in the Liaison HSV-2 IgG screening assay. In addition, to assess the relative roles of HSV-1 components versus cell line components in non-specific inhibition by HSV-1 lysate, the 12 samples with an IND result in the inhibition assay and sufficient volume were tested in a modification of the inhibition assay that included pre-incubation of specimen aliquots with uninfected cell lysate, as well as specimen diluent (control), HSV-1-infected cell lysate, and HSV-2-infected cell lysate.

2.2. HSLT HSV-2 IgG screening assay

Sera submitted for HSV-2 IgG testing were evaluated using the HSLT HSV-2 IgG EIA (Diasorin Molecular, Cypress, CA) according to the manufacturer's instructions. If requested, positive samples (index value ≥1.10) were then tested in the HSV-2 IgG inhibition assay.

2.3. HSV-2 IgG inhibition assay

Sera were tested using a modification of the HSLT HSV-2 IgG EIA as previously described (Hogrefe et al. 2002). Briefly, each serum specimen was diluted 1:51 in specimen diluent and 3 aliquots of 0.075 mL were prepared. Aliquots then received 0.075 mL of specimen diluent (control), HSV-1-infected VERO cell lysate (Lysate 1) (ViruSys, Taneytown, MD) diluted in specimen diluent to 0.12 mg/mL, or HSV-2-infected VERO cell lysate (Lysate 2) (ViruSys) similarly diluted in specimen diluent. After an hour at room temperature, all aliquots were tested in the HSLT HSV-2 IgG EIA per the routine procedure. The 3 resulting HSV-2 IgG index values for a given specimen were used to calculate the % inhibition by each of the two lysates using the formulae:

Non-specific inhibition = [1 − (Lysate 1-treated sample index/ control sample index)] × 100.

HSV-2 inhibition = [1 − (Lysate 2-treated sample index/Lysate 1-treated sample index}] × 100.

Interpretation guidelines were as follows:

True positive (TP): non-specific inhibition <60% and HSV-2 inhibition ≥60%.

False positive (FP): non-specific inhibition <60% and HSV-2 inhibition <60%.

Indeterminate (IND): non-specific inhibition ≥60% (HSV-2 inhibition not considered).

To further characterize samples exhibiting non-specific inhibition ≥60% by Lysate 1, 12 IND specimens with sufficient volume were retested in a modification of the inhibition assay, where an additional aliquot of each specimen received uninfected VERO cell lysate (ViruSys) at the same concentration as Lysates 1 and 2. For this experiment only, % inhibition by each of the 3 lysates was determined relative to control (no lysate); the formula was thus:

Inhibition = [1 − (Lysate-treated sample index / control sample index)] × 100.

2.4. Liaison HSV-2 IgG assay

All study specimens (n = 262) were tested for HSV-2 IgG using the Liaison chemiluminescent analyzer (Diasorin, Stillwater, MN). This "start-to-finish" instrument measures IgG binding to recombinant HSV gG2 protein covalently coupled to magnetic beads. All incubations, washing steps, and assessment of chemiluminescence are performed in a closed system. Results are expressed as an index value; indices <0.90 are considered negative, 0.90–1.09 equivocal, and ≥1.10 positive (Maters et al. 2012).

2.5. Statistical analysis

For samples within a given inhibition assay result category (TP, FP, IND), the proportions of samples with a screening index >3.50 in the two HSV-2 IgG screening assays (HSLT versus Liaison) were compared using Fisher's exact test. Index values for the 12 IND samples pre-incubated with different cell lysate preparations were compared using the two-tailed paired t-test. Significance in both statistical tests was defined as $P < 0.01$.

3. Results

3.1. HSV-2 IgG screening index values in relation to HSV-2 IgG inhibition assay results

Among TP specimens, HSV-2 IgG screening index values >3.50 were slightly more common with the HSLT EIA (81%) than the Liaison assay (75%), but the difference was not statistically significant (Table 1). Both the HSLT and Liaison screening assays yielded index values >3.50 in about half of the IND specimens tested, again with no significant difference between screening tests (Table 1). In contrast, among FP specimens, screening index values >3.50 was almost twice as common with the Liaison assay (39%) as with the HSLT assay (20%), and this difference was statistically significant (Table 1).

3.2. Further characterization of samples exhibiting an IND result in the HSV-2 IgG inhibition assay

To determine the relative roles of HSV-associated components versus VERO cell components in non-specific inhibition by Lysate 1, 12 IND samples were tested in a modified version of the inhibition assay that included an additional sample aliquot pre-incubated with uninfected VERO cell lysate. The mean index in the presence of uninfected cell lysate did not significantly differ from the mean index in the absence of lysate (control) (Table 2). In contrast, mean index values for these 12 IND samples were significantly lower in the presence of both Lysate 1 and Lysate 2. Thus, non-specific inhibition by Lysate 1 was due to components associated with HSV infection of the VERO cells.

3.3. Relationship between HSLT and Liaison screening assay indices

Figs. 1-3 show the correlational relationships between index values in the HSLT and Liaison HSV-2 IgG screening assays for samples within the 3 different inhibition assay result categories. The TP and IND groups showed similar relationships (Figs. 1 and 2); the trend line slopes were similar (1.8 for TP, 1.5 for IND) and indicated that, for both groups, index values were higher overall by Liaison than HSLT, reflecting the broader dynamic range of the Liaison assay. However, the coefficient of

Table 1
Proportions of samples with a HSLT or Liaison HSV-2 IgG screening index >3.50 as a function of HSV-2 IgG inhibition assay results.

HSV-2 IgG inhibition assay result	Number	Number (%) with HSV-2 IgG screening index >3.50		P value, Fisher's exact test
		HSLT	Liaison	
True positive	75	61 (81)	56 (75)	0.431
Indeterminate	63	32 (51)	32 (51)	1.000
False-positive	124	25 (20)	48 (39)	0.002*

* Statistically significant ($P < 0.01$).

Table 2
HSV-2 IgG index values of serum aliquots pre-treated with different cell lysates for a subset of 12 IND samples.

Serum pre-treatment	Index, mean + SD	*P* value compared to no lysate
no lysate	4.78 ± 1.84	Not calculated
uninfected cell lysate	4.97 ± 2.09	0.08
HSV-1-infected cell lysate (Lysate 1)	1.16 ± 0.63	<0.001*
HSV-2-infected cell lysate (Lysate 2)	0.67 ± 0.39	<0.001*

* Statistically significant ($P < 0.01$) using the paired *t*-test.

determination (R^2) value was high in both the TP and IND groups (0.82 and 0.70, respectively), indicating strong relationships between the Liaison and HSLT HSV-2 IgG screening assay results. In contrast, the R^2 value for the FP group was much lower at 0.05, indicating a weak relationship between the Liaison screening index and the HSLT screening index. Fig. 3 reveals that the largest divergence of Liaison versus HSLT screening indices for FP samples occurred within the subset of samples with low-positive HSLT screening indices; 27 (27%) of the 99 FP samples with an HSLT screening index of 1.10–3.50 were negative in the Liaison screening assay (index <0.90), and 24 (24%) had a Liaison screening index >6.00.

4. Discussion

Our findings demonstrate that the CDC recommendation to perform confirmatory testing only on samples with an HSLT HSV-2 IgG screening index of 1.10–3.50 is not adequate for identifying FP and IND reactivity in our patient population. We found that a substantial portion of FP and IND samples exhibited an HSLT HSV-2 IgG screening index >3.50. These FP and IND samples would thus be incorrectly interpreted as TP when following the CDC recommendation. This type of error can have significant patient impact; the patient would be incorrectly diagnosed as having an HSV-2 infection, which may lead to unneeded suppressive antiviral therapy, psychological stress, and detrimental effects on relationships with sexual partners.

The CDC recommendation was based in large part on a study demonstrating that only 12% of HSLT HSV-2 IgG EIA positive/WB negative samples (interpreted as EIA FP) had HSLT screening index values >3.50 (Ashley Morrow et al. 2005). In contrast, we found that 20% of FP samples, as determined using our inhibition assay, had HSLT index values >3.50. It is now appreciated that some HSLT HSV-2 IgG screen positive/WB negative samples actually represent WB false negative reactivity associated with earlier detection of seroconversion by HSLT than by WB (Ashley-Morrow, 2003). Thus, the proportion of HSLT EIA FP samples having EIA index values >3.50 would be expected to be different when using a confirmatory assay other than WB.

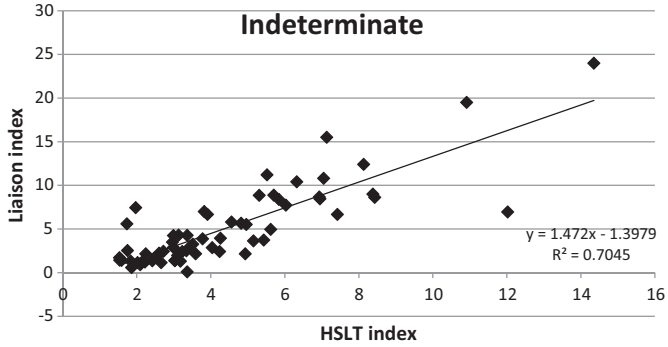

Fig. 2. Correlational relationship between index values in the HerpeSelect (HSLT) and Liaison HSV-2 IgG screening assays for indeterminate samples.

The inhibition assay we use in our laboratory confirms TP reactivity by assessing the ability of HSV-infected cell lysates to inhibit binding of gG2-specific IgG to the recombinant form of gG2 (Ho et al. 1992; Hogrefe et al. 2002). The use of Lysate 1 controls for non-specific inhibition of binding by HSV type-common antigens; thus, when compared to reactivity in the absence of lysates, marked inhibition of binding by Lysate 2, but not Lysate 1, indicates specific blocking of IgG binding by native gG2 protein in Lysate 2 (TP reactivity). Lack of inhibition by both lysates indicates FP reactivity in the screening assay due to IgG recognition of non-gG2-specific epitopes in the screening EIA; the structure of these epitopes remains unclear. Although not described in the initial report of the inhibition assay (Hogrefe et al. 2002), non-specific inhibition of IgG binding by Lysate 1 can also occur; this reactivity leads to an IND interpretation, since Lysate 2 inhibition results are uninterpretable in this setting. As is the case for FP reactivity, the structures found in Lysate 1 that are responsible for IND reactivity have not been characterized; however, as shown by the results presented in Table 2, they appear to be associated with HSV infection, since uninfected cell lysate does not exhibit inhibitory activity.

In recent years additional HSV-2 IgG screening assays have become available, typically with broader dynamic ranges compared to HSLT (Morrow and Friedrich 2006; Maters, 2012). We took advantage of the availability of our panel of specimens with inhibition assay results to assess the relationship between HSLT HSV-2 IgG screening index values and those obtained using the Liaison HSV-2 IgG screening chemiluminescent immunoassay, an assay that demonstrates excellent qualitative agreement with the HSLT HSV-2 IgG screening EIA (Maters, 2012). Despite the broader dynamic range of the Liaison assay, we found a strong correlational relationship between Liaison index values and HSLT index values for TP and IND specimens. This was not the case, however, for FP specimens; Liaison indices for FP samples spanned the entire Liaison dynamic range, from 0.07 to 24.0 (see Fig. 3). These results clearly show that, although most FP samples exhibit low-positive

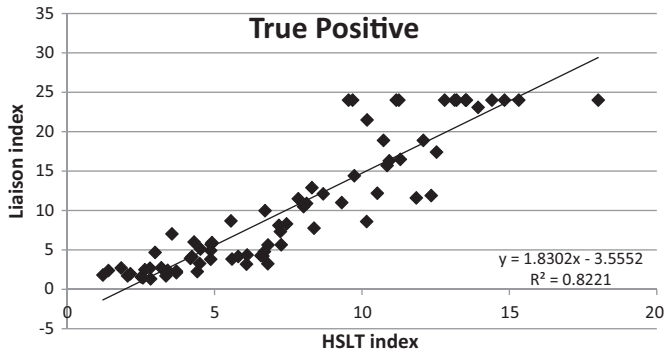

Fig. 1. Correlational relationship between index values in the HerpeSelect (HSLT) and Liaison HSV-2 IgG screening assays for true positive samples.

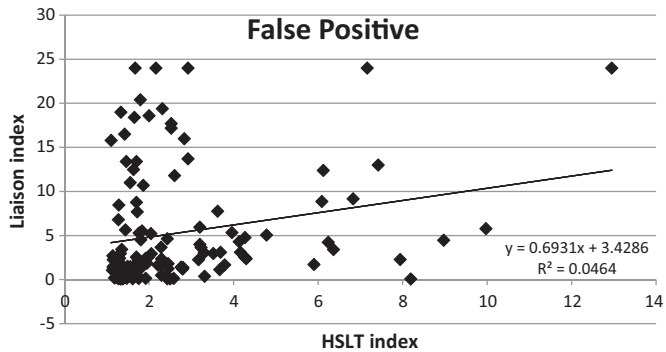

Fig. 3. Correlational relationship between index values in the HerpeSelect (HSLT) and Liaison HSV-2 IgG screening assays for false positive samples.

index values in the HSLT HSV-2 IgG screening EIA, one cannot assume this will be the case for other HSV-2 IgG screening assays. Further studies are needed to determine if this divergence characterizes HSV-2 IgG screening assays other than Liaison, and to identify the factors and mechanisms responsible for FP and IND reactivity.

Consistent with the wide dynamic range of Liaison screening index values for FP samples discussed above, the proportion of FP samples with a Liaison index >3.50 was significantly higher than the proportion with a HSLT index >3.50 (39% versus 20%, respectively). Thus, as is the case for the HSLT screening assay, the CDC recommendation to perform confirmatory testing only on samples with an index of 1.10–3.50 also appears inadequate for identifying non-TP reactivity when using the Liaison assay to screen our patient population. If a laboratory elects to use a screening assay other than HSLT and employ an algorithm where "high index" samples are not reflexed to confirmatory testing, it will need to determine what constitutes a "high index" for that assay.

Acknowledgements

The authors thank Jayson Babilonia, Daniza Co, and Mary Ann Villanueva for expert technical assistance.

References

Ashley R. Performance and use of HSV type specific serology test kits. Herpes 2002;9:38–45.

Ashley Morrow, R., Friedrich, D., Meier, A., Corey, L., 2005. Use of "biokit HSV-2 rapid assay" to improve the positive predictive value of focus HerpeSelect HSV-2 ELISA. BMC Infect Dis 5:84 doi:10.1 186/1471-2334-5-84.

Ashley-Morrow R, Krantz E, Wald A. Time course of seroconversion by HerpeSelect ELISA after acquisition of genital herpes simplex virus type 1 (HSV-1) or HSV-2. Sex Transm Dis 2003;30(4):310–4.

Ashley-Morrow R, Nollkamper J, Robinson NJ, Bishop N, Smith J. 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 2004;10:530–6. https://doi.org/10.1111/j.1469-0691.2004.00836.x.

Centers for Disease Control and Prevention (CDC), 2015. Genital HSV infections. https://www.cdc.gov/std/tg2015/herpes.htm. Accessed January 16, 2019.

Delaney S, Jentsch U, Weiss H, Moyes J, Ashley-Morrow R, Stevens W, et al. Comparison of focus HerpeSelect and Kalon HSV-2 gG2 ELISA serological assays to detect herpes simplex virus type 2 (HSV-2) in a south African population. Sex Transm Infect 2010;86(1):46–50. https://doi.org/10.1136/sti.2009.036541.

Golden MR, Ashley-Morrow R, Swenson P, Hogrefe WR, Handsfield HH, Wald A. 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 2005;32(12):771–7.

Ho DW, Field PR, Sjogren-Jansson E, Jeansson S, Cunningham AL. Indirect ELISA for the detection of HSV-2 specific IgG and IgM antibodies with glycoprotein G (gG-2). J Virol Methods 1992;36:249–64.

Hogrefe W, Su X, Song J, Ashley R, Kong L. Detection of herpes simplex virus type 2-specific Immuoglobulin G antibodies in African sera using recombinant gG2, Western blotting, and gG2 inhibition. J Clin Microbiol 2002;40(10):3635–40.

Maters AW, Wright CV, Lee MT, Schwichtenberg G, Detrick B. Detection of type-specific antibodies to HSV-1 and HSV-2: comparative analysis of a chemiluminescnce immunoassay with a conventional ELISA. Diagn Microbiol Infect Dis 2012;73:273–4. https://doi.org/10.1016/j.diagmicrobio.2012.03.023.

Morrow R, Friedrich D. Performance of a novel test for IgM and IgG antibodies in subjects with culture-documented genital herpes simplex virus-1 or -2 infection. Clin Microbiol Infect 2006;12:463–9.

Ngo TD, Laeyendecker O, La H, Hogrefe W, Ashley Morrow R, Quinn TC. Use of commercial enzyme immunoassays to detect antibodies to the herpes simplex virus types 2 glycoprotein G in a low-risk population in Hanoi, Vietnam. Clin Vaccine Immunol 2008;15(2):382–4.

Smith JS, Bailey RC, Westreich DJ, Maclean I, Agot K, Ndinya-Achola JO, et al. Herpes simplex virus type 2 antibody detection performance in Kisumu, Kenya, using the Herpeselect ELISA, Kalon ELISA, Western blot and inhibition testing. Sex Transm Infect 2009;85(2):92–6. https://doi.org/10.1136/sti.2008.031815.