JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1984, p. 305–306
0095-1137/84/090305-02$02.00/0

Vol. 20, No. 3

Polycarbonate-Coated Microsticks as Solid-Phase Carriers in an Enzyme-Linked Immunosorbent Assay for Rubella Antibody

ISABEL C. SHEKARCHI,[1]* NANCY TZAN,[2] JOHN L. SEVER,[2] AND DAVID L. MADDEN[2]

Microbiological Associates, Inc., Bethesda, Maryland 20814,[1] and Infectious Diseases Branch, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland 20205[2]

Received 9 February 1984/Accepted 29 May 1984

We evaluated the use of microsticks as solid-phase carriers in an enzyme-linked immunosorbent assay for rubella antibody. The microstick enzyme-linked immunosorbent assay was found to be equal in sensitivity to plate and disk enzyme-linked immunosorbent assays and presumably more sensitive than hemagglutination and immunofluorescence assays. The microstick as a solid-phase carrier offers advantages over both plate and bead carriers.

Although the enzyme-linked immunosorbent assay (ELISA) has been shown to be an efficient method for measurement of rubella antibody, quantitation and standardization of the test has been complicated by a number of variables, including variation in the plastic or other support matrix upon which the test is performed. The fabrication of microtiter plates, the most commonly used solid phase, is controlled by manufacturers in an effort to maintain a consistent quality in plates, but lot-to-lot variation still occurs (6). For this reason, each new lot of plates must be tested for binding of each specific antigen. Plastic-coated beads as the solid-phase carrier (8) eliminate much of the expense and loss in time required for pretesting plates, since large numbers of beads can be coated from a single lot of plastic and the conditions of the surface preparation can be monitored by the user. The beads, however, are difficult to handle, especially in tests involving a number of sera with different antigens and antigen controls. Microsticks (7) combine the advantages of a user-coated plastic carrier with a design that permits ease in handling, color coding, and labeling, as well as the economy of using only the number of carriers required for the test.

We have evaluated the use of microsticks in an ELISA for rubella antibody and compared results with those of a standard hemagglutination inhibition (HI) test, a plate ELISA, a disk ELISA, and an immunofluorescence assay (IFA).

MATERIALS AND METHODS

A panel of 100 sera selected for evaluating rubella test methods was used in this study. It included 50 samples with HI titers of <8 and two pairs of acute- and convalescent-phase sera. The samples were drawn from laboratory and hospital personnel and from patients requiring rubella serology. They were aliquoted and stored at −20°C.

Antigen. Sucrose gradient-purified rubella antigen prepared as described earlier (3) was supplied by Preston Dorsett, University of Tennessee, Memphis. Briefly, concentrated culture medium from Vero cells infected with rubella virus HPV-77 was centrifuged in a sucrose gradient. The virion band was collected, dispensed into appropriate working volumes, and stored at −70°C. This antigen was used in both the stick and plate ELISAs.

Microstick ELISA. Polyethylene microsticks (Dynatech Laboratories, Alexandria, Va.) were coated with 1% polycarbonate (Lexan, General Electric Plastics, Pittsfield, Mass.) in methylene dichloride. They were sensitized with antigen, and the test was performed as previously described (7). Briefly, microsticks were sensitized individually in microtiter wells containing 150 μl of antigen per well at 4°C overnight. By use of a microstick transfer plate, the sticks were transferred to phosphate-buffered saline with 0.5% Tween 20 (PBS-Tween), washed three times, and tapped vigorously to remove excess moisture. They were then placed in 1% bovine serum albumin in PBS for 30 min at room temperature to block any remaining binding sites. After being washed as described above, the sensitized microsticks were placed in microtiter plate wells containing twofold dilutions of the serum samples in PBS–1% bovine serum albumin–0.05% edetic acid (100 μl per well). They were incubated for 90 min in a moist chamber at 37°C, washed, and placed in wells containing conjugate diluted in serum diluent for 1 h at 37°C. They were again washed and placed in substrate for 30 min at 37°C. The microsticks were then removed from the wells, and the rubella antibody titer was determined by measuring a color change in the substrate on a Dynatech MicroELISA AutoReader (model MR580).

Microtiter plate ELISA. Flat-bottom polystyrene microtiter plates (Dynatech; Immulon I, lot 112079) were sensitized overnight at 4°C with antigen. They were washed with PBS-Tween and tapped dry, and then twofold dilutions of the sera were added, beginning with 1:8 in serum diluent. After 90 min of incubation at 37°C, the plates were washed and alkaline phosphatase-conjugated anti-human immunoglobulin G was added. After 1 h of incubation at 37°C, they were washed and phosphatase substrate (disodium *p*-nitrophenyl phosphate; 1 mg/ml) (Sigma Chemical Co., St. Louis, Mo.; no. 104-105) was added. The plates were incubated at 37°C for 30 min, and the reaction was stopped with 50 μl of 3 M NaOH per well. Presence of the enzyme, indicated by a color change in the substrate, was measured on an MR580 ELISA AutoReader. High-positive, low-positive, and negative standard sera were included with each test. The endpoint was read as the highest dilution with an optical density equal to or greater than two times the highest reading of the negative control diluted 1:8.

Disk ELISA. An ELISA kit assay, Cordia R-100 (Cordis

* Corresponding author.

TABLE 1. Comparison of HI results with qualitative results by different assay methods for rubella antibody in human sera

HI titer	No. of samples	No. of samples with the indicated result												
		Stick ELISA titer of:				Plate ELISA titer of:				Disk ELISA			IFA	
		<8[a]	≥8	<100[b]	≥100	<8[a]	≥8	<100[b]	≥100	−[c]	Equivalent	+	−[d]	+
<8	50	25	25	49	1	26	24	48	2	40	8	2	50	0
8	3	0	3	0	3	0	3	0	3	0	1	2	2	1
≥16	47	0	47	0	47	0	47	0	47	0	0	47	0	47

[a] A cutoff titer of ≥8 was positive.
[b] A cutoff titer of ≥100 was positive.
[c] Determination from curve with a sample dilution of 1:100.
[d] Determination from a 1:10 dilution.

Laboratories, Miami, Fla.), in which the antigen is bound to a nitrocellulose disk was also used to evaluate the panel. The test was performed according to the manufacturer's directions.

HI test. A standard HI test which employed Kaolin and 1-day-old chicken cells (5) was used to establish base-line titers.

IFA. An indirect fluorescent-antibody kit using a fluorescein conjugate (Electro-Nucleonics Laboratories, Inc., Bethesda, Md.) was used for rubella determinations by IFA. The test was conducted according to the directions of the manufacturer.

RESULTS

A comparison of HI results with qualitative results of the stick, plate, and disk ELISAs and the IFA is presented in Table 1. Complete agreement between HI and all of the other tests was observed with sera having HI titers of ≥16. Of the three samples with an HI titer of 8, all were positive by the ELISAs, while two were negative by IFA. The more sensitive ELISAs detected antibody in nearly half (25 by stick ELISA and 24 by plate ELISA) of the 50 HI-negative samples when an ELISA cutoff titer of 1:8 was used, while a cutoff titer of 1:100 resulted in much closer agreement.

Of the 25 samples negative by HI but positive by stick ELISA, 24 had ELISA titers of ≤64 and one had a titer of 128. Of the 24 samples positive by plate ELISA, 22 had titers of ≤64 and 2 had titers of 128. Of the 50 HI-negative samples, the disk ELISA detected 2 positive and 8 equivocal samples.

All methods detected a significant rise in titer (fourfold or greater) in the two acute and convalescent serum pairs included in the panel.

DISCUSSION

From the data presented, we conclude that polycarbonate-coated microsticks are satisfactory solid-phase carriers for the measurement of rubella-specific antibody by ELISA. The microsticks can be individually labeled and color coded and can be manipulated easily with a minimum of equipment. Diluters, plates, and readers already in use for other ELISAs can be used for the stick ELISA conveniently. Because the carrier surface is prepared in the laboratory, the user not only controls the quality of the surface but also is assured of a relatively unlimited supply of carriers with the same surface characteristics. The use of microsticks eliminates the need for screening of new plate lots and storage of large, uniform lots. Sensitized microsticks can be stored for at least 6 months at −20°C and therefore would have many advantages for use in commercial kits.

Comparison of test results with HI titers showed the microstick ELISA to be more sensitive than HI and IFA and approximately as sensitive as the microplate ELISA. Similar to the findings of others (1), both the stick and plate ELISAs detected antibody to rubella virus in about half of the samples with HI titers of <8. The significance of these low ELISA titers in the assessment of rubella immunity is not clearly understood. Others have stated that ELISA provides more reliable information about the immune status of an individual than HI and that any detectable titer protects against subsequent viremic infection (1, 2). O'Shea et al., however, have reported detection of viremia and viral excretion in vaccinees who had low antibody titers before vaccine challenge (4). The Advisory Committee on Vaccination Practices does not recommend vaccination of persons with HI rubella antibody titers of ≥8, and most commercial kits for rubella antibody detection adjust the cutoff between positive and negative to correspond to this level. A rubella cutoff titer of ≥100 for the plate or stick ELISA described in this paper would give a sensitivity comparable to an HI cutoff of ≥8. Perhaps after further clinical data are coupled with HI and ELISA data, the significance of low rubella antibody titers can be evaluated.

LITERATURE CITED

1. **Buimovici-Klein, E., A. J. O'Beirne, S. J. Millian, and L. Z. Cooper.** 1980. Low level rubella immunity detected by ELISA and specific lymphocyte transformation. Arch. Virol. **66**:321–327.
2. **Centers for Disease Control.** Rubella prevention 1981. Morbid. Mortal. Weekly Rep. **30**:37–47.
3. **Gravell, M., P. H. Dorsett, O. Gutensen, and A. C. Ley.** 1977. Detection of antibody to rubella virus by enzyme-linked immunosorbent assay. J. Infect. Dis. **136**(Suppl.):300–303.
4. **O'Shea, S., J. M. Best, and J. E. Banatvala.** 1983. Viremia, virus excretion and antibody responses after challenge in volunteers with low levels of antibody to rubella virus. J. Infect. Dis. **148**:639–647.
5. **Sever, J. L., D. A. Fuccillo, G. L. Gitnick, J. R. Huebner, M. R. Gilkeson, A. C. Ley, N. Tzan, and R. G. Traub.** 1967. Rubella antibody determinations. Pediatrics **40**:789–797.
6. **Shekarchi, I. C., J. L. Sever, Y. J. Lee, G. Castellano, and D. L. Madden.** 1984. Evaluation of various microtiter plates with measles, toxoplasma, and gamma globulin antigens in enzyme-linked immunosorbent assays. J. Clin. Microbiol. **19**:89–96.
7. **Shekarchi, I. C., J. L. Sever, L. A. Ward, and D. L. Madden.** 1982. Microsticks as solid-phase carriers for enzyme-linked immunosorbent assays. J. Clin. Microbiol. **16**:1012–1018.
8. **Smith, K. O., and W. D. Gehle.** 1977. Magnetic transfer devices for use in solid phase radioimmunoassays and enzyme-linked immunosorbent assays. J. Infect. Dis. **136**(Suppl.):329–336.