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Determination of Varicella-Zoster Virus (VZV) Immune Status with the VIDAS VZV Immunoglobulin G Automated Immunoassay and the VZV*Scan* Latex Agglutination Assay

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The VIDAS varicella-zoster virus (VZV) immunoglobulin G immunoassay and the VZV*Scan* latex agglutination (LA) test were compared for the detection of VZV antibody in patient sera. Of 625 samples tested, 554 were positive for VZV antibody by one or both methods. Five hundred thirteen (82.1%) samples were positive by both methods, 71 (11.4%) were negative by both methods, and there were 41 (6.6%) discrepant samples. Statistically significant differences in sensitivity and specificity were not found ($P = 0.37$); however, several observations were made. All 23 VIDAS repeat equivocal samples were LA positive. Fifty-four samples showed prozone effects with the LA assay, all of which resolved as positive.

Varicella-zoster virus (VZV) is the etiologic agent of two different clinical syndromes; chicken pox (varicella), the primary infection; and shingles (zoster), which is reactivation of latent VZV (12). In most instances, chicken pox is a mild childhood illness resulting in mild fever and malaise followed by a vesicular rash. Adults with chicken pox can develop severe complications such as pneumonia. Shingles or zoster generally occurs in adults from reactivation of latent VZV. Reactivation can be linked to stress, trauma, or certain types of drug therapies, although often no precipitating factors can be identified (12).

Several methods are available for detection of VZV antibody from patient sera (1, 7, 10, 11, 13). However, rapid, sensitive, and easy-to-perform assays are needed clinically in determining the immune status of individuals with no prior history or memory of varicella infection (2). Assays of this nature would be particularly useful in monitoring individuals before and after immunization with the varicella vaccine, as well as those individuals who are at high risk for varicella (health care workers and pregnant women), whose immune status needs to be known on an immediate basis following exposure. For these reasons, we compared two rapid and simple methods, the VZV*Scan* latex agglutination (LA) assay (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and the VIDAS VZV immunoglobulin G automated immunoassay (VZG; bioMerieux/Vitek, Rockland, Mass.), for determining immune status of VZV antibody in patient sera.

A total of 625 serum samples were tested for VZV immune status. Samples were obtained from patients undergoing prenatal screening workups, health care workers and children in the Providence Child Center, and individuals undergoing preemployment physicals at Providence Portland Medical Center.

The VIDAS VZG assay is an enzyme-linked fluorescent immunoassay performed within the automated VIDAS instrument in 40 min. The assay was performed according to the manufacturer's instructions. In brief, the assay uses a 10-well, self-contained reagent strip, which includes all of the buffers and reagents needed for the assay. A solid-phase receptacle coated with VZV antigen is placed into the VIDAS for each sample and control being tested. A minimum of 100 μl of serum is required to perform the assay, and this is added to the first well of the reagent strip. All assay steps including reagent additions, washes, and readings are accomplished automatically within the VIDAS instrument (the actual interaction of the reagent strip and solid-phase receptacle for the VIDAS instrument has been previously published [6]). When the VIDAS VZG assay is completed, the results are analyzed automatically by the computer. For final result interpretation, test values from the patient and control samples are compared with a set of threshold values stored in the VIDAS computer which represent a low-positive varicella antibody titer. Interpretation of the test value thresholds is as follows: <0.60, negative; ≥ 0.60 to ≤ 0.90, equivocal; and ≥ 0.90, positive.

The VZV*Scan* test kit is a passive LA assay for the qualitative and quantitative detection of total VZV antibodies in human serum. Included in the kit were latex reagent sensitized with VZV antigen; buffered diluent; and low-positive, high-positive, and negative control sera. All samples tested by VZV*Scan* were screened by the qualitative screening procedure as outlined by the package insert and previously described (2, 10). Briefly, sera were diluted 1:2 and 1:40 in buffered diluent, and 25 μl of the serum dilutions was added to different wells of a black serologic card. One drop (≈15 μl) of the LA reagent was then added to each of the wells and mixed. A moist cover was placed over the card and then mixed on a serologic rotator for 10 min at 100 rpm. The wells were examined for agglutination under a high-intensity incandescent lamp and compared with the control wells for a positive or negative reaction. Tests of sera giving discrepant results were repeated by both assays. Final results were statistically calculated by McNemar's chi-square test (5).

Of 625 samples tested, 554 (88.6%) were positive for VZV antibody by one or both methods. The two methods had an agreement of 93.4%. A total of 513 (82.1%) were positive by both methods, 71 (11.4%) were negative by both methods, and there were 41 (6.6%) discrepant samples. By the VZV*Scan* assay, there were 544 positive (87.0%) and 81 negative (13.0%) samples. By the VIDAS VZG assay, there were 523 positive (83.7%), 79 negative (12.6%), and 23 equivocal (3.7%) sam-

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TABLE 1. Results of VZV antibody detection by VIDAS VZG and VZV*Scan* for 625 serum samples

VIDAS result	No. of samples with VZV*Scan* result	
	Positive[a]	Negative
Positive	513	10
Negative	8	71
Equivocal	23	0

[a] Includes samples reactive with VZV*Scan* at the 1:2 and/or the 1:40 dilution.

ples (Table 1). Repeat testing of the 41 discrepant serum samples showed no change in the initial test results. Of these, 10 samples were positive only by VIDAS VZG, with threshold values ranging from 0.90 to 2.27. Eight samples were VZV*Scan* positive only, seven being positive at the 1:2 dilution and one being positive at both the 1:2 and 1:40 dilutions. There were 23 serum samples that tested as equivocal with the VIDAS VZG assay, and all 23 were positive by the VZV*Scan* assay (17 positive at the 1:2 dilution and 6 positive at both the 1:2 and 1:40 dilutions). With removal of the equivocal samples, VZV*Scan* had a sensitivity of 98% and a specificity of 90% compared with the VIDAS VZG assay with no equivocal results. Conversely, the VIDAS VZG assay had a sensitivity of 98.5% and a specificity of 87.7% compared with the VZV*Scan* assay.

Rapid and sensitive screening methods are needed for determining past infection and immune status to VZV. The fluorescent-antibody-to-membrane-antigen assay is considered to be the most sensitive method for these determinations. However, the test requires the use of live, unfixed, VZV-infected cells and is very labor-intensive to perform (1, 13). In a study performed in 1991, Steinberg and Gershon tested 878 serum samples and showed that VZV*Scan* was nearly as sensitive as the fluorescent-antibody-to-membrane-antigen assay and was more sensitive than a commercial enzyme immunoassay (EIA) (10). These findings were further confirmed by Gershon and coworkers when testing a group of well-defined VZV acute and convalescent sera (2). Martins et al. in an independent study (9) showed that VZV*Scan* had an excellent sensitivity compared with an indirect fluorescent antibody assay and with a standard EIA. However, in the study, VZV*Scan* seemed to lack specificity because of false-positive interpretations of the assay (9). Two other studies also have shown good correlation between EIA and VZV*Scan* (8, 11). Both of these studies, however, had minor problems with false-negative reactions and prozone effects with VZV*Scan*. There is not a clear explanation for these observed differences in methods. For VZV-*Scan*, it is recommended by the manufacturer that sera negative at the 1:2 dilution be retested at a 1:40 dilution to identify samples exhibiting the prozone phenomenon. Additionally, LA and indirect fluorescent antibody methods (including fluorescent-antibody-to-membrane-antigen assay) can suffer from subjective interpretation in determining specific and significant agglutination or fluorescence (3). As has been seen with other viral serological methods, various tests will have discrepant results with a small number of samples (4). Therefore, in these patients true antibody status may need to be defined with additional antibody testing or other clinical information.

In this study, there were no significant differences between the two methods (chi-square, $P = 0.37$); however, there were several observed differences. (i) All 23 repeat VIDAS VZG-equivocal samples were VZV*Scan* positive. (ii) Fifty-four samples (9%) showed prozone reactions. That is, these samples were considered negative at the 1:2 dilution with VZV*Scan*. These resolved and were all positive upon retesting at the 1:40 dilution. (iii) Of the above 54 samples, 53 were VIDAS VZG positive and 1 was VIDAS VZG negative. (iv) There was one apparent false-positive sample with the VIDAS VZG assay. This sample was a low positive with the VIDAS VZG assay and negative with VZV*Scan*. The individual was diagnosed with chicken pox 2 weeks later.

Our study design did not include testing samples with the VZV*Scan* between the two recommended dilutions of 1:2 and 1:40. As the data show, this study had a total of 23 samples that were positive only with VZV*Scan* (Table 1). Gershon et al. (2) recently showed that 4% of serum samples considered negative in their patient population reacted at the 1:2 dilution but upon further testing were negative at a 1:4 dilution. Gershon and coworkers concluded that screening with the VZV*Scan* kit should begin at the 1:4 dilution to avoid false-positive reactions (2). Therefore, we cannot rule out the possibility that a small percentage of the 23 samples positive by VZV*Scan* in this study are false positive. Ten samples were VIDAS VZG positive only with low threshold values (0.90 to 2.27). Without further testing or clinical information, we cannot rule out the possibility that some of these samples may be false positive as well.

Both methods were easy to perform and to incorporate into the laboratory work flow. The VIDAS VZG assay is performed on the VIDAS instrument, which is fully automated and requires little result interpretation. However, 23 samples were equivocal with the VIDAS after repeat testing. We have observed this problem before with the VIDAS RBG rubella immunoglobulin G assay (9a). Since the assay is performed in a closed automated system and results are generated by the VIDAS instrument, it is difficult to ascertain any specific component or reagent problem with the assay. The VZV*Scan* assay has no equivocal reactions, but interpretation of agglutination patterns can be subjective. Additionally, serum samples that test negative at the 1:2 dilution with VZV*Scan* must be retested at the 1:40 dilution to eliminate false-positive results caused by prozoning. For those laboratories using EIA or automated systems such as the VIDAS, equivocal or indeterminant reactions and negative results from high-risk patients should be tested by a second method, such as VZV*Scan*, for accurate determination of patient immune status.

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