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Measurement of Antibodies to Varicella-Zoster Virus by Using a Latex Agglutination Test

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We evaluated a rapid, simple-to-perform assay for detecting antibodies to varicella-zoster virus by latex agglutination (LA); dilutions of test sera were added to latex particles coated with varicella-zoster virus antigen. We tested 878 serum specimens by LA and with fluorescent antibody to membrane antigen; of these, 227 were also tested by a commercially marketed enzyme-linked immunosorbent assay (ELISA). LA was almost as sensitive as the fluorescent antibody to membrane antigen test and more sensitive than ELISA. No cross-reacting antibodies were detected by LA, and false-positive reactions were rare.

A rapid, sensitive, simple-to-perform assay for antibodies to varicella-zoster virus (VZV) would have great clinical value for testing hospital personnel for immunity to varicella and for evaluating the success of immunization against chicken pox. We therefore evaluated a latex agglutination (LA) test for its sensitivity and specificity with regard to other standard assays for antibodies to VZV.

A total of 130 acute- and convalescent-phase serum specimens were tested. These sera included specimens obtained from 87 children and adults who had no history of varicella and were taken within several weeks after development of the infection. Included in these acute- and convalescent-phase specimens were also sera from 43 children enrolled in a study of acyclovir for the treatment of chicken pox (with the kind permission of the Burroughs Wellcome Study Group); these sera were obtained on the day of onset of the rash of varicella and 28 days later.

Sera from a group of 197 patients (54 healthy children, 48 healthy adults, and 95 leukemic children) with no history of varicella were studied, both before and several months after immunization against varicella with the experimental live attentuated virus vaccine (produced by Merck Sharp & Dohme, West Point, Pa.) that is currently being used in clinical trials. A subset of 15 of the preimmunization specimens from healthy adults were tested for antibodies to other herpesviruses.

In addition, we tested sera that were consecutively submitted to our laboratory for testing for VZV antibodies from 224 individuals, including 18 serum specimens from adults with a known history of varicella in childhood. Of these serum specimens, 131 were obtained from adults and 93 were from children; of these, 114 had received varicella vaccine (34 adults and 80 leukemic children) and 86 were from adults who wished to have their immune status to varicella tested. A subset of 61 of these 86 serum specimens was selected at random and tested by LA, fluorescent antibody to membrane antigen (FAMA) assay, and enzyme-linked immunosorbent assay (ELISA), and another subset of 56 (all seropositive) was tested by end point dilution for VZV antibodies by LA and the FAMA assay.

Sera were obtained and shipped to the laboratory at room temperature. Upon receipt, they were stored at −20°C prior to testing. Except for the 18 serum specimens from adults with varicella in childhood, most sera were stored for only a few weeks or months prior to testing.

For the LA test, serum was added to a suspension of latex particles coated with VZV antigen and then observed for agglutination as an indication of the presence of specific VZV antibodies. LA was performed according to the instructions of the manufacturer (Viral Antigens, Inc., Memphis, Tenn.). The shelf life of this product is believed to be up to 1 year. To perform a test, all ingredients, which were stored at 4°C, were warmed to room temperature. Doubling dilutions of sera were made in 1-cm-diameter flat circles on a wax-coated card (10 by 12 cm) supplied by the manufacturer, and the serum was spread evenly over the circle. A 0.025-ml drop of latex emulsion (tagged with VZV antigen) was added to each circle directly from the dropper bottle. The card was briefly rotated by hand and placed on a mechanical rotator for 10 min at 90 to 110 rpm in a moist chamber. Circles were then examined for agglutination (obvious clumping of the latex particles) with a high intensity desktop lamp.

Antibodies to VZV were measured by two additional methods. These included the FAMA assay (5), which employs unfixed human embryonic lung cells infected about 48 h previously with VZV. A commercial ELISA (Varicella STAT) manufactured by Whittaker-MA Bioproducts, Walkersville, Md., was also used. To test for antibodies to other antigens for evaluation of specificity, the following tests were used: herpes simplex virus (HSV) LA (Wampole Laboratories), cytomegalovirus (CMV) SCAN (Becton Dickinson), and EBV-VCA-FIAX (Whittaker Bioproducts).

Statistical analyses were made by chi-square analysis with continuity correction with Statview software on a Macintosh computer.

Antibodies to VZV were not detected in serum specimens from 130 patients prior to or on the day of onset of varicella by either the FAMA assay or LA. In convalescent-phase serum specimens from the same individuals, 130 (100%) were positive by the FAMA assay and 125 (96%) were positive by LA.

Antibodies to VZV were not detected in serum specimens from 54 healthy children, 95 leukemic children, and 48 healthy adults patients prior to receipt of varicella vaccine by the FAMA assay, LA, or ELISA. After vaccination, we detected VZV antibodies in 61% by LA, in 77% by the

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TABLE 1. Seropositivity to VZV after immunization with live attenuated varicella vaccine determined by three serologic assays

Serum source	No. of samples positive[a]/no. tested (%) by:		
	LA	FAMA assay	ELISA
Healthy children	41/54 (76)	50/54 (93)	17/28 (61)
Leukemic children	54/95 (57)	68/95 (72)	45/93 (48)
Healthy adults	25/48 (52)	33/48 (69)	16/45 (36)
Totals	120/197 (61)	151/197 (77)	78/166 (47)

[a] All preimmunization sera were seronegative by all assays. The differences in positivity rates are significantly different by chi-square analysis with contingency correction between all of the assays (ELISA versus FAMA assay, $P = 0.0001$; LA versus FAMA assay, $P = 0.001$; LA versus ELISA, $P = 0.01$).

FAMA assay, and in 47% by ELISA (Table 1). The seropositivity rates of these three tests were significantly different.

Two hundred-twenty-four serum specimens that had been consecutively submitted to our laboratory were tested by LA and the FAMA assay; these data are presented in Table 2. Positive results were detected by the FAMA assay in 64% and by LA in 65% of the specimens. A subset of 61 serum specimens was also tested by ELISA (Tables 3 to 5). Of the 61 serum specimens tested by all three assays, the FAMA assay was positive in 44 (72%), LA was positive in 44 (72%), and ELISA was positive in 33 (54%). There were no significant differences in rates of seropositivity among the three assays in the subset of 61 serum specimens (Tables 3 to 5).

Combining the data from Tables 1 to 5 indicates that 265 of 421 serum specimens (63%) were positive by LA, 295 of 421 (70%) were positive by the FAMA assay, and 111 of 227 (49%) were positive by ELISA. The sensitivity of LA compared with that of FAMA was 90% (63%/70%), and that of ELISA compared with that of FAMA was 70% (49%/70%).

A comparison of the end point titers of 56 serum specimens tested by FAMA and LA is shown in Table 6. In 38 serum specimens, end points were the same or within one dilution of each other. In nine serum specimens, titers were more than one dilution greater by the FAMA assay, and in nine serum specimens, titers were more than one dilution greater by LA.

Fifteen serum specimens from adults with no history of varicella were also tested for antibodies to HSV, CMV, and Epstein-Barr virus (EBV) by the methods listed in Table 7. None had detectable antibodies to VZV, although most had detectable antibodies to the other herpesviruses (Table 7). In some instances, high antibody titers to the other viruses were present.

Sera with high (1:256), medium (1:32), low (1:4), and negative (<1:2) LA titers were tested on five separate occasions to determine the reproducibility of LA. In each instance the titer remained the same. Three different lots of latex VZV antigen were tested with these same sera, and the titers remained the same. This testing was performed on a single day.

Results of LA and FAMA testing for all of the sera tested are presented in Table 8. There were 92% agreement (44%/48%) between LA and the FAMA assay with regard to positive titers (sensitivity) and 93% agreement (52%/56%) between LA and the FAMA assay with regard to negative titers (specificity).

TABLE 2. Comparison of VZV antibody determinations by LA and the FAMA assay in sera from 224 consecutively tested patients

LA result	No. of FAMA results		
	Positive	Negative	Total
Positive	134	11	145 (65%)
Negative	10	69	79
Total	144 (64%)	80	224

TABLE 3. Comparison of VZV antibody determinations by LA and the FAMA assay in sera from 61 patients[a]

LA result	No. of FAMA results		
	Positive	Negative	Total
Positive	40	4	44 (72%)
Negative	4	13	17
Total	44 (72%)	17	61

[a] After 224 serum samples were tested by LA and the FAMA assay (Table 2), a subset of 61 samples was tested by all three assays (see Tables 4 and 5). For the subset, there are no significant differences between the rates of seropositivity between any of the three assays ($P > 0.05$).

TABLE 4. Comparison of VZV antibody determinations by the FAMA assay and ELISA in sera from 61 patients[a]

ELISA result	No. of FAMA results		
	Positive	Negative	Total
Positive	32	1	33 (54%)
Negative	12	16	28
Total	44 (72%)	17	61

[a] After 224 serum samples were tested by LA and the FAMA assay (Table 2), a subset of 61 samples was tested by all three assays (see Tables 3 and 5). For the subset, there are no significant differences between the rates of seropositivity between any of the three assays ($P > 0.05$).

TABLE 5. Comparison of VZV antibody determinations by LA and ELISA in sera from 61 patients[a]

ELISA result	No. of LA results		
	Positive	Negative	Total
Positive	33	0	33 (54%)
Negative	11	17	28
Total	44 (72%)	17	61

[a] After 224 serum samples were tested by LA and the FAMA assay (Table 2), a subset of 61 samples was tested by all three assays (see Tables 3 and 4). For the subset, there are no significant differences between the rates of seropositivity between any of the three assays ($P > 0.05$).

A rapid, simple, and sensitive assay for detecting antibodies to VZV would have many practical uses. For example, such an assay could be employed to screen potential candidates for vaccination and to determine whether vaccination has been successful, to plan strategy for management of potential nosocomial spread of varicella, to decide whether to administer passive immunization to adults, and to perform serological surveys. Our data indicate that the LA assay fulfills many of these criteria. It can be completed in 15 min and requires no specialized equipment. LA appears to approach the FAMA assay in sensitivity, and we found that

TABLE 6. Comparison of end point titers of VZV antibody by FAMA and LA in 56 serum specimens[a]

Assay	No. of serum samples with endpoint titers[b] of:							
	4	8	16	32	64	128	256	512
FAMA	1	4	3		13	13	15	7
LA		3	5	5	7	19	8	9

[a] In 38 serum specimens, end points were the same or within one dilution of each other. In nine serum specimens, titers were more than one dilution greater by the FAMA assay, and in nine serum specimens titers were more than one dilution greater by LA.

[b] Titers are presented as reciprocals of the end point dilution.

TABLE 8. Summation of the comparison of VZV antibody determinations by LA and the FAMA assay from this entire study of 878 serum specimens[a]

LA result	No. of FAMA results		
	Positive	Negative	Total
Positive	379	11	390 (44%)
Negative	46	442	488
Total	425 (48%)	453	878

[a] The rate of positivity is not significantly different between these assays ($P > 0.05$).

false-positive latex determinations appeared to be minimal. Only 11 of 453 (2%) serum specimens were FAMA negative and LA positive, and of these, 5 were from serum specimens obtained after immunization, so they may not actually represent false-positive LA antibody determinations (the preimmunization sera were negative by LA). In addition, we did not demonstrate any false-positive VZV antibody titers due to cross-reactions with other herpesviruses, and there was no demonstrated lot-to-lot variability. Interestingly, LA tests have been employed successfully to detect antibodies against two other herpesviruses, HSV (2) and CMV (3, 6).

Assays for VZV antibodies vary in their sensitivity. FAMA tests (5) and ELISAs (4) performed in some laboratories appear to be extremely sensitive. However, commercially available ELISAs have been found to lack sensitivity. In an earlier study from our laboratory, a commercially available ELISA showed a sensitivity of 86% compared with the FAMA assay (1). In the present study, the FAMA assay was found to be significantly better at detecting positive VZV antibody titers after immunization than the ELISA, and the sensitivity of the commercial ELISA compared with the FAMA assay was only about 70%. Possibly this was lower than that of our previous study since a number of serum specimens from vaccinated persons were included in the current study and none were studied in the earlier study. In the postimmunization sera from the present study, the FAMA test performed significantly better than the LA assay, but the LA assay was significantly better than the ELISA. In the sense that vaccine-induced immunity is rarely as strong as naturally induced immunity, these findings may not be surprising.

Our comparison of LA and the FAMA assay showed a sensitivity and specificity of about 90%, although for assessment of postvaccine immunity the FAMA test was significantly better than the LA assay. After natural disease and when all of the sera tested were considered, however, there was no significant difference between positive reactions as measured by the FAMA assay or LA. Experience teaches us that no serologic test is perfect. LA and the FAMA assay, however, compare very favorably with each other, and given the ease of performance of LA, it has the potential to become a useful laboratory test for measuring antibodies to VZV.

TABLE 7. Analysis of possible cross-reacting antibodies to other herpesviruses in 15 serum samples from adults with no history of varicella[a]

Serum no.	Antibody titer[b] to:		
	HSV	CMV	EBV
1	<2	<2	Positive
2	≥16	<2	Equivocal
3	<2	<2	Equivocal
4	≥16	≥16	Positive
5	≥16	≥16	Positive
6	≥16	<2	Positive
7	≥16	<2	Positive
8	≥16	<2	Positive
9	<2	≥16	Positive
10	≥16	<2	Positive
11	≥16	<2	Positive
12	≥16	<2	Positive
13	≥16	≥16	Positive
14	<2	<2	Positive
15	≥16	≥16	Positive

[a] Seraa were tested for the presence of antibodies to HSV, CMV, and EBV by HSV LA (Wampole Laboratories), CMV SCAN (Becton Dickenson), and EBV-VCA-FIAX (Whittaker Bioproducts). Despite high antibody titers to some of these other viruses, the VZV antibody titers were negative (<2) as determined by FAMA and LA.

[b] Numerical titers are given as reciprocals.

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