Comparison of Three Commercially Available Assays for Detection of Varicella-Zoster Virus Antibody

THOMAS B. MARTINS,4* TROY D. JASKOWSKI,1 CARL SCHRODER,1
BRUCE STREETER,1 AND HARRY R. HILL1,2

Associated Regional and University Pathologists1 and the Department of Pathology,
University of Utah School of Medicine,2 Salt Lake City, Utah 84108

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Three commercially available diagnostic assays for the detection of antibodies to varicella-zoster virus were evaluated to determine which would be the most suitable for our clinical laboratory. Three different methods were examined: an enzyme-linked immunosorbent assay (ELISA), latex agglutination (LA), and an indirect fluorescent-antibody technique. For the 141 serum specimens tested, the ELISA had an agreement of 90.1% and LA had an agreement of 92.2% with the indirect immunofluorescent-antibody technique. The ELISA had a lower sensitivity (85.6%) than LA (100.0%), but LA suffered from a low specificity (78.4%) compared with the ELISA (98.0%).

It is often necessary to determine prior exposure to varicella-zoster virus (VZV) in health-care workers and closed populations exposed to individuals with varicella infections. The test using fluorescent antibody to membrane antigen (FAMA) is said to be the most effective test for determining immune status. Unfortunately, this test requires the use of live tissue culture cells infected with VZV and is not available in hospital laboratories or in the major reference laboratories in this country. Instead, a standard indirect immunofluorescent-antibody (IFA) assay employing fixed infected tissue culture cells is used. We are often asked to determine the prior exposure and immune status of an individual on a “stat,” or immediate, basis. For these reasons, we evaluated three commercially available assays for detecting antibodies to VZV, including a latex agglutination (LA) assay (Becton Dickinson Microbiology Systems, Cockeysville, Md.), an IFA method (Virgo; Schiapparelli Biosystems, Inc., Columbia, Md.), and an enzyme-linked immunosorbent assay (ELISA) (Sigma Diagnostics, St. Louis, Mo.). Each of the three methods had distinct advantages. The IFA method is based on the traditional FAMA method, which is often considered the “gold standard” for VZV testing. IFA methods have had good agreement with FAMA (2). ELISA compares well with FAMA, and in some studies it is even more specific (3). The ELISA has also eliminated the use of live cell cultures and is readily automated (1). LA is a rapid, easy-to-perform assay which has been reported to be almost as sensitive as FAMA (4). While both the IFA test and ELISA are suitable for routine serological testing of exposed health-care workers and closed populations exposed to VZV, we were interested in the suitability of LA to meet our needs as a stat test for determining exposure status of patients and organ and bone marrow donors.

MATERIALS AND METHODS

Clinical samples. A total of 141 specimens, which were submitted to our laboratory for VZV antibody testing, were included in this study. Of these 141 samples, 130 were from adults and 11 were from children; 101 were from female patients, 39 were from male patients, and 1 was unknown. Patient age varied from 2 months to 81 years, with a mean of 29.2 years. All samples were shipped to the laboratory on ice and stored at 4°C until tested. Samples were then stored at −20°C until additional testing was required. All discrepant samples were retested by all three methods to verify results.

LA procedure. LA was performed according to the instructions of the manufacturer (Becton Dickinson Microbiology Systems). In brief, a 1:2 serum dilution was mixed with polystyrene latex particles coated with partially purified VZV antigens, and the mixture was then observed for visible agglutination (clumping) as an indication of the presence of VZV antibodies. Samples were considered positive for VZV antibody when clumping was visible. In the absence of agglutination, the specimen was considered negative.

ELISA procedure. For the ELISA, the manufacturer’s product insert (Sigma Diagnostics) was followed. One hundred microliters of a 1:41 serum dilution was added to the antigen-coated (inactivated, purified Ellen strain) microtiter well. The plate was read at 405 nm with a microplate reader (Sigma Diagnostics). For interpretation of the optical density values, a calibrator sample which was used to convert the optical densities into arbitrary absorbance units (AU) per milliliter was run. By this method, the following ranges were established: >20 AU/ml, positive; 15–20 AU/ml, equivocal; and <15 AU/ml, negative.

IFA procedure. For IFA testing, Virgo kits (Schiapparelli Biosystems, Inc.), which employ infected and uninfected fixed cells, were used. The assay was performed according to the instructions provided by the manufacturer, with a 1:8 serum dilution used for screening. The reacted slides were read at a magnification of ×400; results were reported as positive if the VZV-infected substrate cells displayed a + or greater fluorescence (up to 4+) and as negative if there was no fluorescence or only barely visible fluorescence in the infected substrate cells.

As mentioned above, the FAMA is often considered the gold standard for determining immune status with regard to VZV. The standard IFA test, using fixed rather than live tissue culture cells, is the test most closely related to FAMA. For these reasons, the IFA test was used as the standard with which
the ELISA and LA were compared. The data from all 141
serum samples were then used to calculate agreement, sen-
sitivity, and specificity for the investigated assays (ELISA
and LA) compared with the IFA test. Calculations were
based on the following correlations: investigated assay and
IFA test results both positive, A; investigated assay result
positive and IFA test result negative, B; investigated assay
result negative and IFA test result positive, C; investigated
assay and IFA test results both negative, D; investigated
assay result equivocal and IFA test result positive, E; investi-
gated assay result equivocal and IFA test result negative, F.
Determinations were made as follows: agreement = (A + D)/(A + B + C + D + E + F); sensitivity = (A)/(A + C + E); specificity = (D)/(B + D + F).

RESULTS

Comparison of ELISA and LA with IFA for the detection
of VZV antibody. The ELISA for VZV antibody performed well
compared with the IFA test: the ELISA had an agreement
of 90.1%, a sensitivity of 85.6%, and a specificity of 98.0% (Table
1). Of the 51 samples which were negative by the IFA test, the
ELISA missed one, identifying it as an equivocal rather than a
negative sample. Of the 90 samples identified as positive by
IFA testing, the ELISA misidentified 13, with 10 samples
having negative results and the remaining 3 having equivocal
results.

We next compared the LA and IFA tests. (Table 2) LA had
an agreement of 92.2%, a sensitivity of 100.0%, and a speci-
ficity of 78.4%. Thus, of the 51 negative results obtained by
IFA testing, LA gave false positives for 11, but it correctly
identified all 90 of the positive results obtained by IFA testing.

In order to quantify the antibody detection limits of the
investigated assays, four samples, which were identified as
positive by all assays, were titrated. The results in Table 3 show
that the ELISA was the most sensitive, identifying one sample
as positive at a 1:2,048 dilution, while the IFA titer was only
1:512 and the LA titer was 1:128. The ELISA also had titers
equal to or greater than those in the IFA test and LA for the
remaining three samples tested.

DISCUSSION

The data indicate similar levels of agreement between the
ELISA (90.1) and LA (92.2) in detecting antibodies to VZV in

patients’ serum when compared with the IFA test. In contrast,
the ELISA proved to be a more specific test than LA but
lacked the sensitivity displayed by LA, even though the ELISA
was able to detect lower antibody concentrations in tested
samples (Table 3). This could be due to the purified nature of
the antigen used to coat the ELISA plates, which may not
include all the epitopes present on the virus. Therefore, the
whole-cell and partially purified antigens utilized by the IFA
test and LA may be responsible for the increased sensitivity
of these assays.

When compared to the IFA test, the ELISA showed fair
total agreement and had better specificity than LA. With the
ELISA, there is no subjectivity in the determination of results,
which are based strictly on optical density readings. The ease in
running larger numbers of samples and the capacity for
automation were also distinct advantages of the ELISA. For
the purposes of our laboratory in determining the immune
status of patients and bone marrow and organ donors, a higher
specificity is more important than a higher sensitivity, since it is
better to erroneously determine that the patient or transplant
does not have antibody to VZV than to incorrectly assume that
the patient or transplant is immune. Thus, a few false-negative
results are more tolerable than false-positive results. Since LA
does not distinguish between immunoglobulin G (IgG) and
IgM antibodies, the 11 samples testing false positive by LA
were tested for IgM antibody. This was done to eliminate the
remote possibility that these patients had just started to
produce IgM antibody and had IgG levels too low to be
detected with the IgG-specific ELISA and IFA test. This, in
theory, could account for the false-positive results seen with
LA. All 11 samples were negative for IgM antibodies when
tested with an IgM-specific ELISA. LA had the advantage of
being rapid and simple to perform, which would be valuable in
stat testing of samples. However, the interpretation of results
obtained by LA suffers from the subjective determination of
significant agglutination.

The IFA test has the advantage of incorporating whole cells
with all antigens present. The reacted slides were clear and
easy to read for those with experience in IFA techniques. A
disadvantage is subjectivity in determining specific and signif-
ificant fluorescence, and therefore in interpreting results.

Considering these aspects of VZV IgG antibody testing and
the needs of our laboratory, the ELISA and IFA test appeared
to be the best choices for general serological testing. This
judgment is based on the statistical analysis of test perfor-
man ce and the ease of performance and the reproducibility
of the assays. The ELISA had the additional advantage of a lack
of subjectivity in reporting results. LA is a rapid and easy-to-
perform assay, but the low specificity requires all samples with
positive results to be held for confirmation by ELISA or IFA
testing. Since the LA test demonstrated 100% sensitivity, all
negative results could be reported on a stat basis for organ and
bone marrow transplants.

For these reasons our laboratory favors using the IFA test
and ELISA for routine serological testing and LA on a stat

| Table 1. Comparison of VZV antibody detection by ELISA with IFA test results for 141 serum samples |
| ELISA result | No. of samples with IFA test result |
|              | Positive | Negative |
| Positive     | 77       | 0        |
| Negative     | 10       | 50       |
| Equivocal    | 3        | 1        |

| Table 2. Comparison of VZV antibody detection by LA with IFA test results for 141 serum samples |
| LA result   | No. of samples with IFA test result |
|            | Positive | Negative |
| Positive   | 90       | 11       |
| Negative   | 0        | 40       |
| Equivocal  | 0        | 0        |

<p>| Table 3. Titers of four positive samples |</p>
<table>
<thead>
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<th>Sample</th>
<th>ELISA</th>
<th>IFA test</th>
<th>LA</th>
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<tr>
<td>96</td>
<td>1:64</td>
<td>1:64</td>
<td>1:16</td>
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<tr>
<td>97</td>
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<td>1:512</td>
<td>1:128</td>
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<td>104</td>
<td>1:512</td>
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basis for identifying patients who have not been exposed and are at risk for infection.

REFERENCES