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Comparison of Latex Agglutination Test with Enzyme-Linked Immunosorbent Assay for Detection of Antibody to Varicella-Zoster Virus

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A new latex agglutination (LA) test (VZVscan; Becton Dickinson, Cockeysville, Md.) was compared with an enzyme-linked immunosorbent assay (ELISA) (Varicella STAT; Whittaker Bioproducts, Walkersville, Md.) for detection of varicella-zoster virus (VZV) antibody. Of 165 samples tested, 126 (76%) were positive by LA, 123 (73%) were positive by ELISA, and 35 (21%) were negative by both methods. Six samples (4%) were LA positive and ELISA negative or equivocal; three samples (2%) were ELISA positive and LA negative. However, LA failed to detect seroconversions in four adults with varicella in samples obtained 14 to 17 days after the onset of rash. These same samples had ELISA values in the high-positive range. In addition, the recommended LA screening dilution (1:2) is not supported by published data and should be changed.

The availability of a rapid and sensitive test for determination of an individual's immune status to varicella-zoster virus (VZV), particularly for health care workers and immunocompromised patients, would be a great benefit to clinical virology laboratories. The fluorescent-antibody-to-membrane-antigen test is the "gold standard," but it is too laborious for routine clinical use (6, 7). The enzyme-linked immunosorbent assay (ELISA) has been found to be reasonably sensitive and specific (3, 4); it is well-suited to routine screening of large numbers of samples and requires approximately 2 to 3 h for its completion. ELISA is not optimal when single-sample testing and rapid turnaround times are preferred. Thus, the report of a sensitive, specific, and rapid latex agglutination (LA) assay for VZV antibody (5) was enthusiastically received. A similar LA test is used in our laboratory and others for determination of cytomegalovirus antibody and has been found to be very sensitive and specific (1). Thus, the LA test was compared with ELISA for the detection of VZV antibody in our laboratory, and the results are described in this report.

Serum samples from the hospital Personnel Health Service, various outpatient clinics, and hospital wards were submitted to the Clinical Virology Laboratory for determination of VZV antibody and were tested by both ELISA and the LA test for the purposes of the evaluation described here. Approximately 78% of samples tested were from adults, and the majority of these were from immunocompetent hosts. Only 14% of specimens submitted were from children, of whom about 60% were immunocompromised. Samples were assayed by ELISA (Varicella STAT; Whittaker Bioproducts, Walkersville, Md.) according to the instructions of the manufacturer. Absorbance values were determined with a spectrophotometer. A predictive index value (PIV) of 1.00 or greater was considered positive for VZV antibody, a value of 0.80 to 0.99 was considered equivocal, and a value of <0.80 was considered negative. Samples were tested by the LA test by using VZVscan (Becton Dickinson, Cockeysville, Md.) according to the instructions of the manufacturer. Serum specimens were screened at a 1:2 dilution. If agglutination was observed, samples were reported as positive for VZV antibody. If no agglutination was observed at the 1:2 dilution, a 1:20 dilution was prepared and the sample was retested to exclude a prozone phenomenon. Samples showing agglutination at the 1:20 dilution were reported as positive. Additional serum dilutions were tested by the LA test for serum samples from four adults with documented primary infection with VZV.

In late 1991, 79 patient samples were tested in parallel by ELISA and the LA test in preparation for the introduction of the LA test for routine use in the clinical laboratory. In July 1992, after the LA test failed to detect seroconversions in convalescent-phase serum samples from several adult patients with varicella, an additional 86 patient samples were tested by both ELISA and the LA test. These results were combined and are presented in Table 1. Results of the two tests agreed for 155 of 165 samples tested (94%). Six samples (4%) were LA test positive but ELISA equivocal or negative. Three samples (2%) were ELISA positive and LA test negative. ELISA values for these discrepant samples are given in Table 2. With the exception of one sample (PIV, 2.18), ELISA values were close to the positive cutoff. A clinical correlation was known for only one of these discrep-

TABLE 1. Comparison of LA test with ELISA for determination of VZV immune status

LA test result	No. (%) of serum specimens with the following ELISA result:			
	Positive	Negative	Equivocal	Total
Positive[a]	120	1	5	126 (76)[b]
Negative	3	35	1	39
Total	123 (73)[b]	36	6	165

[a] One sample exhibited a prozone phenomenon.
[b] The difference between the two tests was not significant (chi-square, <0.75).

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TABLE 2. ELISA PIVs for discrepant samples

No. of samples (n = 10)	Test result		
	LA	ELISA	ELISA PIV[a]
3	−	+	2.18, 1.01, 1.12
1	−	Equivocal	0.80
1	+	−	0.68
5	+	Equivocal	0.90,0.93,0.86,0.88, 0.93

[a] PIV of 0.80 to 0.99, equivocal; ≥1.00, PIV of positive.

ant samples. A child with Burkitt's lymphoma was reported as VZV antibody positive on the basis of the LA test result. He had no history of chicken pox and subsequently contracted varicella from his only sibling (his mother also developed varicella). The patient's sample was subsequently found to be in the equivocal range by ELISA (PIV, 0.93). Three additional ELISA-negative, LA test-positive samples were identified from children with malignancies. One patient had received varicella-zoster immune globulin, and one had received varicella vaccine. The third patient had no history of varicella.

In June 1992, we became aware of three adult patients with documented varicella who were persistently negative by the LA test 2 weeks or more after the onset of clinical disease (Table 3). Two (patients 1 and 2) were hospitalized because of complications of VZV infection. Patient 1 developed varicella pneumonia and suffered a miscarriage. Patient 2 died from varicella-associated cerebral vasculitis. Patient 3 had two children who sequentially developed varicella. Since she was pregnant and had no history of varicella, a serum sample was tested and found to be negative for VZV antibody by the LA test. She subsequently developed varicella, and on her follow-up visit to the clinic, a convalescent-phase serum sample was tested, but it remained negative for VZV antibody by the LA test. The clinic notified the laboratory of the unexpected result. Serum samples from these three patients were then retested by the LA test at multiple additional dilutions to detect a prozone reaction, but none was detected. Samples were then tested by ELISA, and all convalescent-phase serum samples were found to have ELISA values in the high-positive range (≥2.40). Subsequently, acute- and convalescent-phase serum samples from a fourth adult who developed varicella were submitted. These samples were also tested by both the LA test and ELISA (Table 3).

Thus, although the LA test was found to be a rapid and sensitive test for immune status testing for VZV antibody, two problems became apparent with the LA assay and are presented in this report.

First, four adults with documented varicella failed to demonstrate seroconversion by the LA test 14 to 17 days after the onset of illness, when antibody should have been detectable. Serial serum dilutions up to 1:40 (patient 1), 1:256 (patient 2), and 1:640 (patients 3 and 4) failed to reveal a prozone as the cause. Although it is possible that higher serum dilutions may have revealed a prozone, the reasons for this discrepancy have not been established. No serum remained for further testing by more specific methods such as Western blotting (immunoblotting), and sera were not collected later in the convalescence phase. In a previously published report (5), was stated that 130 acute- and convalescent-phase serum specimens from both adults and children with varicella were tested. However, for a subgroup of those patients, it was stated that convalescent-phase sera were obtained 28 days after the onset of rash. For the remaining patients, the timing of convalescent-phase serum sample collection was not stated. The results for these samples as a distinct group and the tests that were applied were not specifically mentioned in the results.

Second, the manufacturer's instructions for VZVscan inadvertently do not accurately reflect the published data but, rather, recommend screening at a 1:2 dilution. This error was discovered when a patient reported as positive by the LA test tested and whose serum was tested only at a 1:2 dilution, according to the manufacturer's directions, developed varicella. The correlation of lack of protection from infection with antibody levels is difficult to assess in immunocompromised patients, and in fact, in two other discrepant samples in our laboratory, it appeared that the LA test was more sensitive than ELISA for the detection of low antibody levels. However, upon careful rereading of the previous reports on the LA test and the fluorescent-antibody-to-membrane-antigen test (2, 5), it was evident that samples

TABLE 3. Antibody to VZV detected by LA and ELISA in four adults with varicella

Patient no.	Sex[a], age (yr)	Underlying condition	VZV-associated complication	Day that sample was obtained[b]	Test results by[c]		Dilutions tested by LA test
					ELISA (PIV)[d]	LA	
1	F, 25	28 wk pregnant	Pneumonia	0	Neg (0.23)	Neg	1:2 and 1:20
				4	Neg (0.37)	Neg	1:2 and 1:20
				14	Pos (7.46)	Neg	1:2 through 1:40
				17	Pos (7.28)	Neg	1:2 through 1:40
2	M, 45	None	Cerebral vasculitis	15	Pos (3.69)	Neg	1:2 through 1:256
3	F, 21	15 wk pregnant	None	−28[e]	Neg (0.49)	Neg	1:2 and 1:20
				14	Pos (3.60)	Neg	1:2 through 1:640
4	F, 21	11 wk pregnant	None	−14[f]	Neg (0.23)	Neg	1:2 and 1:20
				16	Pos (3.64)	Neg	1:2 through 1:640

[a] F, female; M, male.
[b] Day after the onset of varicella.
[c] Neg, negative result; Pos, positive result.
[d] An ELISA PIV of ≥1.00 is considered positive; a PIV of ≥2.40 is considered a high positive.
[e] Serum was obtained when the first of the patient's two children developed varicella, 28 days before the onset of the patient's rash.
[f] Serum was obtained when the patient's child developed varicella.

negative for VZV antibody at a 1:2 dilution were called negative (i.e., <1:2 dilution) and samples positive at a 1:4 dilution were considered positive. Importantly, the disposition of samples positive at a 1:2 dilution only was not specifically mentioned.

As the manuscript of this report was being completed, we received results of a proficiency survey of the College of American Pathologists (set VR3-D, specimen VR3-19, 1992). In that survey, a serum sample for VZV antibody, supposedly from an adult with varicella, was detected as positive by all 147 laboratories by the ELISA methodology but by only 4 of 16 laboratories by the LA test methodology. Of the laboratories that used a variety of other non-ELISA methods, 114 of 115 reported detection of antibody to VZV, reporting titers as high as 1:1,280. The reasons for the low detection rate by the LA test were unknown.

Thus, in this report, we would like to suggest that further evaluation of the LA test for its ability to detect seroconversions within the first 2 to 3 weeks after the onset of illness is needed, because this is the most common time for collection of convalescent-phase serum samples in clinical settings. Furthermore, the observation of agglutination at a 1:2 screening dilution is not sufficient to designate a sample as antibody positive, according to previously published data. Laboratories that use the LA test should be aware of this discrepancy, and the manufacturer's instructions should be modified accordingly.

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