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Comparison of the Hemagglutination Inhibition Test and an Indirect Fluorescent-Antibody Test for Detection of Antibody to Rubella Virus in Human Sera

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Correlation between FIAX and hemagglutination inhibition methods for presence or absence of rubella viral antibody was good at high hemagglutination inhibition titers, but only fair at low or negative titers.

An indirect fluorescent antibody test (FIAX test) for detection of antibody to rubella virus is available in the form of a kit from International Diagnostic Technology, Santa Clara, Calif. The kit contains all the reagents necessary for completion of the test. Rubella viral antigen is immobilized on one side of a paddle-like stick (designated a StiQ sampler). The other side of the StiQ is uncoated and serves as a control for nonspecific binding of the serum sample to the surface of the StiQ. The two surfaces of the StiQ sampler are reacted with a 1:40 dilution of the serum sample by immersing the StiQ in the diluted sample. The StiQ sampler is incubated and washed and then immersed in a fluorescein-labeled anti-human immunoglobulin conjugate. After an additional washing step, the StiQ is placed in the sample-viewing stage of the specially designed FIAX fluorometer, and the fluorescent signals are measured, first from the antigen-coated surface and then from the noncoated surface. The difference in the two signals is calculated either manually or by computer and is related to a titer by reference to a calibration curve. A new calibration curve is determined in each run by measurement of a set of four sera of known rubella virus antibody titer as determined by hemagglutination inhibition (HAI) assay. These sera are supplied with the kit.

We recently evaluated the FIAX test for use as a premarital test for determining the immune status of women to rubella virus infection. Sera were specimens submitted to our diagnostic serology section for that purpose.

A problem in any method is the ability of the method to detect low levels of antibody and to distinguish between such samples and negative samples. In our selection of sera for study we therefore placed emphasis on sera with <1:8, 1:8, 1:16, and 1:32 titers by HAI. HAI titers were determined by a standardized method using heparin manganous chloride for removal of inhibitors (1). Duplicates of serum samples were evaluated for reproducibility of FIAX results on tests done within the same run (158 serum samples), for reproducibility of FIAX results on tests done in different runs (206 different serum samples), and for agreement of FIAX results with HAI results. Three control sera (HAI titers <1:8, 1:16, 1:128) were included in each run. For the purpose of determination of immunity status, FIAX results were evaluated either as antibody present (FIAX titer ≥8) or antibody not detected (FIAX titer <8). This interpretation was based on International Diagnostic Technology's evaluation of their test in their package insert, where it is stated that a FIAX titer of less than 8 indicates that the patient is probably not immune to rubella and that accordingly a FIAX titer of 8 or greater indicates that the patient has been infected with rubella (virus).

Results on reproducibility of FIAX results are given in Table 1. There was very good reproducibility of results on duplicate samples tested in the same run. Results were less reproducible on duplicate samples tested in different runs. Since FIAX titers are calculated from a continuous

TABLE 1. *Reproducibility of results by FIAX test*

Difference (fold) in duplicate titers by FIAX	% of duplicate FIAX assays[a]	
	Same run (n = 158)	Different runs (n = 206)
≥2[b]	0	2.9
≥2[c]	1.9	1.9
≤2[c]	2.5	11.7

[a] Percentage of duplicate assays showing the indicated differences in the results of the duplicate samples. n, Number of sera.

[b] Difference in titer caused no change in interpretation of results from either antibody present to antibody absent or vice versa.

[c] Difference in titer caused a change in interpretation of results from either antibody present to antibody absent or vice versa.

scale, a 1-point deviation in titer from 8 to 7 becomes of importance in interpretation of results. The main lack of reproducibility occurred in the range around a titer of 8, i.e., from 5 to 10 or 12.

Based on FIAX scoring of "antibody present" or "antibody not detected," there was excellent agreement beteween FIAX and HAI results with sera with HAI titers of ≥1:64 (Table 2). Correlation on sera with HAI titers from ≤1:8 to 1:32 was not as good. Percent agreement between the two tests in this range was from 74 to 92% (Table 2). Serum samples with discrepant results between the FIAX and HAI tests in one or both duplicate assays were checked by passive hemagglutination assay (Rubacell; Abbott Laboratories, Chicago, Ill.). With only three sera (HAI titers <1:8) were the passive hemagglutination assay and HAI results in disagreement. It thus appears that the FIAX test is less sensitive or less accurate, or both, in diagnosis of immune status of individuals with negative or low antibody titers by HAI. Part of the insensitivity of the FIAX test at low HAI titers may be due to high background fluorescence (nonspecific adherence of serum to the noncoated surface of the StiQ). A prudent course, therefore, in interpretation of results is to evaluate the background fluorescence and, if it is excessively high, to consider an interpretation of "inconclusive" or "unsatisfactory" for the result on that particular serum sample. To obtain an estimate of the percentage of individuals whose HAI titers would be ≤1:32, HAI titers on 1,839 sera that had been submitted to our laboratory over a period of 1 year were evaluated. A total of 19% had titers of <1:8, and 21% had titers from 1:8 to 1:32. Figure 1 compares HAI titers with FIAX titers. Titers were not strictly comparable. An occasional serum had a low HAI titer and a high FIAX titer and vice versa.

The lack of a specificity control on the antigen per se in the FIAX test is of concern to us. It is usual in indirect fluorescent-antibody tests and similar methods for assaying viral antibodies, such as enzyme-linked immunoassay or radioimmunoassay, to have a control antigen prepared from noninfected cells in the same manner and from the same lot of cells as the specific antigen. All of these tests are similar in theory and differ only in the nature of the tag used to prepare the anti-immunoglobulin conjugate. Viral antigens prepared from infected tissue cultures are contaminated to some extent with products from the tissue culture, with constituents of the medium used for growth of the culture, such as fetal bovine serum, and with any adventitious microorganisms, such as mycoplasma, which may infect the culture. Sera from different patients react differently to these contaminants. A control antigen prepared from the same lot of cells used in making the viral antigen, therefore, should be tested with each patient's sera. The magnitude of the reaction with the viral antigen is then evaluated in relation to that with the control antigen.

The advantages of the FIAX test are as follows: the test can be performed rapidly (results are obtained within 0.5 day); no prior treatment of the sera is required; if the directions are followed closely, danger of technical error by the operator is minimal, and an objective measurement is obtained.

TABLE 2. *Agreement of FIAX test[a] with HAI test: results from duplicate assays*

HAI titers	% agreement of duplicate assays					
	Same run			Different runs		
	n	Result 1	Result 2	*n*	Result 1	Result 2
<1:8	75	88.0	85.4	99	86.9	92.0
1:8	19	84.3	89.5	27	74.1	81.5
1:16	36	86.1	83.3	36	83.4	86.2
1:32	8	87.5	87.5	20	85.0	85.0
≥1:64	20	100.0	95.0	24	100.0	100.0

[a] FIAX results scored as either negative (FIAX titers < 8) or positive (FIAX titers ≥ 8). *n*, Number of sera.

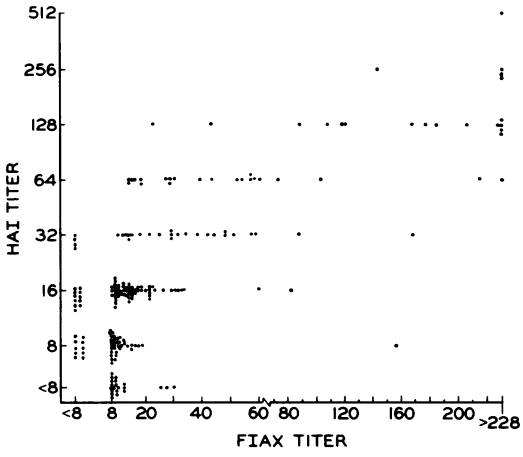

FIG. 1. *Comparison of FIAX titers with reciprocals of HAI titers. Results on the 152 serum samples with antibody not detected by both methods are not plotted. The plotted data are the results of the first assay of each duplicate sample.*

We thank International Diagnostic Technology for supplying us with reagents and equipment to evaluate their test.

LITERATURE CITED

1. **Center for Disease Control.** 1970. A procedural guide to the performance of the standardized rubella hemagglutination-inhibition test. Center for Disease Control, Atlanta, Ga.