Antibody Assays for Varicella-Zoster Virus: Comparison of Enzyme Immunoassay with Neutralization, Immune Adherence Hemagglutination, and Complement Fixation

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An enzyme immunoassay (EIA) was adapted for detection of antibody to varicella-zoster virus, and its sensitivity and specificity were compared with those of neutralization, immune adherence hemagglutination (IAHA), and complement fixation tests. Test sera showed little nonspecific reactivity in the EIA system, and valid results could usually be obtained at serum dilutions as low as 1:8. Demonstration of the presence or absence of varicella-zoster viral antibody by EIA showed 94% correlation with results obtained in neutralization tests, but EIA titers were 2- to 16-fold higher than neutralizing antibody titers. Results by IAHA showed 87% correlation with those obtained by neutralization. No false positive IAHA results were seen, but a number of false negative IAHA results were seen at the 1:8 serum dilution, particularly in older individuals. With increasing age (>40 years), and presumably increased time from varicella infection, neutralizing antibody levels generally declined to 1:8 or 1:16, EIA levels fell to 1:128 or 1:256, and IAHA and complement fixation antibody titers were usually <1:8 or 1:8. EIA and IAHA were as reliable as the neutralization and complement fixation tests for serodiagnosis of varicella and zoster infections. All tests demonstrated heterotypic varicella-zoster antibody titer rises in selected patients with initial herpes simplex virus infections, but fewer heterotypic responses were seen by EIA than by the other methods. EIA offers a rapid, sensitive, and specific method for varicella-zoster antibody assay that is applicable to use in a clinical setting.

Sensitive and specific assays for detecting antibody to varicella-zoster (V-Z) virus are needed both for serodiagnosis of infection and for determining immunity status in high-risk, immunocompromised individuals who are exposed to V-Z infection. In the latter situation, serological findings may serve as a guide for passive immunization.

Serodiagnosis can generally be accomplished by the complement fixation (CF) test. However, the heterotypic titer rises to V-Z virus which sometimes occur in individuals with herpes simplex virus (HSV) infections who have previously been infected with V-Z virus (12, 18, 19) may make interpretation difficult. These heterotypic antibody titer rises are also seen in neutralization (18), indirect fluorescent antibody (18), and immune adherence hemagglutination (IAHA) (8, 23) systems.

The presence of neutralizing antibodies to V-Z virus would be expected to be the most reliable indication of past infection and of presumed immunity to varicella, but neutralization tests are too complex and slow to yield results to be

useful for routine clinical application. It is well recognized that the CF test is not adequately sensitive for detecting V-Z viral antibody elicited by past infection. Immunofluorescence staining of membrane antigens in V-Z virus-infected cells has been proposed as a more sensitive method than CF for determining immunity status (22), but, despite the recent availability of stable, noninfectious V-Z-infected target cells (24), this method is still rather cumbersome and is not generally available in most laboratories. More recently, IAHA has been reported as a relatively simple, rapidly performed method which is more sensitive than CF for detecting antibody to V-Z virus (7, 8, 11, 23). Neither assay of antibodies to membrane antigen nor IAHA has been compared to neutralization tests for sensitivity and specificity in detecting V-Z viral antibody.

Enzyme immunoassay (EIA) (3, 4) is a recently developed technique which has shown high sensitivity for assay of certain viral antibodies (13, 20), and on the basis of versatility, stability of reagents, ease of performance, and adaptability to automation it would appear to

offer promise as a sensitive and useful method for routine determination of V-Z viral antibody levels. Here we report the adaptation of an EIA for detection of antibodies to V-Z virus and compare its sensitivity and specificity with those of neutralization, IAHA, and CF tests.

MATERIALS AND METHODS

Sera examined. Several groups of sera were tested in these comparative studies. One was comprised of single sera from 121 individuals ranging in age from 1 to 88 years who had no clinical evidence of a current infection with V-Z virus. Serum pairs from six mothers and their newborn infants (5 days to 1 month) were examined. The infants had clinical evidence of congenital disease, but not of HSV or V-Z virus infection. Paired sera were tested from six individuals with a clinical diagnosis of varicella and from five patients with a clinical diagnosis of herpes zoster. Paired sera were also tested from seven young adults with an initial HSV infection; the infecting virus was determined by a radioimmunoassay method for typing HSV antibody (5), and HSV neutralizing antibody was assayed as described previously (15). All of the HSV patients had previously experienced infection with V-Z virus, as evidenced by the presence of antibody in their acute-phase sera.

Neutralization tests. Neutralizing antibody assays for V-Z virus were performed by a plaque reduction technique developed in this laboratory (16). Tests were conducted with approximately 10 hemolytic units of fresh guinea pig complement in the serum-virus mixtures, since complement has been shown to enhance neutralization of V-Z virus (16).

CF tests. CF antibody for V-Z virus was assayed by the routine procedure of this laboratory (14).

IAHA. IAHA tests were performed by the method of Gershon et al. (7) using "processed" microtiter "V" plates (Linbro Scientific, Inc., Hamden, Conn.). The human group O erythrocyte suspension was used at a concentration of 0.5% to give more clear-cut patterns. Erythrocytes were obtained from a local hospital and represented a pool from at least three different donors each week. These gave reproducible antigen and antibody titers with our reference reagents, and thus it was not necessary to screen for suitable donors. Antigen was prepared as described by Gershon et al. (7) using the CaQu strain of V-Z virus (17). The appropriate working dilution of antigen was determined in block titrations against both a serum from a current herpes zoster infection and a lower-titered serum from a past V-Z virus infection; this dilution represented approximately 4 agglutinating units against the lower-titered serum and 8 agglutinating units against the high-titered serum. Uninfected control antigen was prepared in the same manner and used at the same dilution. Complement was from a commercial source (Microbiological Associates, Bethesda, Md.).

EIA. V-Z viral antigen was prepared from the CaQu strain propagated in the L-645 line of human fetal diploid lung (HFDL) cells developed by Jack H. Schieble of this laboratory. Monolayer cultures in roller bottles were infected with trypsin-dispersed V-Z virus-

infected cells at a ratio of 1 infected cell to 6 to 10 uninfected cells in the monolayer (17). When the cultures showed a 3- to 4-plus viral cytopathic effect, the volume of medium was reduced to 10 ml, and the cells were dislodged by shaking with glass beads and then pelleted by centrifugation at $700 \times g$ for 15 min. The supernatant fluid was removed, and the cells were resuspended in 0.01 M tris(hydroxymethyl)amino-methane buffer with 0.15 M NaCl and 0.002 M ethylenediaminetetraacetic acid (pH 9.0), using 0.5 ml of buffer for the harvest from each roller bottle. Cells were disrupted by sonic treatment with a Biosonik II apparatus with the probe intensity set at 50% output for 1 min. After centrifugation at $700 \times g$ for 15 min, the supernatant fluid (antigen) was removed and stored at -70°C . Uninfected control antigen was prepared in the same manner from the same lot of HFDL cells. Antigen was assayed in a block titration against a V-Z immune serum of human origin, and a working dilution was selected which gave maximum reactivity with the positive antiserum, and at which the same dilution of uninfected control antigen showed no reactivity with the reference antiserum. For the present studies antigen was used at a 1:1,000 dilution. The CF titer of the antigen, based upon a block titration with the same antiserum, was 1:512.

Assays were conducted in MicroELISA substrate plates (Dynatech Laboratories, Alexandria, Va.) with round-bottom wells. The optimal dilution of V-Z antigen or control antigen in 0.06 M bicarbonate buffer (pH 9.5) was added to the wells in a volume of 0.2 ml, and plates were held at 4°C overnight. Unadsorbed antigen was removed by vacuum suction with a washer-aspirator (Dynatech Laboratories), and the wells were washed once with 0.01 M phosphate-buffered saline (PBS) (pH 7.3) containing 0.05% Tween 20. Protein adsorption sites were then saturated by adding 0.35 ml of a 5% bovine albumin solution in PBS and incubating for at least 4 h at room temperature. The fluids were aspirated, and the wells were washed once with PBS. Antigen-sensitized plates were used immediately, or they could be stored at 4°C for up to 4 weeks without loss of activity.

An alkaline phosphatase conjugate was prepared from goat antiserum to human gamma globulin, obtained from Antibodies, Inc., Davis, Calif. After the immunoglobulin G fraction of the serum was separated by three precipitations with $\frac{1}{2}$ saturated ammonium sulfate and passed through a diethylaminoethyl-cellulose column, antibodies specific for human immunoglobulin G were isolated and purified by immunoadsorption as described previously (6). These antibodies were then labeled with alkaline phosphatase by the method of Avrameas (1). The optimal dilution of the conjugate for EIA, as determined by block titration with antigen-coated wells and varying dilutions of V-Z antiserum, was 1:1,200. A horseradish peroxidase conjugate against human immunoglobulin G from a commercial source (Miles Laboratories, Elkhart, Ind.) could be used at the same dilution and gave similar results in EIA for V-Z viral antibodies.

For the test proper, serial twofold dilutions of serum starting at 1:8 were prepared with 0.05-ml microdiluters in plates coated with V-Z antigen and with control antigen. PBS in a volume of 0.05 ml was added to each

well, and tests were incubated overnight at room temperature. Controls included known positive and negative sera and wells incubated with diluent instead of serum. The following day the contents of the wells were aspirated, and the wells were washed three times with PBS. An optimal dilution of enzyme-labeled antibodies to human immunoglobulin G prepared in 5% bovine albumin in PBS was then added in a volume of 0.1 ml, and tests were incubated for 2 h at room temperature. The conjugate was aspirated from the wells, and they were washed three times with PBS. The enzyme substrate for the alkaline phosphatase system was *p*-nitrophenylphosphate (1 mg/ml) in diethanolamine (10%) buffer (pH 9.8) with 10^{-3} M $MgCl_2$ (20). The enzyme substrate for the horseradish peroxidase system consisted of ortho-phenylenediamine (0.1 mg/ml) and 0.003% hydrogen peroxide, freshly prepared in distilled water. These were added in a volume of 0.1 ml, and tests were incubated for 30 min at room temperature. The reaction in the alkaline phosphatase system was stopped by the addition of 0.05 ml of 3 N NaOH per well, and that in the horseradish peroxidase system was stopped by 0.05 ml of 8 N H_2SO_4 ; results were read by visual inspection against a white background. Antibody end points were taken as the highest serum dilution showing a visible color, and reactions were considered to be specific only if the corresponding wells containing uninfected control antigen showed no color. Initially readings were made both visually and with a Beckman DB spectrophotometer, using an optical density at 450 nm of ≥ 0.06 , compared with a negative reading of ≤ 0.03 in the nonspecific control, as the antibody end point. Titers determined by spectrophotometric readings were generally twofold higher than those determined by visual reading. The titers reported in this study were based upon visual reading.

RESULTS

Specificity of EIA and IAHA reactions. Table 1 summarizes the nonspecific reactions, i.e., reactions of sera with uninfected control antigen, for all of the sera tested by EIA and IAHA. In neither system did titers of nonspecific reactivity exceed 1:16. More sera reacted nonspecifically in the EIA system than in IAHA, but titers were low, and 96% of the sera tested had EIA titers against the uninfected control antigen of $\leq 1:8$.

V-Z viral antibody status as demonstrated by the neutralization test was used as the base line for evaluating specificity of the reactions seen in EIA and IAHA systems against the V-Z antigens. Table 2 compares the detection of antibody by neutralization and by EIA and IAHA for the 121 sera from individuals without evidence of current V-Z virus infection. There was 94% correlation between results obtained by EIA and neutralization, and 87% correlation between neutralization and IAHA results. Only four of the sera showed EIA results which might be considered false positive reactions on the basis of the

TABLE 1. *Comparison of reactivity against uninfected control antigens in the EIA and the IAHA systems*

Test system	V-Z antibody	No. of sera	No. (%) of sera showing non-specific titers		
			<1:8	1:8	1:16
EIA	Negative	70	54	11	5
	Positive	103	83	18	2
	Total	173	137 (80)	29 (16)	7 (4)
IAHA	Negative	86	81	2	3
	Positive	84	81	3	0
	Total	170	162 (95)	5 (3)	3 (2)

TABLE 2. *Correlation between detection of V-Z viral antibody by neutralization and by EIA and IAHA^a*

Antibody	Sera with V-Z neutralizing antibody:		Correlation (%)
	Positive (53) ^b	Negative (68)	
EIA			
Positive ^c	50	4	94
Negative	3	64	
IAHA			
Positive ^c	37	0	87
Negative	16	68	

^a Sera from individuals without evidence of a current V-Z virus infection.

^b Titer $\geq 1:4$. Parentheses indicate number of sera.

^c Titer $\geq 1:8$.

neutralization results. The few discrepant findings between neutralization and EIA or IAHA are described more fully below.

Sensitivity of antibody assay systems. Figure 1 compares V-Z viral neutralizing antibody levels with titers obtained by EIA, IAHA, and CF for the 121 sera from individuals without evidence of current V-Z virus infection. With few exceptions, antibody titers obtained by EIA were 2- to 16-fold higher than those demonstrated by neutralization. Whereas some of the IAHA titers were higher than neutralization titers, there was a greater tendency for them to be lower, and 14 false negative results were seen with IAHA at the 1:8 serum dilution. These comparisons confirm the low sensitivity of CF for detecting V-Z viral antibody from past infections.

Discrepant results seen between neutralization and EIA or IAHA tests are shown in detail in Table 3. The first 7 patients showed discrepant neutralization and EIA results, and the next 13 showed antibody by neutralization and EIA, but titers of $<1:8$ by IAHA. The low EIA titer of the first patient is of questionable specificity. The neutralizing antibody titer for the second

FIG. 1. Comparison of V-Z neutralizing antibody titers with titers obtained by EIA, IAHA, and CF. Tested were 121 sera from individuals without evidence of current V-Z virus infection.

TABLE 3. Discrepant results obtained in V-Z neutralization, EIA, or IAHA tests

Patient		Age (yrs)	Clinical impression	V-Z antibody titers by:			
No.	Name			Neutralization	EIA	IAHA	CF
1	J. Ca	2	Measles	<8	8	<8	
2	C. Jo	3	Measles	32	<8	<8	<8
3	V. Al	3	Leukemia	<4	16	<8	<8
4	A. De	7	Leukemia	<4	32	<8	<8
5	A. Ha	34	Not given	32	<8	256	<8
6	H. Eh	62	Influenza in immunosuppressed patient	<4	16	<8	<8
7	J. Fr	70	Leukemia	16	<16	16	<8
8	B. Br	4	Leukemia, chickenpox exposure	8	128	<8	<8
9	R. Ab	17	Influenza	4	32	<8	<8
10	J. Wi	20	Mother of infant with congenital disease	8	32	<8	<8
11	M. We	22	Mother of infant with congenital disease	8	32	<8	<8
12	R. At	28	Guillain-Barre syndrome	16	256	<8	<8
13	W. Ke	33	Not given	16	32	<8	<8
14	P. Du	44	Influenza	16	32	<8	<8
15	W. Jo	47	Guillain-Barre syndrome	16	128	<8	<8
16	E. Sc	59	Influenza	8	128	<8 ^a	<8
17	S. Sc	69	Pneumonia	8	128	<8	<8
18	R. Ad	71	Meningitis	16	128	<8	<8
19	M. Sc	72	Acute hearing loss	16	128	<4	<8
20	J. Jo	80	Pneumonia	16	256	<8 ^a	<8

^a Weak positive reaction at 1:4 dilution.

patient was reproducible, but its specificity is uncertain in the face of the negative EIA titer; other sera with neutralizing antibody titers of this level had high EIA titers. Four of the leukemic patients had low titers by one test and negative titers by the other; it is uncertain whether this was due to immunosuppression or to lack of specificity of the positive reactions. The negative EIA titer (obtained in duplicate testing) for patient no. 5 can probably be interpreted as a false negative result. The individuals for whom IAHA titers were negative (<1:8) and neutralization and EIA results were positive

were all adults, and some of them were elderly, suggesting that IAHA is not as sensitive as the other two methods for detecting V-Z antibody elicited by long-past infection. This is further supported by data from Fig. 2.

Figure 2 relates antibody levels to age for the patients without evidence of current V-Z virus infection. With few exceptions, positive titers in the younger age groups were high in all tests; the low titers were seen in the "problem cases" presented in Table 3. With increasing age (>40 years) and presumably increased time past varicella infection, neutralizing antibody titers gen-

erally fell into the range of 1:8 to 1:16, and EIA titers into the range of 1:128 to 1:256. However, IAHA and CF titers of the same individuals were generally at levels of <1:8 to 1:8.

For five of the mother-newborn serum pairs,

64			1		2				
32		3	1	1	1			CF	
16		1		3	3	3			1
8			1		4	4	3	2	
<8	42*	20	6	2	4	2	4	7	

1024				1					IAHA
512			1		1				
256					1	1			
128		2		1	1	1			
64				2	3	1			
32		1	1		3	3			
16			1			1	1	2	
8			1		2	1	3	1	
<8	42	20	6	2	3	1	3	7	

2048				1					EIA
1024		1			1	1			
512		2	2	2	4	1			
256				1	4	2	1	3	
128		1	1		3	2	4	5	
64						1	1		
32			1	2	1	1	1		
16			1					1	
8	1								
<8	41	18	5		1	1		1	

256		1		1	1				NEUT
128		1	1	1	3	1			
64		2	1	2	5	4	1	1	
32		1	1		2	2	1		
16					1	2	3	6	
8		1		1	1		2	2	
4				1					
<4	42	18	6		1			1	

	1-2	3-4	5-8	10-20	21-30	31-40	41-60	61-88
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V-Z viral antibody titer

Age group (years)
* Number of sera with indicated titer

FIG. 2. Distribution of neutralizing, EIA, IAHA, and CF antibody titers to V-Z virus by age.

V-Z viral antibody titers were identical or differed by no more than twofold by neutralization, EIA, and IAHA. One mother had a low titer of 1:8 by neutralization, 1:32 by EIA, and <1:8 by IAHA, and no antibody was demonstrable by any method in the newborn. These results indicate that EIA is as suitable as neutralization for detecting maternally acquired V-Z viral antibody.

Reliability of antibody assay methods for serodiagnosis of V-Z virus infections. Paired sera from six patients with a clinical diagnosis of varicella and five with a clinical diagnosis of herpes zoster were tested by the four assay methods. Changes in titer between acute- and convalescent-phase sera demonstrated by each method are shown in Fig. 3. All of the patients showed significant ($\geq$ fourfold) antibody titer rises by each procedure, with the exception of a single zoster patient with a stationary IAHA titer of 1:128. This stationary titer was reproducible upon retesting. In comparing EIA, IAHA, and CF antibody levels with neutralizing antibody titers, it was found that EIA titers of both acute- and convalescent-phase sera tended to be 2- to 16-fold higher, IAHA titers were generally the same or 2-fold lower than neutralization titers, and CF titers were 2- to 16-fold lower. The higher titers demonstrable by EIA in the acute-phase sera did not result in a loss of sensitivity for detecting titer increases (Fig. 3), since convalescent-phase EIA titers were extremely high.

Heterotypic antibody titer rises to V-Z virus in HSV infections. Table 4 compares

FIG. 3. Comparison of acute- and convalescent-phase antibody titers obtained by neutralization, EIA, IAHA, and CF in varicella and zoster patients.

TABLE 4. *Heterotypic antibody responses to V-Z virus in patients with an initial HSV infection*

Patient	Infecting virus	HSV antibody titers			V-Z virus antibody titers			
		Neutralization		CF ^a	Neutraliza- tion	EIA	IAHA	CF
		HSV-1	HSV-2					
B. Go	HSV-1	<8	<8	<8	32	128	128	16
		256	64	128	128	128	2,048	128
F. Al	HSV-2	<8	<8	<8	32	32	64	8
		128	128	64	512	64	1,024	256
A. Al	HSV-2	8	8	<8	256	128	512	128
		256	128	128	1,024	128	2,048	512
S. Ba	HSV-2	<8	8	<8	64	128	128	16
		16	64	64	128	128	2,048	256
L. Ca	HSV-2	<8	<8	<8	32	16	32	8
		128	32	64	512	32	1,024	128
P. Pa	HSV-2	<8	<8	<8	8	8	64	<8
		128	64	64	256	64	4,096	256
L. Wi	HSV-2	<8	<8	<8	32	64	64	8
		128	64	128	256	256	2,048	256
No. of $\geq 4 \times$ titer rises		7	7	7	6	2	7	7

^a CF tests against an HSV-1 antigen.

the heterotypic V-Z viral antibody titer increases demonstrated in each of the assay systems for seven patients, with initial HSV infections, showing $\geq$ fourfold CF antibody titer rises to V-Z virus. The patients all showed heterotypic V-Z antibody titer rises by IAHA as well as by CF, and six showed significant V-Z neutralizing antibody increases. However, only two of the patients showed fourfold or greater titer increases to V-Z virus by EIA.

DISCUSSION

The EIA described herein proved to be a specific and sensitive method for assay of V-Z viral antibody which is applicable both to serodiagnosis of infection and to determination of immunity status. Compared with some of the other EIA methods that have been described for assay of viral antibody (2, 9, 10, 21), this EIA showed remarkably few nonspecific reactions with lower dilutions of test serum. This permitted a more valid comparison of the sensitivity of the method with that of neutralization and other tests. When sera show nonspecific reactivity in EIA systems at dilutions lower than 1:50 or 1:100, it may be difficult to determine whether high levels of reactivity with the viral antigens are actually due to increased sensitivity for viral antibody, or to an additive effect of nonspecific reactivity and specific antibody activity.

Most of the sera from individuals with past V-Z virus infections had EIA titers of 1:64 or greater. Titers of $\leq 1:32$ were occasionally seen in individuals without corresponding neutraliz-

ing antibody for V-Z virus; these were found for the most part in immunocompromised persons. Thus it is possible that the greater sensitivity of EIA permitted demonstration of V-Z antibody in some individuals in whom antibody demonstrable by neutralization was depressed to undetectable levels. However, such low EIA titers would have to be interpreted with caution regarding their specificity, and it is also questionable whether specific antibody at such low levels would confer immunity to varicella in high-risk patients.

Although EIA demonstrated fewer heterotypic titer rises to V-Z virus in patients with HSV infection than were detected by neutralization, IAHA, and CF, it is uncertain whether in testing larger numbers of sera the technique would show marked advantages for serodifferentiation of HSV and V-Z viral infections, since some heterotypic titer rises were seen in the EIA system.

The EIA for V-Z viral antibody would be adaptable for routine use in a clinical setting, and tests could be conducted on any scale desired. Results can be obtained about as quickly as with the CF tests, and antigen-coated plates are stable for up to 4 weeks at 4°C whereas antigen is stable indefinitely at -70°C. Results can be read accurately by visual inspection, and parallel testing showed that systems using alkaline phosphatase or horseradish peroxidase as the conjugated enzymes gave comparable results. The latter enzyme produces a colored reaction product that is more distinct for visual reading, but equally accurate visual readings can

be made of tests employing an alkaline phosphatase enzyme system.

Although results can be obtained more rapidly by IAHA than by EIA, the test was somewhat less sensitive for detecting antibody from past infections, and a number of false negative IAHA results were obtained at the 1:8 serum dilution. Failure of IAHA to demonstrate V-Z viral antibody was most striking in individuals over 40 years of age, i.e., in those with presumed long-past varicella infections. Wong et al. (23) also studied IAHA antibody levels to V-Z virus in individuals of different ages. For age groups up to 40 years, the mean positive IAHA antibody titers were remarkably similar in our two studies. However, our mean titers were considerably lower in individuals past that age. Wong et al. examined more sera in each group than we did, and it is possible that with wider sampling chances are increased of selecting sera from individuals whose titers have been boosted by recent episodes of herpes zoster.

In contrast to some workers (7, 11, 23), but in agreement with Gillani and Spence (8), we did not find it necessary to select erythrocyte donors for V-Z IAHA tests, since erythrocytes pooled from different donors each week gave reproducible antigen and antibody titers. The use of pooled erythrocytes may actually minimize batch-to-batch variation in susceptibility to agglutination, since erythrocytes from individual donors are reported to be unsatisfactory from time to time (11). At any rate, it appears that use of the IAHA test for V-Z virus need not be limited by the requirement for a special erythrocyte donor. We encountered little nonspecific reactivity with uninfected control antigen in the IAHA test, but the sera had not been stored for prolonged periods, which has been reported to enhance nonspecific activity in the IAHA system (7). We did find, however, that prozones (inhibition of agglutination) occurred in regions of antigen and antibody excess in block titrations, and also with lower dilutions of high-titered sera in tests against a working antigen dilution. This should cause no problems if sera are titrated to expected end points rather than being screened at one or a few low dilutions.

We also confirmed the findings of Wong et al. (23) and Gillani and Spence (8) that IAHA tests do not avoid the heterotypic titer increases to V-Z virus seen in some individuals with HSV infections who have experienced previous infections with V-Z virus. It is not clear whether other workers (7) who have not seen heterotypic IAHA titer rises to V-Z virus in HSV infections actually examined sera which showed heterotypic titer rises by CF.

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