Use and Limitations of Varicella-Zoster Virus–Specific Serological Testing to Evaluate Breakthrough Disease in Vaccinees and to Screen for Susceptibility to Varicella

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A plethora of tests for determining the presence of antibodies to varicella-zoster virus (VZV) have been developed over the years, with a wide range of performance standards. There is general agreement that the presence of VZV antibodies in serum indicates immunity to varicella and protection from chickenpox, although the role of specific antibody in mediating protection remains unclear. Both antibodies and cellular immunity probably interact to mediate immunity to the virus. In any case, VZV-specific serum antibody is a useful indicator of protection against chickenpox in patients and persons at high risk of exposure, whether they have been immunized or naturally infected. Serological tests are also a useful implement for evaluating the length of time that immunity to varicella persists after vaccination and whether waning of vaccine-induced immunity occurs. The purpose of this review is to contrast the strengths and weaknesses of currently available VZV antibody assays. Although several of these methods are useful for various specific applications, simpler and more accurate tests to measure antibodies to VZV are a high priority for future research and development.

The presence of antibodies to varicella-zoster virus (VZV) in serum correlates both with a history of varicella and with protection against subsequent infection [1–3]. With the licensing and widespread use of Oka varicella vaccine in the United States, a number of serological tests have been used to determine the susceptibility of individuals to varicella and to assess their response to immunization. Antibody tests with low sensitivity may result in unnecessary vaccinations, which are costly, and a poor correlation between test results and true immune status after vaccination can erode public confidence in an immunization program. Assays with low specificity that are used to screen for susceptibility to varicella are prone to producing false-positive results, placing persons mistakenly identified as immune at risk of infection.

INFECTED CELL AND BEAD-BASED METHODS

The fluorescent antibody to membrane antibody (FAMA) test is the most extensively validated assay and correlates best with susceptibility to and protection against clinical varicella [1–9]. For this reason, FAMA is widely regarded as the standard against which other tests should be measured. The assay can be performed using either fixed or unfixed VZV-infected human fibroblast diploid lung cells or a melanoma cell line...
FAMA titer (serum [17, 18]. Bound antibody is detected using fluorescein VZV-infected cells fixed in acetone before the addition of test positive results have been documented [19].

Comparison of commercial ELISAs against FAMA revealed the former to be less sensitive, detecting between 43% and 76% of naturally infected individuals with positive FAMA results [20]. A more recent study showed that between 83% and 92% of naturally infected subjects with positive results of a modified FAMA assay were also found to be seropositive by use of 2 different commercial ELISAs [21].

LA results appeared to correlate better with FAMA than did commercial ELISAs [7]. However, the subjective nature of LA and reports of both false-positive and false-negative results with adverse clinical consequences cast the reliability of this test for routine screening in some doubt [13, 14]. Although antibody detected by FAMA is the best measure of seroconversion to wild-type infection and protection against clinical disease, rare second symptomatic infections have been documented among FAMA-positive individuals [26–28].

An alternative approach has been to evaluate commercial ELISAs against panels of serum samples from healthy individuals, using a mixture modeling approach to estimate a true cutoff. By this method, 3 populations of results are defined:
seronegative, seropositive, and equivocal. The adjusted optical density values for serum in each category are distributed normally. Fitting the data to this model facilitates an estimation of the most probable cutoff values and allows the characteristics of each test to be analyzed and compared with others. One study that compared 4 commercial ELISAs by use of this approach concluded that, although agreement between several of the commonly used commercial assays is good, sensitivity is generally low compared with that of FAMA, ranging from 60.4% to 91.8% [22]. Better agreement was observed between FAMA and ELISAs, with respect to specificity [22]. More than half of specimens that tested negative by the most sensitive ELISAs had low-positive antibody levels by use of the modified FAMA [22], which is consistent with data from studies of healthy individuals [7]. Results from commercial assays can be modeled to permit comparison for epidemiological purposes and can be used to screen for VZV susceptibility, particularly among adults at elevated risk of exposure. This is because the risk from vaccinating a person with a false-negative test result is substantially lower than the risk from natural infection in an individual falsely identified as seropositive [13]. Therefore, for this application, reduced assay sensitivity is preferable to reduced specificity. More studies may be needed to establish the optimal approach to screening for VZV susceptibility.

**ANTIBODY TESTING OF IMMUNIZED INDIVIDUALS**

The use of serological assays to detect vaccine-induced antibody has generated even greater interest. In one study, levels of antibody to VZV glycoproteins in immunized healthy children exhibited geometric mean titers that were lower by a factor of 10 than those of naturally infected control children [29]. However, another study reported that levels of neutralizing and antiglycoprotein antibody in vaccinated children were comparable to those in healthy adults [30]. Seroconversion with a FAMA titer $\geq 1:4$ after vaccination was associated with protection against varicella. Sixty percent (3/5) of health care workers who did not seroconvert after vaccination developed breakthrough varicella, compared with 8% (9/115) of those with antibody titers $\geq 1:4$ at some time after vaccination [2]. Seroconversion rates of 98% and 94%, as detected by FAMA, were reported among immunized children and adults, respectively [31, 32]. A positive FAMA titer after vaccination is not necessarily predictive of long-term protection, because some vaccinees may lose antibodies detectable by FAMA over time. Although $>98\%$ of children in one study seroconverted to the Oka vaccine strain, as detected by FAMA, as many as 5% of them were reported to have lost detectable antibody during 6–10 years of follow-up [30]. Among vaccinated health care workers, 36% lost detectable FAMA antibody during 11 years of follow-up, and 17% of them experienced breakthrough infection [2].

The overall incidence of breakthrough infection (vaccine failure) among children is $\sim 2\%$–$3\%$ per annum [2, 31, 33–35]. These figures may comprise a combination of primary and secondary vaccine failures and reflect the results obtained by FAMA. Notwithstanding, $\sim 80\%$ of vaccinated persons of any age are protected against chickenpox. In prelicensure studies, a modified FAMA using dried infected cells was used to demonstrate that varicella vaccine–induced immunity persists for up to 10 years in the majority of immunized children. In addition, although the erosion of antibody titers with time in a small percentage of vaccinees was observed in some studies, others reported increasing titers with time, presumably as a result of exogenous boosting [7, 36, 37]. Naturally, this effect would be expected to diminish as varicella incidence declines with increased levels of vaccine coverage. Persons with positive FAMA titers ($\geq 1:4$) at or near the time of exposure are extremely unlikely to develop varicella [2, 7, 8, 27, 38, 39].

**GLYCOPROTEIN ELISA METHOD**

The gpELISA developed by Merck has been used extensively in studies of children immunized with the Varivax Oka vaccine [40]. This highly sensitive ELISA detected seroconversion at a rate approaching 100% among immunized children. A titer of 5 gpELISA units/mL (equivalent to 10 mIU/mL, by the international reference standard) at 6 weeks after immunization was associated with a high degree of protection against breakthrough infection during 7 years of follow-up [41]. Although the gpELISA demonstrated the persistence of specific antibody for up to 5 years after immunization in several hundred children, the precise gpELISA reading that correlates with protection against infection is not known; a number of breakthrough infections have been observed in children with values that were 5 gpELISA units/mL 6 weeks after vaccination [31, 40–45]. Moreover, because few long-term prospectively data are available for gpELISA, there is little information concerning these antibodies just before development of breakthrough varicella in vaccinees. A careful head-to-head comparison of FAMA with gpELISA is in the planning stages but has yet to be performed. A study comparing FAMA and WC ELISA revealed that $\sim 23\%$ of specimens (a combination of acute- and convalescent-phase pairs from patients with suspected primary varicella and serial specimens from immunocompromised recipients of intravenous VZV immunoglobulin) that tested negative by WC ELISA were positive by FAMA (D.S.S., unpublished observations). Among the serum samples received at the Centers for Disease Control and Prevention that test negative or equivocal by VZV WC ELISA, between 15% and 21% test positive by gpELISA, depending on the stringency of the cutoff used. Whether
gpELISA and FAMA perform similarly is currently being investigated.

Commercial ELISAs are considerably less sensitive than either FAMA or gpELISA for the detection of seroconversion after vaccination. One commercial ELISA performed with a sensitivity of 76% and a specificity of 89%, compared with FAMA [2]. Two other commercial tests had sensitivities of 66% and 62.5%, compared with a modified FAMA, when equivocal results were considered to be negative [46]. The sensitivity of these assays improved, however, when equivocal test results were interpreted as positive (90% and 82%), but with a concomitant decline in specificity to 98% [46]. LA test results have also been shown to correlate well with FAMA results, but the same concerns regarding false-positive and -negative results apply in this context [20].

**SUMMARY**

Serological testing remains a critical component of any program for the prevention and diagnosis of VZV infection, particularly in the context of immunization. Among the tests currently in use, only FAMA has been shown to correlate well with protective antibodies from both natural and vaccine-induced immunity. In general, although a number of commercial tests have improved in their sensitivity and specificity and are quite suitable for determining immunity from natural infection, commercial ELISAs still lack sufficient sensitivity to reliably detect vaccine seroconversion. Although LA is sufficiently sensitive for that purpose, the assay has limitations in specificity and technical difficulties in judging positive reactions that make this method less desirable both for screening for varicella susceptibility and for documenting vaccine seroconversion. In addition, it cannot be automated. The gpELISA method appears to perform comparably to FAMA, but care must be taken to establish realistic cutoff values for positive, negative, and equivocal test results. Although it cannot be automated, it would and should be possible to test subsets of serum samples by FAMA when evaluating new varicella vaccine products, such as quadrivalent measles-mumps-rubella-varicella vaccine. Important information indicating protection against infection of vaccinees would emerge, because FAMA is the best approach to identifying an immune correlate of protection that is now available. Unfortunately, however, FAMA is impractical for widespread use as a screening tool, and gpELISA has not been made commercially available. More-robust VZV commercial serological assays amenable to automation are needed.

**Acknowledgments**

Supplement sponsorship. This article was published as part of a supplement entitled “Varicella Vaccine in the United States: A Decade of Prevention and the Way Forward,” sponsored by the Research Foundation for Microbial Diseases of Osaka University, GlaxoSmithKline Biologicals, the Sabin Vaccine Institute, the Centers for Disease Control and Prevention, and the March of Dimes.

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