JOURNAL OF CLINICAL MICROBIOLOGY, July 1982, p. 82–85
0095-1137/82/070082-04$02.00/0

Vol. 16, No. 1

Comparison of Several Test Systems Used for Determination of Rubella Immune Status

ALICE S. WEISSFELD,[1]†* WARREN D. GEHLE,[2] AND ALEX C. SONNENWIRTH[1]

Departments of Pathology and Microbiology and Immunology, Washington University School of Medicine, and Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, St. Louis, Missouri 63110,[1] and Litton Bionetics, Kensington, Maryland 20795[2]

Received 2 December 1981/Accepted 29 March 1982

Hemagglutination inhibition (HAI) is currently the most widely used technique for the determination of rubella immune status. However, two new methods, enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (FIAX), have also been adapted for this purpose. In comparing a commercially available ELISA system (BIO-BEAD, Litton Bionetics) with an HAI system (RUBA-tect, Abbott Laboratories), some ELISA-positive sera were found to be rubella antibody negative by the HAI system. To determine which of these results more accurately reflected the immune status of the patient, 74 RUBA-tect-negative sera were retested by ELISA BIO-BEAD, FIAX (International Diagnostic Technology) and by modified HAI, employing fresh erythrocytes (using Flow Laboratories reagents). Eleven RUBA-tect-negative sera (15%) were positive by ELISA, FIAX, and modified HAI. Two sera were positive only by ELISA and FIAX, two sera were positive by ELISA and HAI, four sera were positive by ELISA only, and one serum was positive by FIAX only. Neutralization assays were subsequently performed on sera positive by only one or two of the procedures to determine the presence of protective rubella antibodies in these sera; all but three of the sera were positive for neutralizing antibody. Commercial ELISA and FIAX systems appear to be more sensitive indicators of rubella immune status than are commercial HAI kits which use stabilized erythrocytes. Neither ELISA nor FIAX require extraction of serum; moreover, the ELISA BIO-BEAD test assay can be performed without an expensive instrument for reading.

Rubella is a common, usually benign childhood disease. The most important complication of rubella infection is a variety of fetal anomalies that result from disease in early pregnancy; women who acquire the illness at this time may elect to undergo a therapeutic abortion. For this reason, the serological determination of an individual's immune status is especially important.

Several methods have been used for the determination of immunity to rubella virus. These include hemagglutination inhibition (HAI), neutralization, complement fixation, and passive hemagglutination. HAI is currently the most widely used laboratory test to screen for rubella immunity. However, other tests are now available to detect rubella antibody; these include an enzyme-linked immunosorbent assay (ELISA) and an indirect immunofluorescence technique (FIAX).

We evaluated one of the new commercially available ELISA kits (BIO-BEAD, Litton Bionetics) by comparing it with the commercial HAI kit (RUBA-tect, Abbott Laboratories) used in the Serology Laboratory of The Jewish Hospital of St. Louis. During the course of this assessment it was noted that several sera that were rubella antibody negative by the HAI procedure were positive when tested by ELISA. To determine whether these specimens were false negatives by HAI or false positives by ELISA, we tested a series of RUBA-tect-negative sera by several different methods. This report is a summary of our findings.

MATERIALS AND METHODS

Experimental protocol. Sera submitted to the Serology Laboratory at The Jewish Hospital of St. Louis for rubella immune status determination were routinely screened using the RUBA-tect HAI test. These same sera were used to evaluate a new ELISA test kit for determining rubella immune status. During the evaluation, several HAI-negative, ELISA-positive sera were observed. To determine which test more accurately reflected the immune status of the patient, the next 74 sera submitted for routine screening that were negative by HAI were studied further. ELISA testing was

† Present address: Division of Clinical Pathology, Cedars-Sinai Medical Center, Los Angeles, CA 90048.

performed at The Jewish Hospital of St. Louis, FIAX and modified HAI tests were done at Bionetics Medical Laboratories, Kensington, Md., and neutralization tests were performed at Litton Bionetics, Kensington, Md., on coded and split specimens sent to each participating laboratory. Frozen sera were shipped on dry ice by overnight mail and stored at −70°C upon receipt in Kensington, Md. All assays were performed within 30 days of receipt of the sera.

RUBA-tect HAI test. RUBA-tect HAI test kits (Abbott Laboratories, North Chicago, Ill.) were used according to the manufacturer's instructions. Briefly, serum specimens were extracted with heparin-$MnCl_2$ for 15 min at 4°C and absorbed with stabilized chicken erythrocytes for 60 min at 4°C (with shaking every 15 min). After centrifugation, serial twofold dilutions of treated sera (1:10 to 1:640) were placed in wells of a V-type microtiter plate, and rubella antigen containing four hemagglutinating units was added to each well. After incubation for 1 h at 4°C, standardized 0.4% stabilized chicken cells were added to each well (with shaking on a mechanical vibrator). The plate was incubated at 4°C, and the hemagglutination pattern was read after 90 min. The dilution of serum which completely inhibited hemagglutination was taken as the endpoint. Results were evaluated either as antibody present (titer, ≥1:10) or antibody not present (titer, <1:10).

Modified HAI test. The modified HAI test was performed according to the standard Centers for Disease Control protocol (5). Reagents for the test were purchased from Flow Laboratories, McLean, Va. Test sera were extracted with heparin-$MnCl_2$ for 15 min at 4°C and absorbed with 50% fresh chicken erythrocytes for 1 h at 4°C. After centrifugation, serial twofold dilutions of treated sera (1:8 to 1:1,024) were made in wells of a V-type microtitration plate, and rubella antigen containing four hemagglutinating units was added to each well. After incubation for 1 h at 4°C, standardized 0.25% fresh chicken cells were added to each well (with shaking on a mechanical vibrator). The plate was then incubated at 4°C, and the hemagglutination pattern was read after 90 min. The dilution of serum which completely inhibited hemagglutination was taken as the endpoint. Results were evaluated either as antibody present (titer, ≥1:8) or antibody not present (titer, <1:8).

ELISA. ELISA testing was performed by using the Rubella BIO-BEAD screen test kit (Litton Bionetics, Kensington, Md.), according to the manufacturer's instructions. In this system, rubella virus is bound to the surface of metal beads. Virus-coated beads were placed in a single 1:100 dilution of the test serum for 90 min at 37°C; this dilution is approximately equivalent to a 1:8 serum dilution, using the HAI test. After incubation in test sera, the beads were washed to remove unbound human serum protein and were then released into a reaction mixture containing peroxidase-conjugated anti-human globulins for 90 min at 37°C. After the beads were washed to remove unbound conjugate, they were placed in a substrate solution for 10 min at 25°C. The appearance of a green color (read visually) indicated the presence of human antibody to the rubella antigen on the bead. Negative control beads, prepared from uninfected cell cultures similar to those used to prepare the rubella antigen, were included in the kit to detect nonspecific reactions. The results of the test are qualitative and are reported as either positive or negative for rubella antibodies.

FIAX. Indirect immunofluorescence testing was performed according to the manufacturer's instructions, using the FIAX anti-rubella antibodies test kit (International Diagnostic Technology, Santa Clara, Calif.). In this system, individual dipsticks called STiQ are used; one side of the STiQ contains immobilized rubella viral antigen and the other side (control) contains no viral antigen. To perform the test, the STiQ was immersed in a 1:40 dilution of the test serum and shaken for 30 min. The STiQ was then washed and shaken for 30 min in fluorescein isothiocyanate-labeled anti-human immunoglobulins. After the STiQ was washed to remove unbound antibody, the STiQ was read in a fluorometer. The amount of bound fluorescein on the control side of the STiQ was subtracted from the amount bound on the side containing rubella antigen. The titer for each sample was then obtained by interpolation from a standard curve. Results were evaluated either as antibody present (titer, ≥8) or antibody not present (titer, <8).

Neutralization test. Neutralization assays were performed as described previously (3, 6). Sera were heated at 56°C for 30 min, and dilutions were made in Eagle basal medium containing penicillin and streptomycin. Equal volumes of diluted serum and rubella virus (prepared in Eagle basal medium containing 4% guinea pig serum, penicillin, and streptomycin) were mixed and incubated at 37°C for 1 h. Monolayers of RK-13 rabbit cells were inoculated, in duplicate, with 0.2 ml of each mixture and incubated for 60 min (with shaking every 15 min). The inoculum was then removed, the cells were washed, 0.25% special Noble agar (diluted in Eagle basal medium containing 10% calf serum) was added, and the culture was incubated for 5 days at 37°C in an atmosphere containing 5% CO_2. A fluorescent focus inhibition assay was used to observe the number of virus-infected cells. Monolayers were washed and fixed with 95% methanol, and human serum containing a high titer of rubella antibody was allowed to absorb to the cells for 30 min. The monolayer was then washed, and fluorescein-conjugated goat anti-human immunoglobulins were added for 30 min. After the cells were washed, they were examined at a magnification of ×24 with a Zeiss RA microscope equipped with an Osram HBO 100-W mercury lamp; a BG12 primary filter was used in combination with an OG1 secondary filter. The percentage of reduction in the number of fluorescent foci in each test specimen was calculated by comparing it with the number of fluorescing cells in the negative control, i.e., virus mixed with a serum known to be negative for rubella antibody. Neutralizing antibody was determined to be present if there was a greater than 50% reduction in the number of foci of fluorescent cells.

RESULTS

Of the 74 sera tested in this study 54 (73%) were negative by ELISA, FIAX, and modified HAI, and 11 (15%) were positive by all three of these tests. Two sera were positive by ELISA and FIAX, two sera were positive by ELISA and modified HAI, four sera were positive by

ELISA only, and one serum was positive by FIAX only.

Neutralization tests were performed on sera positive by only one or two of the three tests to determine whether these specimens actually contained protective rubella antibody (Table 1). One specimen positive by FIAX only (number 35), one serum positive by ELISA and FIAX (number 43), and one serum positive by ELISA only (number 42) did not contain neutralizing antibody.

We were particularly interested in serum 42, since this patient had received rubella immunization approximately 5 weeks before the submission of this specimen. Since antibodies detectable by ELISA may appear before either HAI or neutralizing antibodies, we postulated that specimen 42 was drawn before the HAI or neutralizing antibodies had reached a detectable titer and that a second specimen drawn over a year later would show evidence of rubella antibody by any of the serological methods used. In fact, this later specimen was positive by ELISA, FIAX, and modified HAI and showed a 64% reduction in the number of fluorescent foci, indicating the presence of neutralizing antibody. Unfortunately, we were unable to obtain any clinical information or follow-up serum for patient 35 or 43.

DISCUSSION

Several test procedures (e.g., HAI, ELISA, and FIAX) are now commercially available for the determination of rubella immune status. One way in which investigators can evaluate different test kits is by comparing their ability to detect low levels of antibody. Recent studies indicate that sera which are rubella antibody negative by HAI are often positive when newer, more sensitive methods are used (2, 4, 7, 8). In this series, approximately 24% of the sera were shown to be false negative by RUBA-tect, a commercial HAI test kit which used stabilized erythrocytes. Of interest is the fact that most of the RUBA-tect-negative sera were positive when tested by a modified HAI test which used fresh erythrocytes. Similar findings were recently reported by Castellano et al. (2), who compared several commercially available diagnostic test kits for rubella.

The results we obtained with one commercial ELISA test kit (BIO-BEAD) agree with those reported by other investigators who have used in-house ELISA tests, i.e., ELISA was more sensitive than HAI (7–9). A recent report comparing HAI, ELISA, and lymphocyte transformation suggested that ELISA may be a better indicator of prior exposure to rubella virus than is HAI (1). The additional evidence presented in this study indicating that most sera positive by ELISA, FIAX, or both do in fact contain neutralizing antibody (which is thought to be protective [6]) should allow serologists to state with a greater degree of certainty that HAI-negative, ELISA-positive, FIAX-positive, or both ELISA- and FIAX-positive sera actually do contain rubella antibody. The failure to detect neutralizing antibody in sera 35, 42, and 43 may be due to (i) technical problems encountered during shipping or handling, (ii) submission of specimens before neutralizing antibodies had reached a detectable level (as is postulated for specimen 42) or (iii) false positive ELISA or FIAX tests. More detailed studies must now be performed to unequivocally determine the most accurate method for determining an individual's immune status to rubella virus.

TABLE 1. Determination of the presence of neutralizing antibody in discrepant sera

Serum	% Reduction[a]	Neutralizing antibody
ELISA and FIAX positive		
43	29	−
46	52	+
ELISA and modified HAI positive		
70	63 (1:10)	+
79	59 (1:10)	+
ELISA only positive		
19	67	+
26	59	+
33	56	+
42	10	−
FIAX only positive		
35	34	−
Negative control	0	−
Low positive	66	+
High positive	80	+

[a] Fluorescent focus assay; sera diluted 1:4 except where notated.

ACKNOWLEDGMENT

The technical expertise of Jeanne Ruperto is greatly appreciated.

LITERATURE CITED

1. **Buimovici-Klein, F., 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. **Castellano, G. A., D. L. Madden, G. T. Hazzard, C. S. Cleghorn, D. V. Vails, A. C. Ley, N. R. Tzan, and J. L. Sever.** 1981. Evaluation of commercially available diagnostic test kits for rubella. J. Infect. Dis. 143:578–584.
3. **Field, A. M., E. M. Vandervelde, K. M. Thompson, and D. N. Hutchinson.** 1967. A comparison of the haemaggluti-

nation-inhibition test and the neutralization test for the detection of rubella antibody. Lancet ii:182–184.

4. **Frisch, C., and T. Whiteside.** 1981. A comparison of the FIAX and hemagglutination tests for IgG antibodies to rubella virus. J. Clin. Lab. Automation 1:33–37.
5. **Palmer, D. F., J. J. Cavallaro, and K. L. Herrmann.** 1977. A procedural guide to the performance of the rubella hemagglutination-inhibition tests, p. 1–37. Centers for Disease Control, Atlanta, Ga.
6. **Schluederberg, A., D. M. Horstmann, W. A. Andiman, and M. F. Randolph.** 1978. Neutralizing and hemagglutination-inhibiting antibodies to rubella virus as indicators of protective immunity in vaccinees and naturally immune individuals. J. Infect. Dis. 138:877–883.
7. **Shekarchi, I. C., J. L. Sever, N. Tzan, A. Ley, L. C. Ward, and D. Madden.** 1981. Comparison of hemagglutination inhibition test and enzyme-linked immunosorbent assay for determining antibody to rubella virus. J. Clin. Microbiol. 13:850–854.
8. **Van Loon, A., J. T. van der Logt, and J. van der Veen.** 1981. Enzyme-linked immunosorbent assay for measurement of antibody against cytomegalovirus and rubella virus in a single serum dilution. J. Clin. Pathol. 34:665–669.
9. **Vejtorp, M., and J. Leerhoy.** 1980. Comparison of the sensitivity of ELISA and haemagglutination-inhibition test for routine diagnosis of rubella. Acta Pathol. Microbiol. Scand. Sect. B 88:349–350.