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NOTES

Performance of Two Commercial Glycoprotein G-Based Enzyme Immunoassays for Detecting Antibodies to Herpes Simplex Viruses 1 and 2 in Children and Young Adolescents

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In 61 patients 1 to 14 years of age, the Gull/Meridian enzyme-linked immunosorbent assay (ELISA) had a sensitivity of 100% for herpes simplex virus type 1 (HSV-1) and specificities of 74% for HSV-1 and 48% for HSV-2. In 128 similarly aged patients, the HerpeSelect ELISA (Focus Technologies) showed sensitivities of 80% for HSV-1 and 88% for HSV-2, and specificities of 97% for HSV-1 and 100% for HSV-2.

Herpes simplex virus type 1 (HSV-1) infection is common in childhood and may be indistinguishable from viral or bacterial stomatitis, otitis media, and upper respiratory tract infections (6, 11, 14). HSV-2 infections are unusual after the neonatal period and before sexual debut; seroprevalence begins to rise in late adolescence (9). Virologic methods for diagnosing HSV infection in children are limited by the need to collect samples early in the clinical course and by the need to perform the vigorous swabbing that is necessary to obtain infected cells from mucosal surfaces or from lesions. Serologic tests to detect HSV antibodies are available commercially. Some tests can distinguish HSV-1 from HSV-2 antibodies on the basis of type-specific antigens of glycoprotein G-1 (gG-1) and gG-2, respectively (1, 3). The first such test to be approved by the Food and Drug Administration was an enzyme-linked immunosorbent assay (ELISA) from Gull Laboratories, Salt Lake City, Utah that was sold under the Premier brand by Meridian Diagnostics (Cincinnati, Ohio) (termed the Gull/Meridian ELISA). This ELISA was both sensitive and specific in a premarket evaluation of adult sera (4).

To assess the accuracy of the Gull/Meridian ELISAs for children and adolescents, we tested blood samples from healthy children from southern Texas (n = 61; mean age, 7.4 years; range, 1 to 13 years) with kits purchased from Gull Laboratories and compared these results to those obtained by Western blotting (WB), a well-validated "gold standard" (2, 3). Later, when the Gull/Meridian ELISAs were withdrawn from the market, we extended this study to evaluate the HerpeSelect HSV-1 and HSV-2 ELISAs from Focus Technologies (formerly MRL) on pediatric sera (n = 128; mean age, 5.7 years; range, 1 to 13 years) that had been sent to the University of Washington Virology Laboratory for HSV type-specific serology. Our comparison studies revealed substantial differences among the performances of these ELISAs.

The seroprevalence of HSV-1 determined by WB in Texas-based patients was 49% (30 of 61 positive); no patient was positive for HSV-2. Of the sera from Seattle, 46 of 128 (36%) were seropositive for HSV-1 by WB and 8 of 125 (6%; ages 11 days to 14 years) were positive for HSV-2.

All eight samples with discordant results for HSV-1 by the Gull/Meridian ELISA and WB were false positive by the ELISA. The sensitivity for the HSV-1 Gull/Meridian ELISA was 100%, with a negative predictive value (NPV) of 100% (Table 1). The specificity for HSV-1 was 74%, with a positive predictive value (PPV) of 79%. The sensitivity of the Gull/Meridian HSV-2 ELISA could not be evaluated (none were HSV-2 WB positive). Thirty-two of 61 samples (52%) were positive by the Gull/Meridian HSV-2 ELISA, giving a specificity of 48% and a PPV of 0%.

The results for 5 of 128 pediatric sera tested by the HerpeSelect HSV-1 ELISA were equivocal, and the sera could not be classified as negative or positive for comparison. For the remaining 123 sera, the HerpeSelect HSV-1 ELISA had a sensitivity of 80% and a specificity of 97%, with a PPV and an NPV of 95 and 89%, respectively (Table 1).

Two sera had equivocal results with the HerpeSelect HSV-2 ELISA. Neither was positive by WB for HSV-2; one was positive for HSV-1 antibody by both WB and the HerpeSelect HSV-1 ELISA. In addition, three sera had atypical HSV-2 results by WB and could not be scored by that test as either negative or positive. Of the 123 evaluable result sets for HSV-2 antibody, HerpeSelect HSV-2 ELISA had a sensitivity of 88%, a specificity of 100%, a PPV of 100%, and an NPV of 99% (Table 1).

Thus, two commercial ELISAs had very different performance characteristics with pediatric sera. The Gull/Meridian ELISA (based on immunoaffinity-purified gG-1 and gG-2) suffered from very low specificity and unacceptably low PPVs, especially for HSV-2. At least one widely used reference test,

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TABLE 1. Results of WB and two ELISA for HSV-1 and HSV-2 antibodies in patients aged 1 to 14 years from Texas and Washington

Virus	ELISA	n^a	No. of WB-positive patients (%)	No. of patients with indicated results for WB/ELISA				ELISA sensitivity (%)	ELISA specificity (%)	ELISA PPV (%)	ELISA NPV (%)
				+/+	+/−	−/+	−/−				
HSV-1	Gull/Meridian	61	30 (49)	30	0	8	23	100	74.2	78.9	100
	HerpeSelect	123[b]	46 (36)	37	9	2	75	80	97.4	95	89
HSV-2	Gull/Meridian	61	0 (0)	0	0	32	29	Indeterminate	47.5	0	100
	HerpeSelect	123[b]	8 (6)	7	1	0	115	87.5	100	100	99

[a] *n*, number of patients. Gull/Meridian tests were used for patients from Texas; HerpeSelect tests were used for patients from Washington.
[b] Equivocal HerpeSelect test results or indeterminate WB results are not included.

the gG-1 and gG-2 immunodot enzyme assay, is based on antigens similar to those in the Gull/Meridian ELISAs (9, 12, 13). The HerpeSelect ELISAs had high specificity for both HSV-1 and HSV-2 but surprisingly low sensitivity for HSV-1 (80%). Our study raises important issues about the use of these gG-based HSV type-specific serologic tests in children.

The very low specificities of the Gull/Meridian ELISAs were surprising in light of previous data for adults (4). Although these tests could be more sensitive than WB, this higher sensitivity (if true) appears to apply only to children (15). Alternatively, some young people may have circulating factors that nonspecifically bind glycoprotein G in this particular ELISA. A previous report may have given an early, unrecognized warning of a unique problem with Gull/Meridian ELISAs for pediatric sera (7). It is unclear whether the specificity problem with Gull/Meridian ELISAs for pediatric sera is restricted to the Gull antigen. Studies using immunoaffinity-purified HSV-2 antigens in gG-2-based tests other than the Gull/Meridian ELISA suggest a reasonably low HSV-2 prevalence rate in children (8, 10, 12); these studies give no insight into ELISA performance for antibodies to HSV-1 in pediatric sera.

The HerpeSelect HSV-1 and HSV-2 ELISAs are based on baculovirus recombinant gG-1 and gG-2 (5). Unlike the Gull/Meridian ELISAs, these tests with pediatric sera gave no indication of excessive false-positive HSV-1 results (two of 77 sera) or HSV-2 results (0 of 116 sera) (Table 1). However, the sensitivity for HSV-1 was only 80%. Two of nine false-negative sera were from infants under 1 year of age and could have represented low-titer maternal antibodies. The other seven sera were from children with a median age of 4.5 years (range, 3 to 12) who might have been in the process of seroconverting, based on the appearance of the WB profiles (data not shown).

The accuracy of HSV serologic testing for children appears questionable with current ELISAs. False-positive HSV-1 results may lead to inappropriate treatment or to unnecessary antiviral prophylaxis in immunosuppressed patients. Positive HSV-2 tests for children suggest the occurrence of sexual abuse. Our limited testing of the HerpeSelect ELISAs provides cautious optimism that these tests are reasonably accurate for children. However, prospective studies using virologic diagnosis of infection as the gold standard are needed.

A serologic diagnosis of HSV infection in children should be made with caution. A negative test should be followed by testing in 6 to 8 weeks to detect seroconversion. Positive results for HSV-2 antibodies should be confirmed by a second type-specific test, such as WB or the Focus HerpeSelect immunoblot (5).

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