Evaluation of a New Latex Test and a New Enzyme Immunoassay for Determination of Rubella Immunity

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A new enzyme immunoassay (Rubenostika; Organon Teknika, Turnhout, Belgium), a new latex agglutination test (Rubalex; Orion Diagnostica, Espoo, Finland), and three other accepted methods for the determination of rubella immunity were compared with a standard hemagglutination inhibition assay. Of 224 serum samples tested, 54 (24%) were nonreactive and 24 (11%) were low titered. All procedures were very specific (94 to 100%). Rubenostika was the least sensitive method (88%), and Rubalex was the most sensitive (98%).

The hemagglutination inhibition (HAI) assay is considered the standard method for the detection of rubella antibodies (6, 9). However, other less tedious and more rapid procedures have been found to be acceptable alternatives to the HAI assay for rubella immunity screening. Rapid methods that have been developed for rubella serology testing include enzyme immunoassay (1, 2, 5, 8, 13), latex agglutination (2, 3, 5, 7, 10–13), passive hemagglutination (2, 4, 9), fluorescence immunoassay (1, 4, 9), and radioimmunoassay (4, 9). The purpose of this study was to evaluate five rapid commercial tests, including a new enzyme immunoassay (Rubenostika; Organon Teknika, Turnhout, Belgium) and a new latex agglutination procedure (Rubalex; Orion Diagnostica, Espoo, Finland), and compare them with a standard HAI assay for the determination of rubella immunity.

A total of 224 serum samples were tested by all methods. The sera had been submitted for the determination of rubella immunity, frozen at −70°C, and thawed once prior to testing. Sera were selected to include 54 (24%) nonimmune specimens and 24 (11%) samples with low titers of antibody (1:8 to 1:16) as determined by HAI assay. Using these sera, we evaluated two commercial enzyme immunoassay kits (Rubenostika, Organon; Rubzyme, Abbott Laboratories, North Chicago, Ill.), two latex agglutination procedures (Rubalex, Orion; Rubascan, BBL Microbiology Systems, Cockeysville, Md.), and a rapid passive hemagglutination procedure (Rubaqwick; Abbott), and compared them with a standard HAI assay (RubeHIT; Behring, Marburg, Federal Republic of Germany).

The HAI assay was done following pretreatment of sera with kaolin, using reagents, controls, and stabilized human erythrocytes supplied by the manufacturer. Sera that were positive only at a titer of 1:8 by HAI assay were retested to ensure that this was not a false-negative reaction. For all other procedures, sera were tested without pretreatment, using reagents and controls supplied by the manufacturer, according to the instructions of the manufacturer. Latex agglutination with Rubascan was done by using sera diluted 1:10, as recommended by the manufacturer to approximate the level of sensitivity obtained with HAI assay methods, and also by using undiluted serum samples. All tests were done blind; sera were coded, and comparative results were unknown until the tabulation of all data. The sensitivity, specificity, and positive and negative predictive values were calculated for each test procedure for comparison with the HAI test results.

A comparison of HAI assay results with those obtained with the other test kits is shown in Table 1. Rubalex and Rubascan (undiluted) had the greatest sensitivities (98% each). Rubenostika was the least sensitive method (88%). Undiluted serum specimens improved the sensitivity of Rubascan, but with some loss of specificity, compared with the use of sera diluted 1:10. All assays, except for Rubascan (undiluted), were found to be very specific.

The sensitivities of the test procedures for sera with high (HAI titer, ≥1:32) compared with low levels of antibody are shown in Table 2. All the tests performed well if sera had high levels of rubella antibody, although two high-titered serum samples were positive with Rubascan with sera diluted 1:10 but were negative with Rubascan with undiluted sera. The majority of false-negative reactions occurred with sera with low-positive antibody titers (HAI titer, 1:8 to 1:16). With low-positive sera, Rubascan (with undiluted sera) and Rubalex performed better (sensitivities of 92 and 88%, respectively).

The critical requirement of a diagnostic test for the determination of rubella immunity is that it be highly specific. False-positive results imply immunity in susceptible individuals or seroconversion in someone who has not been infected. This evaluation of five commercially available test kits for detecting rubella antibodies, including two that have only recently been introduced in North America (Rubenostika and Rubalex), found that all methods were very specific. There was greater variability in the sensitivity of these tests. With all of the procedures, the majority of false-negative results occurred with sera containing low levels of antibody (Table 2). These results suggest that when rubella antibody tests are being evaluated, large numbers of low-titered serum samples should be included.

False-negative results occurred most often with Rubenostika and Rubascan with sera diluted 1:10. Results obtained with Rubascan with sera diluted 1:10 have been found to correlate best with HAI assay results (3, 12). Although the use of undiluted sera may increase the sensitivity of the test, it is not certain that rubella antibody titers corresponding to HAI titers of less than 1:8 are protective. Moreover, in this study, two high-titered serum samples were positive with Rubascan when diluted 1:10 but negative when undiluted. The cause of this prozonelike phenomenon is unknown, but it has been previously described with Rubascan (3, 5). It has been suggested (3) that when Rubascan is used, sera should

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initially be tested undiluted; nonreactive sera should then be retested at a 1:10 dilution to maximize the sensitivity of the test while avoiding prozone reactions. However, on the basis of the results of the present study (Rubascan undiluted specificity, 94%), this approach cannot be recommended. The high sensitivity of Rubalex may also be problematic if it is true that very low levels of rubella antibody are not protective. However, as Rubalex was compared with a standard HAI procedure in this evaluation, we believe that the Rubalex results can be considered an accurate reflection of rubella immune status. The ideal rubella screening test would be highly sensitive and specific, give reproducible results, and require no special equipment. It would be rapid, technically simple to perform, and inexpensive. Rapid agglutination tests and enzyme immunoassays for the detection of antibodies have been found to meet these requirements. As in previous studies (2, 5, 7, 8, 10, 12, 13), Rubazyme, Rubascan, and Rubaquick gave acceptable results for rubella screening. In this study, the newer enzyme immunoassay, Rubenostika, was found to be a relatively insensitive procedure. The Rubaquick latex agglutination test gave the most accurate results compared with the HAI assay, and it was also the most sensitive test for low-titered sera. The procedure used undiluted serum, was easy to perform and interpret, and provided results in 3 min. The assays evaluated in this study cannot be used for the diagnosis of rubella, but they do provide suitable alternatives to the HAI assay for the determination of immunity.

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LITERATURE CITED


