

University of Groningen

**Comparison of two enzyme-linked immunosorbent assays and one rapid immunoblot assay for detection of herpes simplex virus type 2-specific antibodies in serum**

Groen, J; Van Dijk, G; Niesters, Hubert; Van Der Meijden, W I; Osterhaus, A D

*Published in:*  
Journal of Clinical Microbiology

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
1998

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Groen, J., Van Dijk, G., Niesters, H. G., Van Der Meijden, W. I., & Osterhaus, A. D. (1998). Comparison of two enzyme-linked immunosorbent assays and one rapid immunoblot assay for detection of herpes simplex virus type 2-specific antibodies in serum. *Journal of Clinical Microbiology*, 36(3), 845-847.

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

## Comparison of Two Enzyme-Linked Immunosorbent Assays and One Rapid Immunoblot Assay for Detection of Herpes Simplex Virus Type 2-Specific Antibodies in Serum

JAN GROEN,<sup>1\*</sup> GRIETJE VAN DIJK,<sup>2</sup> HUBERT G. M. NIESTERS,<sup>1</sup> WILLEM I. VAN DER MEIJDEN,<sup>2</sup>  
AND ALBERT D. M. E. OSTERHAUS<sup>1</sup>

*Institute of Virology<sup>1</sup> and Department of Dermatology and Venerology,<sup>2</sup>  
University Hospital Rotterdam, 3015 GD Rotterdam,  
The Netherlands*

Received 16 July 1997/Returned for modification 19 August 1997/Accepted 24 November 1997

**The sensitivities and specificities of three immunoassays for the detection of herpes simplex virus type 2 (HSV-2)-specific immunoglobulin G antibodies in serum, including the one-strip rapid immunoblot assay (RIBA; Chiron Corporation) and two indirect enzyme immunoassays (EIA; Gull Laboratories and Centocor), were compared by testing a panel of 1,250 serum samples from individuals attending an outpatient clinic for sexually transmitted diseases. A qualitative agreement among the three assays was observed with 1,080 serum samples (86.4%); 291 of the serum samples (23.3%) were positive, 789 samples (63.1%) were negative, and 170 serum samples (13.6%) gave a discordant result. Results were considered conclusive when a concordant result was obtained with two of three assays. The sensitivities and specificities of the RIBA, the Gull EIA, and the Centocor EIA proved to be 99.2, 99.7, and 89.9% and 97.1, 96.7, and 99.3%, respectively. These results indicate that the Chiron RIBA and the Gull EIA are especially useful and reliable for the detection of HSV-2-specific antibodies in serum.**

Ranking after infections with *Chlamydia trachomatis* and human papillomavirus, genital herpes is the third most common sexually transmitted disease (4). The majority of recurrent genital herpes infections are caused by herpes simplex virus type 2 (HSV-2). Seroepidemiological studies of the prevalence of HSV-2-specific antibodies are especially important to determine the impact of this infection among risk groups. Furthermore, adequate identification of HSV-2-infected individuals is important to prevent transmission to partners and neonates and to identify asymptomatic HSV-2 infections (9). Most of the epidemiological studies and clinical diagnoses of HSV infections are based on virus isolation, PCR, and Western blot (WB) (2, 3, 8) analyses. Both PCR and virus isolation are of limited value, since they give positive scores only during active infection. Serological diagnosis of HSV-2 infections has been difficult, since distinction between HSV-1- and HSV-2-specific antibodies in serum is complicated by the high degree of cross-reactivity. Several assays for the detection of HSV-1- and HSV-2-specific antibodies in serum have been described, including WB analysis (2), immunodot blot analysis (6), and enzyme immunoassay (EIA) analysis (5, 7, 10). However, the "gold standard" for HSV-1- and HSV-2-specific serology to date is WB analysis (2), which is performed predominantly in specialized laboratories.

Recently, three rapid immunoassays, one rapid immunoblot assay (RIBA) and two EIAs, have become available.

The RIBA (Chiron Corporation, Emeryville, Calif.) is based on nitrocellulose membranes blotted with HSV-1 and HSV-2 recombinant proteins D (gD), two HSV-1-specific antigens (gG1 and gB1), and one specific HSV-2 recombinant antigen (gG2). The Gull HSV-2 immunoglobulin G (IgG) EIA (Gull Laboratories, Salt Lake City, Utah) is based on plates coated with affinity-purified, type-specific HSV-2 glycoprotein G (gG). The Centocor Captia Select HSV-2-G EIA (Centocor, Malvern, Calif.) is based on plates coated with purified HSV-2 recombinant baculovirus-expressed gG. In a retrospective study, we compared the three assays, using a panel of 1,250 serum samples from individuals aged between 15 and 68 years who visited the outpatient clinic for sexually transmitted diseases of the University Hospital Rotterdam between February 1993 and February 1994. After collection, the serum samples were stored at  $-20^{\circ}\text{C}$  until use. All assays were performed according to the instructions provided by the manufacturers. Results were considered true values when they agreed in at least two of the three assays tested. The sensitivities, specificities, and positive and negative predictive values of the three assays were determined in relation to each of the respective assays and against the defined true values. Serum samples with discordant results between the assays were tested by WB as previously described (2). To verify if the measured values between the assays were in agreement with the expected values and not based on a matter of chance, the results were statistically analyzed by the  $\kappa$  method (1).

Table 1 summarizes the results and gives calculations of the overall agreement, sensitivity, and specificity, as well as positive and negative predictive values for each of the respective assays and for the true values. Comparison of the Chiron RIBA and the Gull EIA results shows a concordance of 1,166 (93.3%), with 358 positive samples, 806 negative samples, and 2 inde-

\* Corresponding author. Mailing address: Institute of Virology, University Hospital Rotterdam, dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. Phone: 31.10.4635428. Fax: 31.10.4633441. E-mail: groen@viro.azr.nl.

TABLE 1. Estimation of levels of agreement, sensitivities, specificities, and positive and negative predictive values for three HSV-2 IgG-specific antibody assays<sup>a</sup>

Assay and true value	RIBA							Gull EIA			
	No. of samples			Overall agreement (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	No. of samples		
	Positive	Indeterminate	Negative						Positive	Indeterminate	Negative
RIBA											
Positive								358	9	22	
Indeterminate <sup>b</sup>								9	2	20	
Negative								22	2	806	
Gull EIA				93.3	94.2	97.3	94.2	97.3			
Positive	358	9	22								
Indeterminate <sup>b</sup>	9	2	2								
Negative	22	20	806								
Centocor EIA				87.9	93.6	93.3	83.5	97.6			
Positive	294	0	20						293	3	16
Indeterminate <sup>b</sup>	36	0	6						33	3	6
Negative	58	31	805						63	7	826
True value				95.2	99.2	97.1	93.5	99.6			
Positive	360	0	3						360	3	1
Indeterminate <sup>b</sup>	3	2	0						0	5	0
Negative	25	29	828						29	5	847

<sup>a</sup> Each of the respective assays and the true values were considered the gold standard. PPV and NPV, positive and negative predictive values, respectively.

<sup>b</sup> Indeterminate results were not used to calculate overall agreement, sensitivity, specificity, and positive and negative predictive values.

terminate results. Discordant results were found among 84 serum samples; 22 samples scored positive in the Chiron RIBA and negative in the Gull EIA, 22 scored positive in the Gull EIA and negative in the Chiron RIBA, and 40 samples scored indeterminate in both assays. The measure of agreement between these assays ( $\kappa = 0.852$ ) was very good (1). Comparison between the Chiron RIBA and the Centocor EIA demonstrated an overall agreement of 87.9% (1,099 of 1,250), with 294 positive and 805 negative serum sample results. A total of 151 serum samples proved to be discordant for both assays. The measure of agreement between these assays ( $\kappa = 0.731$ ) was good (1). Between both EIAs, the overall agreement was 89.8% (1,122 of 1,250); 293 samples scored positive, 826 scored negative, and 3 scored indeterminate. Discordant results were detected for 128 samples. The measure of agreement between these assays ( $\kappa = 0.765$ ) was good (1). Concordant results with all three assays were obtained for 1,080 of the 1,250 serum samples (86.4%); a positive result in all three assays was obtained with 291 serum samples (23.3%), a negative result was obtained with 789 samples (63.1%), and a discordant result was found for 170 serum samples (13.6%). Ninety-three serum samples of the 170 samples with a discordant result were tested by WB. Forty-seven (50.4%) of the 93 discordant samples scored positive by WB. Of these 47 WB-positive serum samples, 37 (78.7%) were positive by the RIBA, 23 (48.9%) were positive by the Gull EIA, and 10 (21.2%) were positive by the Centocor EIA. The calculated sensitivities of the Chiron RIBA against the Gull EIA and Centocor EIA were 94.2 and 93.6%, respectively. Sensitivities of 94.2 and 94.8% were obtained with the Gull EIA against the Chiron RIBA and the Centocor EIA, respectively. The sensitivity of the Centocor EIA was slightly lower than those of the Chiron RIBA and the Gull EIA (83.5 and 82.3%, respectively). The variation in specificity between the respective assays was less than 10% (92.9 to 98.1%). When the results of the respective

assays were determined in relation to the defined true values, the sensitivity of the RIBA was 99.2%, that of the Gull EIA was 99.7% and that of the Centocor EIA was 89.9%. The specificities of these assays varied between 96.7 and 98.2%.

Until recently, no commercial assays were available for the detection of HSV-2-specific antibodies in human serum. The WB was the only assay that could discriminate between HSV-1- and HSV-2-specific antibodies but could not be performed routinely in clinical laboratory settings for HSV-1 and HSV-2 serodiagnosis. The availability of HSV-2-specific serological assays offers the opportunity for clinical and peripheral diagnostic laboratories to confirm a clinical diagnosis of HSV-2 infection without using relatively time-consuming and relatively expensive virus isolation methods. Our retrospective studies have analyzed the potential utility of the three HSV-2 assays to ensure correct serodiagnosis of HSV-2 infection and have shown slight differences in sensitivity and specificity. The high degree of sensitivity and specificity of the Chiron RIBA for the detection of HSV-2 antibodies in serum allows an accurate serodiagnosis of HSV-2 infections in clinically suspected cases. The advantage of this assay is that with one nitrocellulose strip both HSV-1 and HSV-2 antibodies in serum can be identified. In contrast with the Chiron RIBA, which is particularly useful for analysis of limited numbers of samples, the Gull EIA provides a rapid tool for large seroepidemiological studies with comparable sensitivity and specificity. Approximately 10% of the samples tested in this study by the Chiron and the Gull immunoassays were not detected by the Centocor assay. The specificity of the Centocor EIA is similar to those of the other assays. This study clearly demonstrates that the results obtained with the Chiron RIBA and the Gull EIA especially coincided very well and that both can be used for serodiagnosis of HSV-2 infections.

TABLE 1—Continued

Overall agreement (%)	Gull EIA				Centocor EIA							
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	No. of samples			Overall agreement (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
					Positive	Indeterminate	Negative					
93.3	94.2	97.3	94.2	97.3	294	36	58	87.9	83.5	97.9	94.5	93.3
					0	0	31					
					17	6	805					
					293	33	63	89.9	82.3	98.1	94.8	92.9
					3	3	7					
					16	6	826					
89.8	94.8	92.9	82.3	98.1								
96.7	99.7	96.7	92.5	99.9	295	30	33	92.5	89.9	98.2	94.9	96.3
					0	3	2					
					16	9	859					

## REFERENCES

1. Altman, D. G. 1991. Practical statistics for medical research, p. 403–408. Chapman & Hall, London, United Kingdom.
2. Ashley, R., and J. Militoni. 1987. Use of densitometric analysis for interpretation HSV serologies based on Western blot. *J. Virol. Methods* **18**:159–168.
3. Aurelius, E., B. Johansson, B. Skoldenberg, and M. Forsgen. 1993. Encephalitis in immunocompetent patients due to herpes simplex virus type 1 and 2 as determined by type-specific polymerase chain reaction and antibody assays of cerebrospinal fluid. *J. Med. Virol.* **39**:179–186.
4. Cunningham, A. L. 1989. Genital herpes, p. 89–104. In R. Richmond and D. Wakefields (ed.), *AIDS and other sexually transmitted diseases*. Harcourt Brace Joanovich Publishers, Sydney, Australia.
5. Ho, D. W. T., P. R. Field, E. Sjogren-Jansson, S. Jeansson, and A. L. Cunningham. 1992. Indirect ELISA for the detection of HSV-2 specific IgG and IgM antibodies with glycoprotein G (gG-2). *J. Virol. Methods* **36**:249–264.
6. Lee, F. K., L. Pereira, C. Griffin, E. Reid, and A. Nahmias. 1986. A novel glycoprotein for detection of herpes simplex virus type 1 specific antibodies. *J. Virol. Methods* **14**:111–118.
7. Parkes, D. L., C. M. Smith, J. M. Rose, J. Brandis, and S. R. Coates. 1991. Seroreactive recombinant herpes simplex virus type 2-specific glycoprotein G. *J. Clin. Microbiol.* **29**:778–781.
8. Peterson, E., O. W. Schmidt, L. C. Goldstein, R. C. Nowinski, and L. Corey. 1983. Typing of clinical herpes simplex virus isolates with mouse monoclonal antibodies to herpes simplex virus types 1 and 2: comparison with type-specific rabbit antisera and restriction endonuclease analysis of viral DNA. *J. Clin. Microbiol.* **17**:92–96.
9. Proper, C. G., and A. M. Arvin. 1989. Genital herpes and pregnant women. *Curr. Clin. Top. Infect. Dis.* **10**:1–26.
10. Svennerholm, B., S. Olofsson, S. Jeansson, A. Vahlne, and E. Lycke. 1984. Herpes simplex virus type-selective enzyme-linked immunosorbent assay with *Helix pomatia* lectin-purified antigens. *J. Clin. Microbiol.* **19**:235–239.