

Comparison of Five Assays for Antibody to Varicella-Zoster Virus and the Fluorescent-Antibody-to-Membrane-Antigen Test

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Three commercially available assays (the Varicelisa Test Kit [Whittaker M.A. Bioproducts, Walkersville, Md.], the VZV Indirect Fluorescent-Antibody Test [Electro-Nucleonics, Inc., Columbia, Md.], and the Litton VZV Bio-EnzaBead Screen Kit [Litton Bionetics, Inc., Charleston, S.C.]) and two enzyme-linked immunosorbent assays used in our laboratory, one using a membrane-associated antigen and the other using a soluble antigen dotted on nitrocellulose paper, were compared with a varicella-zoster virus antibody reference assay, the fluorescent-antibody-to-membrane-antigen test. All of the assays compared favorably to the fluorescent-antibody-to-membrane-antigen test when evaluated for sensitivity (0.95), specificity (0.84), and test-retest reliability (79 to 96%), except for the Litton assay, which demonstrated significantly different results for all of the parameters tested (0.55, 1.0, and 69%, respectively).

The presence of serum antibody to varicella-zoster virus (VZV) has been shown to correlate with immunity to varicella (6, 15). Determination of immune status to varicella is of vital importance in the containment of nosocomial infection and in deciding whether to administer postexposure prophylaxis with varicello-zoster immune globulin (VZIG) (1, 3). It will be important in determining the eligibility of some patients for live attenuated VZV vaccine when it becomes licensed.

In a previous study, we have shown that the prevalence of immunity to VZV in a sample of normal adults with no history of varicella was 0.76 (10). Therefore, using only history of clinical disease as an indicator of immunity to VZV is an insensitive and ultimately costly method in people with no history of varicella (9). Until recently, assays for antibody to VZV have only been available in a few reference laboratories using the fluorescent-antibody-to-membrane-antigen test (FAMA), a radioimmune assay, or an enzyme-linked immunosorbent assay (ELISA). A number of commercial VZV antibody kits are now available. We evaluated three of the commercial kits and two ELISAs currently in use in our laboratory, comparing the sensitivity, specificity, and test-retest reliability with those of the FAMA, which has been the standard for determining immunity to VZV in our laboratory (15).

MATERIALS AND METHODS

Serum samples were collected from 229 consecutive subjects (mean age, 32 ± 11.8 years), most (220/229) of whom could not recall having had varicella. A subset ($n = 24$) of the sample was selected, by using a random-number table, for repeat testing to evaluate test-retest reliability.

The important features of all of the assays are outlined in Table 1. The FAMA, membrane ELISA (M-ELISA), and dot ELISA (D-ELISA) were performed in our laboratory.

The FAMA was performed as previously described (15). The M-ELISA was performed by the method of Cleveland et al. (2) by using VZV (Ellen strain)-infected and control human embryonic lung fibroblasts (Flow Laboratories, Inc., McLean, Va.) fixed and frozen at -70°C by the method of Zaia et al. (16) as an antigen. Viral and control antigens for the D-ELISA were prepared by the method of Kamiya et al. (8) and were kindly provided by Merck Sharp & Dohme, West Point, Pa. The total protein content of the antigen preparations was 4.2 mg/ml (measured with the Protein Assay Kit [Sigma Chemical Co., St. Louis, Mo.]). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated that albumin, added as a stabilizer, accounted for approximately 90% of the total protein. Therefore, the actual antigen concentration was about 0.42 mg/ml. The VZV and control antigens were dotted (0.20 μl) onto nitrocellulose paper (Schleicher & Schuell, Inc., Keene, N.H.) with a 10- μl syringe (The Hamilton Co., Reno, Nev.) and allowed to dry. The amount of antigen to be dotted had been previously determined to be the smallest volume of undiluted antigen which could correctly identify known varicella-immune and -susceptible sera (P. LaRussa, S. Steinberg, E. Waithe, M. Seeman, R. Holzman, and B. Hanna, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 459, 1985). The paper was then incubated in blocking buffer (20% fetal bovine serum, 1% bovine serum albumin in phosphate-buffered saline) overnight on a rocker at room temperature to decrease nonspecific binding. Sera, diluted 1:10 and 1:100 in blocking buffer, were incubated on VZV and control dots for 1 h at room temperature in a 96-well, reusable suction manifold (V&P Scientific, Inc., San Diego, Calif.). The wells were washed with Tris-buffered saline between successive incubations with staphylococcal protein A-horseradish peroxidase diluted 1:2,000 in blocking buffer for 30 min at room temperature and 0.05% 4-chloro-1-naphthol (Sigma)-0.0000883% H_2O_2 (Fisher Scientific Co., Pittsburgh, Pa.) in Tris-buffered saline for 30 min at room temperature, protected from light. The dots were evaluated visually and rated on a scale from negative to +3 on the basis

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TABLE 1. Description of VZV antibody assays

| Test | VZV antigen | Serum dilution | Conjugate ^a | Interpretation |
|--------------|--------------|----------------|-------------------------------|--------------------------|
| FAMA | Live cells | 1:2-1:8 | Fluorescein G-H IgG, IgA, IgM | Membrane fluorescence |
| M-ELISA | Fixed cells | 1:40; 1:160 | SPA-HRP | Optical density change |
| D-ELISA | Soluble | 1:10; 1:100 | SPA-HRP | Visual color change |
| Litton assay | Coated beads | 1:100 | G-H IgG-HRP | Optical density change |
| MAB assay | Fixed | 1:25 | R-H IgG-AP | Optical density change |
| ENI assay | Fixed cells | 1:8 | Fluorescein G-H IgG | Cytoplasmic fluorescence |

^a Abbreviations: G-H Ig and R-H Ig, goat and rabbit anti-human immunoglobulins, respectively; SPA, staphylococcal protein A; HRP, horseradish peroxidase; AP, alkaline phosphatase.

of the intensity of the purple color change. Sera were considered to have specific antibody to VZV when a purple dot was present in the VZV antigen well with no color change evident in the control well. Occasionally (5 of 229 sera), a serum specimen, especially at the 1:10 dilution, gave a very faint dot, equally evident on both the viral and control antigens. This was thought to represent nonspecific binding to control antigen. These sera were considered to be VZV antibody negative.

The three commercial kits were provided by the manufacturers. The VZV Bio-EnzaBead Test Kit (Litton Bionetics, Inc., Charleston, S.C. [Litton]) and Varicelisa Test Kit (Whittaker M.A. Bioproducts, Walkersville, Md. [MAB]) were performed by technical representatives from the respective companies. The VZV Indirect Fluorescent-Antibody Test (Electro-Nucleonics, Inc., Columbia, Md. [ENI]), was performed by one of us. The methods used in the three commercial kits were those described in the respective product instructions.

The FAMA detects immunoglobulin G (IgG), IgA, and IgM in serum. All of the other assays detect only IgG.

RESULTS

Table 2 shows the percentage of positive results in the sample for each assay. Approximately 76% of the samples were positive when tested by any of the first five assays listed. Only 43% were positive when tested by the Litton assay. Since this result was so different from the results obtained in the other assays, we retested the first 84 sera evaluated in the first run ($n = 135$) by the Litton assay and obtained similar results (33/84 [39%] positive). In this group of 84 sera, the result of the second run agreed with the result of the first run with 61% of the sera. Because it was no longer possible to obtain additional kits, the statistical analysis for the Litton assay was limited to the 135 serum samples analyzed in the first run. The percentage of positive results in the other five assays was still approximately 76% for the 135 sera tested by the Litton assay, indicating that this smaller group was not different from the group overall.

Table 2 also shows the sensitivity and specificity of the individual assays as compared with the FAMA. By definition, the sensitivity and specificity of the FAMA are 1.0. The sensitivity of an assay was defined as the proportion of FAMA-positive sera which were positive in that assay, and

TABLE 2. Comparison of the sensitivity and specificity of the assays with those of the FAMA

| Test | No. of samples | No. (%) positive | Sensitivity | Specificity |
|--------------|----------------|------------------|-------------|-------------|
| FAMA | 229 | 174 (76) | 1.00 | 1.00 |
| M-ELISA | 229 | 178 (78) | 0.97 | 0.84 |
| D-ELISA | 229 | 173 (76) | 0.95 | 0.87 |
| MAB assay | 227 | 170 (75) | 0.94 | 0.84 |
| ENI assay | 207 | 160 (77) | 0.93 | 0.82 |
| Litton assay | 135 | 58 (43) | 0.55 | 1.00 |

the specificity was defined as the proportion of FAMA-negative sera which were negative in that assay. The sensitivity (0.95) and specificity (0.84) were similar for all of the assays except the Litton assay (sensitivity, 0.55; specificity, 1.0).

Figure 1 shows the predictive value (PV) of a positive test. Plotted on the x axis is the prevalence of immunity to VZV in a population (0 to 1.0). Plotted on the y axis is the probability of immunity to VZV given a positive test result (0 to 1.0). The values for the M-ELISA and the MAB assay were similar and resulted in superimposed curves. If the prevalence of immunity to VZV in the population studied (normal adults with no history of varicella) is estimated to be 0.76, that is, the proportion of sera positive in the FAMA assay, then a positive result in any assay but the Litton assay increases the probability of immunity from 0.76 to 0.95. A positive result in the Litton assay improved the probability of immunity to 1.0.

Figure 2 shows the PV of a negative test. Plotted on the x axis is the prevalence of immunity to VZV, and on the y axis is the probability of susceptibility given a negative test result. If the prevalence of immunity in the population of normal adults with no history of varicella is estimated to be 0.76, then a negative result in any of the assays except the Litton assay increases the probability of susceptibility from 0.24 (i.e., $1 -$ the prevalence of immunity) to 0.84. A negative result in the Litton assay increased the probability of susceptibility from 0.24 to 0.45.

Table 3 shows the proportion of correct answers for each assay, defined as the proportion of test results for each assay that agreed with the result of the FAMA. When analyzed by the chi-square test, the D-ELISA, MAB assay, ENI assay,

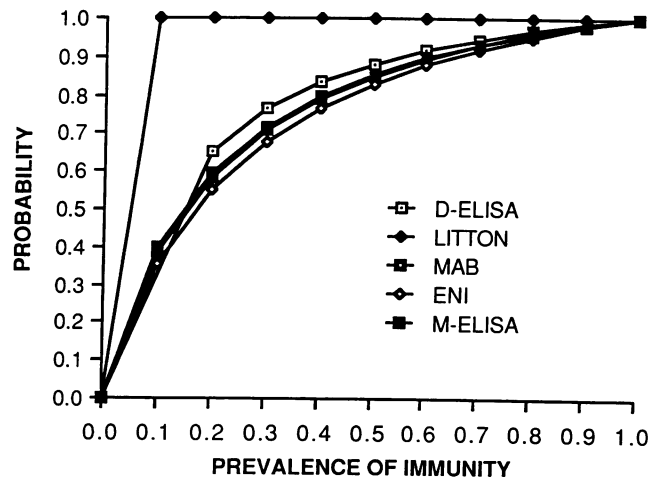


FIG. 1. Predictive value of a positive test.

and M-ELISA formed a homogeneous group ($P = 2.3$), indicating no significant difference among the results of these assays. When the results of the Litton assay were included, the group became heterogeneous, indicating that there was a significant difference between the results of the Litton assay and those of the other four assays ($P < 0.001$). In the subset of 135 sera for which all six assays were performed, there was agreement among all six assays for 79 sera (59%), among five of the six assays for 39 sera (29%), among four of the six assays for 14 sera (10%), and among three of the six assays for three sera (2%). In the group of 39 sera for which there was agreement among five of the six assays, the discordant assay was the Litton assay in 32 cases, the MAB assay in 3 cases, the M-ELISA in 2 cases, and the ENI assay and the FAMA in 1 case each. In the group of 14 sera for which there was agreement in four of the six assays (i.e., 28 discordant results), the discordant assay was the Litton assay in 11 cases, the ENI assay in 7 cases, the FAMA in 5 cases, the MAB assay and the D-ELISA in 2 cases each, and the M-ELISA in 1 case.

Table 4 shows the test-retest reliability for the sample of sera randomly selected to be retested. Limited availability of the Litton assay kits allowed retesting of only 13 of the 24 randomly selected sera. Agreement for test and retest ranged from 100% (FAMA) to 69% (Litton assay). By the type of analysis described above, when the results of the Litton assay were added to the results of the homogeneous group consisting of the other five assays, the group became heterogeneous ($P = 0.03$), indicating a significant difference between the test-retest results of the Litton assay and those of the others.

DISCUSSION

The prevalence of immunity to VZV in our sample of subjects with no history of varicella was 0.76 (Table 1). This is in agreement with previous studies (4, 5, 7, 13). Other authors have reported both higher (0.95) and lower (0.47) prevalences in similar populations (11, 12). This variation in the prevalence of immunity may be a reflection of the mean age of adults, the proportion of people from tropical countries (see below), and the sensitivities of the assays used in the different studies.

To come to a conclusion on the usefulness the various assays, one must first define the population to be studied. In

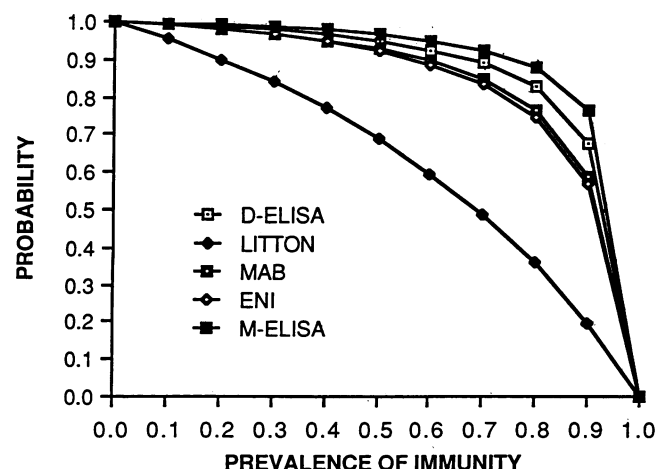


FIG. 2. Predictive value of a negative test.

TABLE 3. Proportion of correct results for each assay

| Test | No. of correct results/total ^a | % ^b |
|--------------|---|-----------------|
| M-ELISA | 215/229 | 94 |
| D-ELISA | 214/229 | 93 |
| MAB assay | 207/227 | 91 |
| ENI assay | 188/207 | 91 |
| Litton assay | 88/135 | 65 ^c |

^a The number of assay results which agreed with the result of the FAMA.
^b The results of the first four assays were not statistically different by the chi-square test.

^c The chi-square test revealed a difference between the results of the Litton assay and those of the others ($P < 0.001$).

the United States and other areas of the world with temperate climates, the overall prevalence of immunity to VZV in the adult population averages 0.95 (5, 12). This includes those with and those without a history of varicella. In this population, if the prevalence is 0.95, Fig. 1 and 2 show that a positive test result in any assay except the Litton assay has a PV of 0.99 and a negative test has a PV of 0.48. Thus, in this population, if one had guessed that a person was immune, one would be correct 95% of the time. A positive test result improves the chance of predicting immunity to 99%. A negative test result improves the chance of predicting susceptibility from 5 to 48%. If, however, a history of varicella is accepted as proof of immunity, the population to be studied consists of only those with no history of varicella, and the prevalence of immunity to VZV is 0.76 (Table 1). In this situation, the respective PVs are 0.95 and 0.84 (Fig. 1 and 2). Therefore, performing the assay improves the chance of predicting immunity from 76 to 95% and that of predicting susceptibility from 24 to 84%. The prevalence of immunity to VZV in adult populations living in tropical climates may be as low as 0.72 in the overall group, without regard to clinical history (14). The utility of the assays in this situation is similar to that described above for the history-negative group from a temperate climate.

In our study, all of the assays except the Litton assay performed comparably when measured against the FAMA. We found the Litton assay to be highly specific but relatively insensitive. In addition, it had the lowest test-retest reliabil-

TABLE 4. Test-retest reliability

| Assay (no. of samples) | Test 1 result | No. with the following result in test 2: | | % Agreement |
|------------------------|---------------|--|---|-------------|
| | | + | - | |
| FAMA (24) | + | 17 | 0 | 100 |
| | - | 0 | 7 | |
| D-ELISA (24) | + | 15 | 0 | 96 |
| | - | 1 | 8 | |
| M-ELISA (24) | + | 14 | 0 | 88 |
| | - | 3 | 7 | |
| MAB assay (23) | + | 16 | 2 | 87 |
| | - | 1 | 4 | |
| ENI assay (24) | + | 15 | 5 | 79 |
| | - | 0 | 4 | |
| Litton assay (13) | + | 3 | 2 | 69 |
| | - | 2 | 6 | |

ity. This was evident both in the larger group of 84 sera retested to verify the results of the first run and in the smaller group of 13 randomly chosen to be retested by all of the assays. For all of the other assays, it would be reasonable to screen for immunity to VZV on the basis of history and test the sera from those with no history of varicella or herpes zoster.

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