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Evaluation and Comparison of Two Assays for Detection of Immunity to Rubella Infection

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Two commercially available rapid screening tests, Rubacell (Abbott Laboratories; passive hemagglutination) and FIAX (International Diagnostic Technology; indirect immunofluorescence) were compared with a standard hemagglutination inhibition assay for detection of immunity to rubella infection. In tests of approximately 300 sera, both rapid assays were specific and sensitive and showed a high predictive value of a positive result. Within-run reproducibility studies were excellent for both tests; however, Rubacell was superior to FIAX with respect to time-cost analysis.

The evaluation of immune status for rubella viral infection is recognized to be important because of the teratogenicity of this infection in certain age groups (6). The standard hemagglutination inhibition test (HI) to detect antibodies to rubella virus, first described by Stewart et al. (9), is sensitive and specific but requires time-consuming incubations, standardization of reagents, and removal of nonspecific inhibitors and agglutinins (8). Consequently, several alternative methods of screening for rubella immunity have been developed, using such varied methodologies as radial hemolysis (1), immunodiffusion (5), and solid-phase radioimmunoassay (10). We have evaluated and compared two such commercially available rapid assays for rubella immune status: Rubacell (Abbott Laboratories) and FIAX (International Diagnostic Technology). The Rubacell test is based on the passive hemagglutination (2) of erythrocytes coated with rubella viral antigen to detect specific anti-rubella antibody. The FIAX test employs a modification of an indirect immunofluorescence assay for rubella antibodies first described by Brown et al. (3). This assay utilizes immobilized rubella viral antigen to bind specific antibody with subsequent fluorometric detection of fluorescein-labeled anti-human immunoglobulin.

MATERIALS AND METHODS

Rubacell. Test kits of 100 determinations each were stored at 4°C. The manufacturer's lyophilized human erythrocytes, sensitized with rubella antigen, were reconstituted with distilled water and used within 1 h. Specimen dilution buffer, 0.1 M phosphate buffer with protein and ion stabilizers, was used as supplied.

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Specimen dilution buffer (25 μl) was added to the wells of the "V" plate included in each kit. Control sera and unknown sera (2 μl) were added by pipette (Oxford) to appropriate wells and were mixed as specified by the manufacturer. Reconstituted sensitized erythrocytes (25 μl) were added to each well, and the plate was agitated to mix contents of each well. The plate was sealed and left undisturbed at room temperature for 2 h after which a reader mirror (Dynatech Laboratories, Inc.) was used to detect the presence of hemagglutination (positive) or compact button formation (negative).

FIAX. Test kits of 50 determinations each were stored at 4°C in the dark. The FIAX fluorescent reader, horizontal shaker, and clip were used. Variable pipettors (Finnpipette) were used to make the serum dilutions. The following were supplied in the kit: (i) dual-surfaced sticks coated with antigen on surface 1 and no antigen on surface 2; (ii) fluorescent antibody reagent, fluorescein isothiocyanate-labeled goat antibody to human immunoglobulins, in buffer; (iii) washing buffer; (iv) dilution buffer; and (v) calibrators prepared from human plasma, having assigned values obtained by the manufacturer by using the standard HI assay.

Calibrators and unknown sera were diluted 1/40 with dilution buffer in glass tubes (12 by 75 mm). Dual-surfaced sticks were placed in the tubes, and the tubes were shaken for 30 min in a horizontal shaker. Sticks were transferred to tubes with washing buffer and shaken for 5 min. The reaction sticks were then transferred to tubes with the fluorescent antibody reagent and shaken for another 30 min, after which they were transferred to tubes with washing buffer and shaken for an additional 10 min. The fluorometer was calibrated and measurements were taken immediately; surface 1 was read first, and then surface 2. The fluorometer displayed the fluorescence value as a whole number. The change in fluorescence of each calibrator (surface 1 minus surface 2) was plotted on the linear axis with the stated calibrator value on the log axis on three-cycle semilogarithmic paper. The

best-fit curve was drawn and used to extrapolate unknown titers. Figure 1 demonstrates a typical standard curve.

HI. The procedure used was the standardized Center for Disease Control rubella HI test (7). Dextran sulfate and calcium chloride were used to remove nonspecific inhibitors.

Controls. A recalcified plasma-positive control was supplied with the Rubacell Kit and used in each run. A negative control (Flow Laboratories) and buffer control were also used in the Rubacell test. Negative and positive control sera (Flow Laboratories) were used in each FIAX and HI run.

Test sera. The sera were retrospectively tested by the FIAX and Rubacell tests and compared with previously performed HI test results. Twenty-seven percent of the sera had HI titers of less than 1:8 (negative). Of the positive sera, the range of HI titers was: 1:8, 7.2%; 1:16, 10%; 1:32, 13.6%; 1:64, 16%; 1:128, 9.6%; 1:256, 8.5%; 1:512, 6.4%; and ≥1:1,024, 3%. All sera had been stored at −70°C for less than 6 months before use.

Discrepant results. All discrepant results, i.e., HI versus FIAX or HI versus Rubacell, were retested by both methods.

Interpretation of titers. Patient sera with FIAX or HI titers equal to or greater than 1:8 were considered to have serological evidence of past infection. Serum for the Rubacell test was diluted 1:13.5.

RESULTS

Rubacell evaluation. Of the 301 sera tested by both Rubacell and HI, there was agreement on 289 (96.0%). Using the standard HI as a reference method, the sensitivity of the Rubacell was 94.7% and the specificity was 100%. The predictive value of a positive result was 100%, and that of a negative result was 85.8%. (See Table 1 for definitions and for comparison with FIAX results.) Of the 12 false-negative discrepant sera, 2 were positive on repeat testing. Of the remaining 10 false-negative sera, the HI value of 9 sera was 1:8. There were no false-positive results. A within-run reproducibility study was done by testing the same sera 20 times, using both a positive sample (HI of 1:64) and a negative sample (HI < 1:8). There were no false-negative or false-positive results.

FIAX evaluation. Of the 293 sera tested by FIAX and HI, there was agreement on 276 (94.2). Sensitivity of the FIAX was 93.5% and specificity was 96.2%. The predictive value of a positive result was 98.5%, and that of a negative result was 84.3% (Table 1).

Of the 17 discrepant sera, 7 false-negatives were found to have elevated background fluorescence, which reverted the FIAX test result from positive to negative. In addition, two sera that agreed in positivity with the HI also demonstrated nonspecific background fluorescence. With these sera the result was changed from a

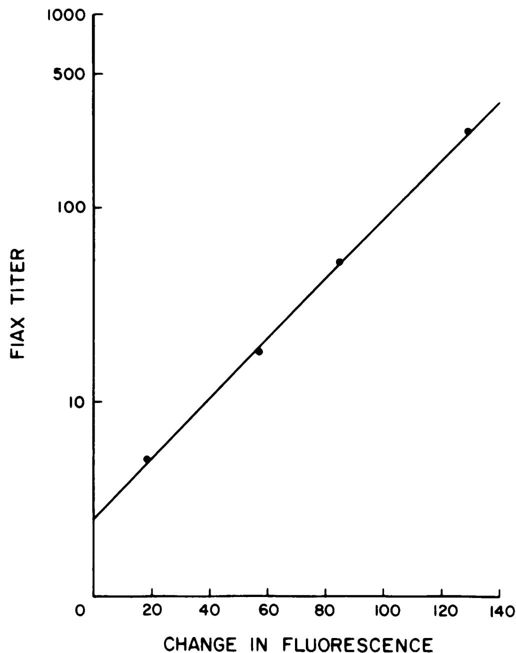

FIG. 1. *Plot of typical curve for FIAX rubella test, using four calibrators. Titers are continuous, corresponding to the reciprocal of the sample dilution. Change in fluorescence was calculated as described in the text.*

high-titered positive to a low-titered positive, thus not altering the overall agreement. One serum that was negative by HI and FIAX also displayed nonspecific background fluorescence by FIAX. Of the eight remaining false-negative sera, five were positive on repeat testing, and two sera remained negative. All were, however, low-titered sera. With one serum showing a false-negative FIAX result, there was insufficient sample for repetition. All of the three false-positive sera had low titers by FIAX and were negative when repeated. Within-run reproducibility studies of both a positive and a negative sample resulted in no errors ($n = 20$).

Comparison evaluation. The accuracy of neither a positive prediction nor a negative prediction was statistically different between the Rubacell and the FIAX tests (χ^2 test).

Time and cost analysis. The time to perform 50 tests by the Rubacell assay was 55 min, not including the 2-h incubation, i.e., a total of 175 min. The initial financial investment for this assay was $95.00. The cost per test including the kit ($125.00/100 tests), disposables, and technologist time, calculated at $6.00 per hour, was $1.39.

The total time necessary to perform 50 tests and reduce the data by the FIAX procedure was

TABLE 1. *Comparison of results obtained with Rubacell and FIAX*[a]

Method	Agreement[b]	Sensitivity[c]	Specificity[d]	Predictive value of positive result[e]	Predictive value of negative result[f]
Rubacell	289/301 (96.0)	216/228 (94.7)	73/73 (100.0)	216/216 (100.0)	73/85 (85.8)
FIAX	276/293 (94.2)	201/215 (93.5)	75/78 (96.2)	201/204 (98.5)	75/89 (84.3)

[a] Each test was compared separately with the standard HI test. Parentheses indicate percent.
[b] True positive + true negative/true positive + false positive + true negative + false negative.
[c] True positive/true positive + false negative.
[d] True negative/true negative + false positive.
[e] True positive/true positive + false positive.
[f] True negative/true negative + false negative.

165 min. Because of the short length of the incubation periods, the incubation time was included in the time analysis. The calculated initial financial investment for performing the FIAX rubella test, including the FIAX fluorometer, shaker and clip, variable pipettors, and test tube racks, was $6,875.40. Neither the manufacturer's pipettor-dilutor nor the microcomputer was used in either the sample analysis or the cost evaluation. The cost per test including the kit ($70.00/50 tests), disposables, and technologist time was $1.85 per test. This figure does not include instrument depreciation.

DISCUSSION

The most important requirement of a test for rubella immunity is a low occurrence of false-positive reactions, because this results in not vaccinating women who are, in fact, susceptible to rubella infection. High predictive value of a positive and high specificity are indicators that the number of false-positive results is low (4). Both Rubacell and FIAX had excellent predictive values of a positive result and high specificity as compared to the standard HI test. However, because of the possibility of incomplete removal of nonspecific inhibitors present in certain sera, the HI test may not always be correct, and additional internal controls for specificity should be considered by the manufacturers. In the Rubacell test, unsensitized erythrocytes might be tested against each serum, and in the FIAX test, a more appropriate background fluorescence control would be antigen prepared from noninfected cells in the same manner as that prepared from infected cells.

The occurrence of false-negative results is another parameter to evaluate, in which the clinical implication is the unnecessary vaccination of a naturally protected woman. The disadvantages would be cost, though minimal, and inconvenience. False-negativity can best be quantitated by using the test sensitivity and the predictive value of a negative result (4). Both of the tests evaluated were sensitive, but the number of false-negatives affected the predictive value of a negative result. With the Rubacell test, 75% of the false-negative sera had HI values of 1:8. Because the Rubacell serum dilution is 1:13.5, there may be samples with antibody titers between 1:8 and 1:13.5 that are considered "false-negatives." With the FIAX test, the occurrence of nonspecific background fluorescence contributed to the problem of false-negative results, affecting nine (3.0%) of the tested sera and changing the results in six (2.0%).

Test performance time and cost analysis, including initial investment and operating costs, are also of consideration in a high-volume screening test. Although the total time required to perform 50 tests by these two methods was comparable, other laboratory work could be completed during the 2-h incubation period of the Rubacell test. The initial investment of $95.00 for the Rubacell test as opposed to $6,875.40 for the FIAX test is certainly a major consideration. However, once purchased, the FIAX fluorometer may be used for many other assays within the clinical laboratory: for example, quantitation of serum immunoglobulins, antinuclear antibody, and cerebrospinal fluid immunoglobulin G and albumin levels.

We feel that both the Rubacell test and the FIAX test are excellent screening methods for rubella immunity as compared to the standard hemagglutination assay; however, Rubacell is superior to FIAX with respect to time and cost analysis. Both assays would be improved by the addition of internal specificity controls.

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