PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 762-765 (1976)

Detection of Antibody to Varicella-Zoster Virus by Immune Adherence Hemagglutination[1] (39302)

ANNE A. GERSHON, ZANE G. KALTER, AND SHARON STEINBERG
(Introduced by William J. Kuhns)

Department of Pediatrics, New York University School of Medicine, New York, New York 10016

A laboratory test for determination of immune status to varicella-zoster (V-Z) virus is essential for rational management of patients exposed to this infection. Individuals at high-risk to severe varicella include susceptible immunocompromised patients, newborn infants, and adults who may be protected from severe varicella by passive immunization with zoster immune globulin (ZIG; 1) or high-titered immune serum globulin (ISG; 2) within 72 hr of exposure. Since supplies of ZIG and ISG are limited, it is important to assess the immune status of individuals shortly after exposure in order to plan rational therapy for them.

The fluorescent antibody to membrane antigen (FAMA; 3) test is an effective assay for evaluation of immunity to V-Z virus. This sensitive, specific test is not practical for managing patients, however, since it is not generally available.

The technique of immune adherence hemagglutination (IAHA) is useful both for the study of bacterial and viral pathogens and for the immune response of the host directed against them (4–7). This assay may be used to detect either antigen or antibody; it seems to be sensitive, relatively free of nonspecific reactions, and simple to perform. This report describes an adaptation of the IAHA test for the detection of V-Z antibody in serum specimens obtained from patients before and after onset of varicella and zoster.

Materials and methods. Reagents. Gelatin veronal buffer (GVB) was prepared by mixing one part of 5× barbital buffer to four parts of 0.1% gelatin in water. Two liters of 5× barbital buffer contains 83 g NaCl, 10.19 g Na 5,5-diethyl barbiturate, 34.6 ml 1 *N* hydrochloric acid, and 5 ml of a solution containing 1 *M* $MgCl_2$ and 0.3 *M* $CaCl_2$ (20.33 g $MgCl_2 \cdot 6H_2O$ and 4.4 g $CaCl_2 \cdot 2H_2O$ in 100 ml H_2O). The pH of this solution was 7.4. VB was a similar solution with gelatin omitted. DTT-VB-EDTA was prepared by mixing 200 ml of 0.10 *M* EDTA, 300 ml VB, and 1.5 g dithiothreitol (DTT; Sigma Chemicals). Alsevers solution was prepared by adding 20.5 g glucose, 8.0 g Na citrate, and 4.2 g NaCl to 1000 ml of H_2O. The pH was adjusted to 6.1 with citric acid if necessary. All solutions were stored at 4° and were stable indefinitely with the exception of solutions containing gelatin which were stable for up to 1 week.

Cells, virus, and antigen. The Ellen strain of V-Z virus was propagated in human embryonic lung fibroblasts (HELF) grown in modified Eagle's medium (3). V-Z antigen was prepared by scraping infected cells into Hanks solution, disrupting the cells by sonication, and clarifying the solution by low-speed (1000*g*) centrifugation. Generally, a 32-oz bottle of infected HELF (90% cytopathic effect) was used to prepare 1 ml of antigen. Antigen was stored frozen at −70° until ready for use. A control preparation was made by sonication of a similar concentration of uninfected cells. The optimal dilution of V-Z antigen in GVB was determined by a standard checkerboard titration of dilutions of antigen against dilutions of V-Z antiserum. Usually a 1:16 dilution of antigen was optimal and was therefore used. The control preparation was used in a similar dilution with each serum to be tested.

Sera. The initial serum tested for V-Z antibody by IAHA was the laboratory's reference antiserum. This serum was obtained from a patient several weeks after onset of zoster. The V-Z FAMA titer of this serum was 1:512 and the V-Z CF titer was 1:128. The cytomegalovirus (CMV) CF titer was 1:4, the herpes simplex virus I (HSV I) CF

[1] Supported by Grant No. AI 12814-01 of the National Institutes of Health.

titer was 1:16 and the herpes simplex virus II (HSV II) CF titer was 1:4. The V-Z IAHA titer was 1:1024.

Sera in most instances had been obtained from patients from 1–17 years previously; sera were stored at −20°. Serum had been obtained from 19 persons at or prior to their exposure to varicella, from 23 patients 1–15 weeks following the onset of varicella, from 15 patients during the first week of zoster and again while recovering from zoster, and from 19 adults with a history of varicella many years previously.

Sera were inactivated by heating to 56° for 30 min. Dilutions were made in GVB in tissue culture grade microtiter V plates (Linbro).

Complement. Guinea pig complement (C′) (Cordis Laboratories) was stored at −70°. It was diluted for use in GVB. Each new lot was titered to find the optimal dilution of C′ to produce 4+ agglutination of RBC with optimal dilutions of V-Z antigen and antibody. Usually a dilution of 1:100–1:120 was used. Complement was kept in an ice bath after dilution and prior to addition to plates.

Red blood cells (RBC). Human type O positive whole blood was collected in twice the volume of Alsever's solution and kept at 4°. RBC could be kept for up to 1 week. Prior to use RBC were washed three times with GVB and adjusted to a 1.5% suspension in GVB. Not all RBC are said to be suitable for the test (5). A donor whose cells gave 4+ agglutination with V-Z antigen and antibody (but not with the control preparation) was used. Of three O positive donors tested all gave good results with the V-Z IAHA technique.

Fluorescent antibody to membrane antigen (FAMA) test. This assay was performed as described previously (3) using unfixed HELF infected with V-Z virus as antigen.

Complement fixation. This assay was performed as described previously (3) utilizing five 50% hemolytic endpoint units of complement.

Performance of the V-Z IAHA test. 1. Serial dilutions of 1:2–1:256 of serum were made in GVB in microtiter plates (0.025 ml/well). Only tissue culture grade plates could be used for the test. Two sets of dilutions of serum were made for each test, one to which V-Z antigen would be added and one to which control antigen would be added.

2. V-Z antigen, 0.025 ml (or control preparation, 0.025 ml), at the previously determined optimal dilution in GVB was added to each well containing serum dilutions. Plates containing sera and antigen were mixed on a vibrator at room temperature for 10 seconds and then incubated at 37° for 1 hr. Timing was critical and incubation times had to be exactly as noted in order to avoid low (decreases of up to three dilutions) or nonspecific "positive" reactions (i.e., 4+ agglutination with the control uninfected antigen).

3. Complement at the appropriate dilution in GVB (0.025 ml) was added to all wells. The plates were again mixed for 10 sec and incubated at 37° for 40 min.

4. DTT-VB-EDTA (0.025 ml) was added to all wells and the plates were mixed on the vibrator.

5. A 1.5% suspension of RBC in GVB (0.025 ml) was added to each well. The plates were mixed for 5 min and left at room temperature for 3 hr for hemagglutination to develop.

6. Observed reactions ranged between a well-demarcated button of RBC (no agglutination) read as O and a uniform pattern of agglutination in which there was no button of RBC visible, read as 4+. Only a 4+ reaction was read as positive. Occasionally a nonspecific reaction was observed with certain sera (4+ agglutination in wells containing control uninfected antigen). This type of reaction was more common in sera that had been stored for 8–10 years at −20°.

Results. Sera from patients susceptible to varicella. Serum that had been obtained from 19 normal individuals prior to onset of varicella were tested by the V-Z IAHA method. As indicated in figure 1 the IAHA V-Z antibody titer of all 19 specimens was <1:2.

Sera from persons convalescing from varicella. Serum that had been obtained from 23 otherwise normal individuals from 1–15 weeks following the onset of varicella were tested for V-Z IAHA antibody. Most of these sera were convalescent specimens from those persons from whom the prevaricella bloods had been obtained. As indi-

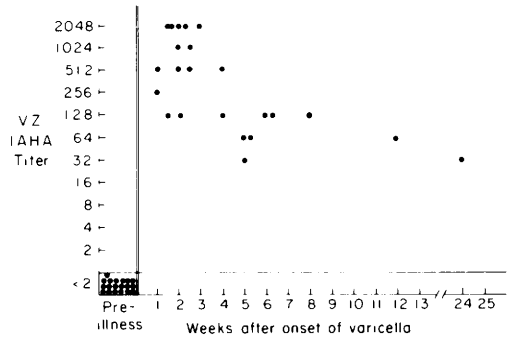

FIG. 1. Graph depicting varicella-zoster (V-Z) immune adherence hemagglutination (IAHA) antibody titers in 22 patients before and after the onset of varicella. Prior to onset all individuals had titers of <1:2. During convalescence from varicella all had detectable V-Z IAHA antibody with titers ranging from 1:32–1:2048.

cated in Fig. 1, V-Z IAHA antibody was detected in each of the 23 specimens tested; the titer ranged between 1:32 and 1:2048.

Sera from persons immune to varicella. Serum had been obtained from 19 adults with a history of varicella 10–20 years prior to sampling. As indicated in Fig. 2, all 19 sera had detectable V-Z IAHA antibody with titers ranging from 1:4 to 1:512.

Patients with zoster. Sera had been obtained from 15 individuals early and late in the course of zoster. As indicated in Fig. 2, V-Z IAHA antibody was detected in all sera. The antibody titers ranged from 1:8 to 1:128 in the acute sera, and 1:128 to 1:8192 in convalescent sera. In each instance, the diagnosis of zoster had been confirmed by virus isolation or by a fourfold increase in V-Z CF titer, or both.

Sera from patients with herpes simplex virus (HSV) and cytomegalovirus (CMV) infections. These sera were tested to determine whether a rise in V-Z titer by IAHA occurs during infection with other herpesviruses closely related to V-Z virus. In four of four cases of CMV infection and in four of four cases of HSV infection in which a significant rise in antibody to the homologous agent could be detected by CF, there was no change in V-Z IAHA titer. Of the patients with HSV infections three had a previous history of varicella and had positive FAMA titers with no increase in V-Z titer between acute and convalescent titers as measured by FAMA and CF as well as IAHA.

Comparison of IAHA, FAMA, and CF titers. Representative samples of these titers are presented in Table I. In serum obtained prior to the onset of varicella antibody to V-Z virus was not detected by any of these techniques. During convalescence from varicella antibody to V-Z virus was detected by all of the techniques. With all techniques a fourfold increase in V-Z titer was noted. Generally IAHA and FAMA titers were within one dilution of each other; CF titers were often three or more dilutions below IAHA and FAMA titers. V-Z antibody titers in immune adults were similar with both IAHA and FAMA tests. In contrast only 25% of immune adults had detectable antibody by CF and only at low dilutions. Following onset of zoster, V-Z antibody was detectable by all three techniques. Again IAHA and FAMA titers were within one dilution of each other and often three or more dilutions higher than CF titers. In patients with zoster a fourfold or greater rise in V-Z antibody titer between acute and convalescent sera was detected by each method.

Discussion. These data suggest that the IAHA technique is a serologic tool that may be useful for the diagnosis of varicella or zoster. High titers of V-Z antibody appear following varicella and the magnitude of the response is even higher following the secondary V-Z infection, zoster. The V-Z antibody titers are greater than those obtained by complement fixation and similar to those

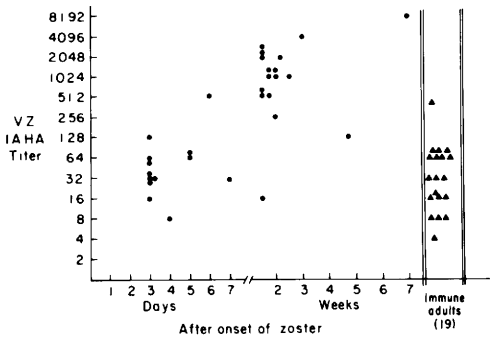

FIG. 2. Graph depicting varicella-zoster (V-Z) immune adherence hemagglutination (IAHA) antibody titers in 15 patients early and late in the course of zoster (○). In all patients initial sera contained detectable V-Z IAHA antibody. In all patients convalescent sera revealed a fourfold or greater rise in V-Z IAHA antibody titer. V-Z IAHA titers of 19 normal adults with a history of varicella years ago are also shown (▲).

TABLE I[a]

Patient	Reciprocal of titer IAHA	FAMA	CF
1. Prevaricella	<2	<2	<4
2.	<2	<2	<4
3.	<2	<2	<4
4.	<2	<2	<4
5. Convalescent varicella (1 week post)	256	128	32
6. (2 weeks post)	1024	1024	128
7. (2 weeks post)	128	128	64
8. (2 weeks post)	512	256	4
9. Immune adult	4	4	<4
10.	8	4	4
11.	8	4	<4
12.	16	16	<4
13. Convalescent zoster (1.5 weeks post)	2048	512	64
14. (2 weeks post)	2048	1024	128
15. (2 weeks post)	1024	512	256
16. (4 weeks post)	128	64	64

[a] Comparison of V-Z antibody titers obtained by immune adherence, fluorescent antibody to membrane antigen, and complement fixation techniques. The former two techniques yielded similar titers which were frequently at least three dilutions higher than titers obtained by complement fixation. In patients with varicella and zoster fourfold or greater differences in V-Z titer were demonstrated using all three techniques.

obtained by FAMA technique. High titers of specific antibody to hepatitis virus have been demonstrated similarly following hepatitis A and B infection using the IAHA test (6, 7). The mechanism underlying this test is that when antigen and antibody combine, the third component of complement (C'3) is activated causing RBC to agglutinate due to their C'3 receptor (4).

Thus far, the V-Z IAHA antibody test appears to be specific since heterologous rises in antibody titer to other herpesviruses were not found in the sera which were tested. The only technical problems noted with the IAHA technique were occasional nonspecific reactions against control antigens, particularly with serum that had been stored for 10 or more years.

Most important, the V-Z IAHA assay is a very practical test. It appears to be sensitive, and sera can be tested in low dilutions so that low levels of antibody may be detected. The test is simple enough to be performed in any routine laboratory and thus within hours one could determine the immune status of an individual with regard to varicella. If an exposed person is susceptible, and is at high-risk to severe varicella, passive immunization may be promptly administered.

The other sensitive method to demonstrate V-Z antibody, the FAMA test, also may be used to determine immune status to varicella (3). However, this test has the serious drawback of limited availability; it utilizes live tissue culture cells infected with V-Z virus as antigen so that it can only be performed in a laboratory in which the virus is being propagated. In contrast, the V-Z antigen used for IAHA is stored frozen at −70°, and it may be shipped to any laboratory for use. The V-Z IAHA antibody assay requires little in the way of equipment whereas equipment for fluorescence microscopy is required for the FAMA test as well as a tissue culture laboratory.

Summary. A serologic test for measurement of antibody to V-Z virus by immune adherence hemagglutination is described. Initial evaluation of the test has shown it to be highly sensitive, specific, rapid, and simple to perform. The V-Z antigen may be stored at −70°, and the test could be performed in any routine serology laboratory.

The authors wish to thank Dr. Saul Krugman for his helpful advice and criticism. The authors would also like to thank Dr. Stephen Millian for supplying serum from patients with CMV infections and Mrs. Rena Rauch for secretarial assistance.

1. Brunell, P. A., Ross, A., Miller, L., and Kuo, B., N. Engl. J. Med. **280,** 1191 (1969).
2. Gershon, A. A., and Smithwick, E., Presented at the Sixth International Congress of Pharmacology in Helsinki, Finland (1975).
3. Williams, V., Gershon, A., and Brunell, P. A., J. Infect. Dis. **130,** 669 (1974).
4. Nelson, R. A. Jr., Science **118,** 733 (1953).
5. Mayumi, M., Okochi, K., and Nishioka, K., Vox. Sang. **20,** 178 (1971).
6. Krugman, S., Friedman, H., and Lattimer, C., N. Engl. J. Med. **292,** 1141 (1975).
7. Miller, W. J., Provost, P. J., McAleer, W. J., Ittensohn, O. L., Villarejos, V. M., and Hilleman, M. R., Proc. Soc. Exp. Bio. Med. **149,** 254 (1975).

Received December 1, 1975. P.S.E.B.M., 1976, Vol. 151.