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Comparison of an Enzyme-Linked Immunosorbent Assay with Indirect Hemagglutination and Hemagglutination Inhibition for Determination of Rubella Virus Antibody: Evaluation of Immune Status with Commercial Reagents in a Clinical Laboratory

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Comparative evaluations of immune status for rubella virus are described for enzyme-linked immunosorbent assay, hemagglutination inhibition, and indirect hemagglutination. A 92.1% agreement between enzyme-linked immunosorbent assay and indirect hemagglutination assay was demonstrated for rubella immune status. Enzyme-linked immunosorbent assay and hemagglutination inhibition demonstrated a 92.6% agreement and were compared in an attempt to define the quantitative usefulness of comparisons of single sera for determining immune status. These data support the relative lack of correlation between single enzyme-linked immunosorbent assay and hemagglutination inhibition quantitative values. Enzyme immunoassay was, however, an acceptable alternative to hemagglutination inhibition for the determination of immune status to rubella virus.

Rubella is a common communicable disease of childhood which is ordinarily benign in children and adults. However, for the developing fetus the infection may be very serious. Effects of congenital rubella may include congenital heart disease, cataracts or chorioretinitis, deafness, hepatosplenomegaly, and thrombocytopenic purpura. The incidence of congenital birth defects varies with the time of maternal infection, decreasing with subsequent trimesters. In part due to the growing list of agents shown to be involved in perinatal infections (e.g., herpes simplex, toxoplasma, cytomegalovirus, treponema, listeria, rubella, chlamydia, echovirus), it is becoming increasingly important to accurately determine the immune status of women of reproductive age and to diagnose and confirm recent infections possibly related to congenital syndromes. Hemagglutination inhibition (HAI) has been the most commonly used technique for the laboratory diagnosis of rubella. However, this test is lengthy, labor intensive, and poorly adaptable to automation. In addition, there may be considerable variation from one laboratory to another.

The use of an enzyme-linked immunosorbent assay (ELISA) has been proposed as an alternative to HAI. However, the precise usefulness of the ELISA methodologies for the determination of rubella antibody levels and the comparability of ELISA and HAI quantitative determinations has not been described.

This report describes a comparative laboratory analysis of indirect hemagglutination (IHA) and ELISA with commercial reagents, with HAI titers determined by several reference laboratories.

MATERIALS AND METHODS

Specimens. A total of 311 serum specimens were studied retrospectively at the University of Texas Medical Branch serology laboratory. A group of 57 serum specimens were received from the Texas Department of Health, Medical Serology Branch, and 139 serum specimens were received from the City of Houston Public Health Laboratories. HAI tests were carried out in each laboratory submitting specimens to the University of Texas Medical Branch. A total of 115 routine serum specimens submitted to the University of Texas Medical Branch serology laboratory from campus hospitals were screened by IHA. All sera were tested by ELISA (M. A. Bioproducts, Walkersville,

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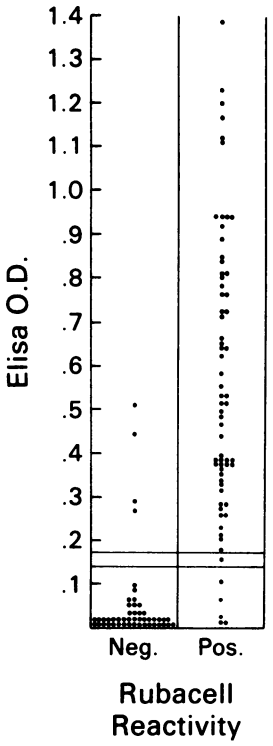

FIG. 1. Comparison of rubella immune reactivities with sera obtained at the University of Texas Medical Branch Hospitals; ELISA versus IHA.

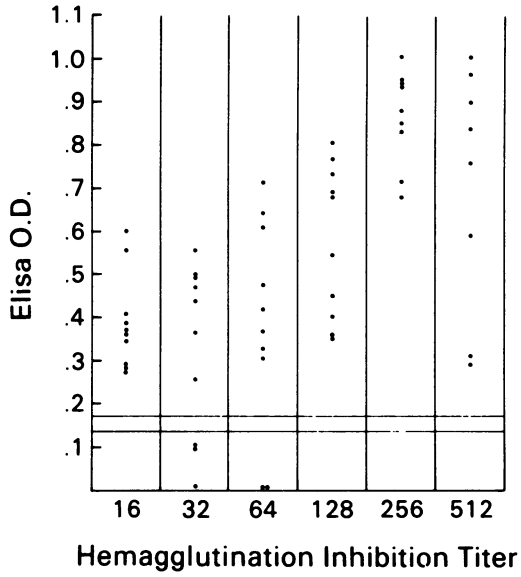

FIG. 2. Comparison of rubella immune reactivities with sera provided by the Texas Department of Health, Medical Serology Branch; ELISA versus HAI.

Md.; a Gilford PR-50 dispenser and spectrophotometer were used.)

HAI. Rubella HAI was performed at the City of Houston Public Health Laboratories, with heparin-MnCl used for the removal of nonspecific inhibitors (2). Rubella HA antigen was obtained from Abbott Laboratories, North Chicago, Ill. Baby chick erythrocytes were obtained from Flow Laboratories, Rockville, Md. All sera obtained from the City of Houston Laboratory were tested in a single day's run. High and low titer and negative serum controls were used to assure accurate HAI results.

HAI performed at the Texas Department of Health laboratory also utilized the heparin-MnCl system, with chick erythrocytes as the indicator system (2).

ELISA. Enzyme immunoassays were conducted on all sera at the University of Texas Medical Branch serology laboratory with the Rubelisa reagents (M. A. Bioproducts). The solid phase component consisted of strips with 10 joined cuvettes. Alternate cuvettes of each strip contained fixed rubella antigen. Control antigen was fixed to remaining cuvettes. Each test serum employed one rubella antigen cuvette and one control antigen cuvette. Optical density due to nonspecific background binding was subtracted from the corresponding test. Cuvettes were incubated for 2 h at room temperature (20 to 25°C) in a humid chamber. After rinsing with buffer, alkaline phosphatase-conjugated rabbit anti-human immunoglobulin G was added and incubated for 2 h at room temperature. The cuvettes were rinsed again, *p*-nitrophenyl phosphate was added, and the cuvettes were incubated at room temperature for precisely 45 min. The reaction was stopped by adding 3 N NaOH. Readings were made at 405 nm. High-positive, low-positive, and negative controls were required to fall within the prescribed range. A net change in optical density (O.D.) of $\leq$0.14 was interpreted as negative, $\geq$0.17 was interpreted as positive, and 0.15 to 0.16 was interpreted as equivocal.

IHA. IHA was performed according to the instructions of the manufacturer (Abbott Laboratories). The test utilized human erythrocytes. Briefly, 25 μl of phosphate buffer was added to V-bottommed microwells. A 2-μl volume of test specimens and positive and negative controls were added and mixed with 25 μl of sensitized erythrocytes. Plates were incubated at room temperature for 2 h. Erythrocytes settling into a sharp, compact button represented a negative test, and a disperse settling pattern represented a positive reaction.

RESULTS

ELISA versus IHA. A 92.1% (105/114) agreement was demonstrated for rubella immune status between ELISA and IHA (Fig. 1). When compared with IHA, ELISA was 92.4% (61/66) sensitive and 91.7% (44/48) specific; 8.3% (4/48) false-positive and 7.6% (5/66) false-negative results were documented when compared with IHA. One specimen was deleted from this analysis due to a borderline ELISA value.

ELISA versus HAI. A total of 139 serum specimens from the City of Houston Public Health Laboratories and 57 serum specimens from the Texas Department of Health laboratory were analyzed for relative values obtained by

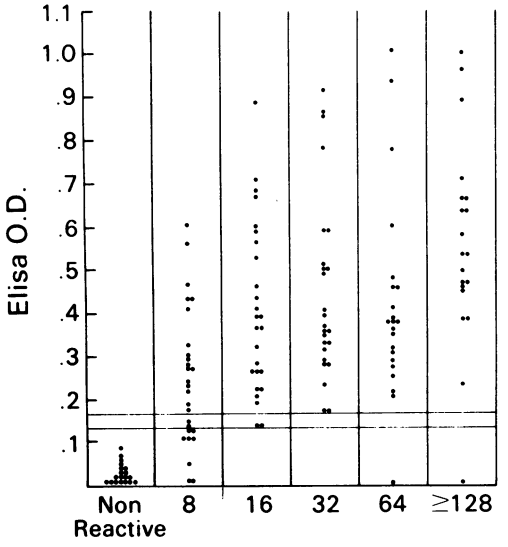

FIG. 3. Comparison of rubella immune reactivities with sera provided by the City of Houston Public Health Laboratories; ELISA versus HAI.

HAI or ELISA. Previous reports have varied with respect to the precise quantitative relationship between viral antibody titers as measured by HAI or ELISA. Several investigators have reported an almost linear relationship between viral antibody titers and ELISA optical densities (1, 5, 6). Recent studies have described the lack of correlation between individual ELISA values and HAI serum titers (3, 4, 7). We corroborated this relative lack of correlation between single ELISA and HAI values (Fig. 2 and 3).

Enzyme immunoassay, however, continues to be an acceptable alternative to HAI for the determination of immune status to rubella virus. Our data demonstrate that when compared with HAI, ELISA was 91.4% (106/116) sensitive and 100.0% (19/19) specific. No false-positive (0/19) and 8.6% (10/116) false-negative results were documented when compared with HAI. A 92.6% (125/135) agreement was demonstrated between ELISA and HAI for rubella immune status. Graphic representation of these data is seen in Fig. 3. Four specimens were deleted from this analysis due to borderline ELISA values.

DISCUSSION

Previous reports have demonstrated that HAI and ELISA were comparable for demonstrating seroconversions, rises in titer, and the presence of antibody to rubella virus (3). However, it appears from these and other data that although comparable for determining immune status, single serum specimens may not be quantitatively comparable with these two methods. In general, serum specimens with higher HAI values demonstrated higher ELISA optical densities. The range, however, was so exaggerated that clear comparisons could not be made. Shekarchi and colleagues have suggested that the two tests may not be dependent upon exactly the same antibody attachments (3). This appears to be a reasonable hypothesis. For this reason it has been proposed that standardization in rubella antibody testing be implemented by determining exactly which antibodies are being measured. International reference standards are needed to allow more meaningful analysis of immune status.

IHA continues to be a reliable method for screening sera for immunity to rubella virus. In our hands the IHA was comparable in sensitivity to ELISA for the determination of immunity.

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