

Comparison of Enzyme-Linked Immunosorbent Assay, Hemagglutination Inhibition, and Passive Latex Agglutination for Determination of Rubella Immune Status

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Hemagglutination inhibition, enzyme-linked immunosorbent assay (ELISA), and passive latex agglutination were used to test 495 human serum samples for determination of rubella immunity. Overall agreements for immune status were as follows: hemagglutination inhibition versus ELISA, 94.7% (469 of 495); hemagglutination inhibition versus passive latex agglutination (1:10 dilution), 99.2% (491 of 495); and passive latex agglutination (1:10 dilution) versus ELISA, 94.7% (469 of 495). Both ELISA and passive latex agglutination are satisfactory for rubella immunity screening, with the reservation that the ELISA method examined yielded a large number of false-negative results.

Extensive immunization programs have led to a marked reduction in the incidence of congenital rubella syndrome in the United States, with fewer than 10 cases per year reported since 1981. However, several hundred cases of rubella are still reported each year, and over 60% of these occur in persons 15 years of age or older (3). Therefore, it is important to continue to monitor the immune status of females of childbearing age and to confirm suspected infections. The availability of reliable, cost-effective laboratory tests is vital to both screening and diagnosis.

Various serological methods are in wide use for the detection and quantification of rubella antibodies. Among these are hemagglutination inhibition (HAI), enzyme-linked immunosorbent assay (ELISA), and passive latex agglutination (PLA). In this study, the ELISA and the PLA were each compared with a standard HAI for sensitivity, specificity, and agreement.

A total of 500 sera submitted consecutively to the New Mexico Scientific Laboratory Division were tested by the three methods. The sera were submitted primarily for the purpose of obtaining assistance in immunization decisions and for monitoring the level of rubella immunity in the New Mexico female population. The sera were first tested by ELISA and then stored at 4°C for no longer than 3 days until subsequent testing by HAI and PLA. All specimens were coded, and comparative results were unknown until tabulation of all data.

The Rubazyme test kit (Abbott Laboratories, North Chicago, Ill.) was used to perform the ELISA test, according to the procedure of the manufacturer for immune status (1). A polystyrene bead coated with rubella antigen was reacted with a 1:200 dilution of patient serum at 37°C for 60 min in a plastic reaction tray. The beads were washed in distilled water, and 200 μ l of anti-human immunoglobulin G-peroxidase conjugate was added. The trays were then incubated at 37°C for 60 min. The beads were again washed in distilled water and transferred to reaction tubes where 300 μ l of *o*-phenylenediamine \cdot 2HCl was added at room temperature and incubated for 30 min. After 30 min the enzymatic reaction was stopped by the addition of 2.0 ml of 1 N HCl.

The contents of the tubes were mixed, and results were read on a spectrophotometer (Quantum II; Abbott Laboratories) at 492 nm. A specimen was considered positive ("immune") if its absorbance was equal to or greater than the mean of two low-positive control values in the same run, i.e., if the Rubazyme Index equaled at least 1.000 (the optical density of the patient specimen divided by the mean optical density of the low-positive controls). For a given test run to be considered valid, a concurrent high-positive control serum sample must have had an absorbance of at least 0.700, being at least 1.80 times as great as the absorbance of the mean of the low-positive controls. Field evaluations by the manufacturer have shown that Rubazyme test results are equivalent to HAI test results in their ability to determine immune status (1).

The HAI test was performed according to the standard method of the Centers for Disease Control (2), with heparin-MnCl₂ treatment for removal of nonspecific inhibitors, and trypsinized human O cells. The initial serum dilution was 1:8. Specimens with titers of \geq 8 were classified as immune. Controls consisted of high-positive, weakly positive, and nonreactive specimens provided by the Centers for Disease Control.

The Rubascan test kit (Hynson, Westcott & Dunning, Baltimore, Md.) was used to perform the PLA test, according to the procedure of the manufacturer for screening sera for rubella antibody at a 1:10 dilution (PLA 1:10) (4). Latex particles sensitized with soluble rubella virus antigen and 25 μ l of a 1:10 dilution of serum were mixed on a Teflon-coated card. The mixture was then incubated at room temperature on a mechanical rotator for 8 min at 100 rpm. After rotation, the card was read visually without magnification. A positive result, that is, immune, was interpreted as any agglutination of the latex antigen. Strongly reactive, weakly reactive, and nonreactive controls were provided by the manufacturer and run concurrently with patient specimens. According to the manufacturer this procedure will approximate the sensitivity level obtained with hemagglutination inhibition methods (4).

Of the 500 serum samples tested, 5 were found to have nonspecific hemagglutination in their serum controls and were discarded from this study. For the remaining 495 specimens, an agreement of 94.7% (469 of 495) was obtained

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for rubella immune status between HAI and ELISA (Table 1). With HAI as a standard, ELISA showed a sensitivity and specificity of 94.7 and 95.3%, respectively. The predictive value of a positive test (PV+) was 99.5% and the predictive value for a negative test (PV-) was 63.1% (Table 2).

A 99.2% agreement (491 of 495) was obtained for rubella immune status between HAI and PLA 1:10 (Table 1). When compared with HAI, PLA showed 99.6% sensitivity and 95.3% specificity. The PV+ was 99.6, and the PV- was 95.3% (Table 2).

A 94.7% agreement (469 of 495) was obtained for rubella immune status between PLA 1:10 and ELISA. When compared with PLA, ELISA showed a relative sensitivity and relative specificity of 94.7 and 95.3%, respectively. The PV+ was 99.5%, and the PV- was 63.1% (Table 2).

The data presented indicated that both the PLA and ELISA were accurate (and reliable) methods for rubella immunity screening, as previously shown by other workers (6-8). Their results are consistent with the observation that rubella antibodies measured by PLA and ELISA appear in parallel with antibodies detected by HAI after both natural infection and immunization (7).

In the present study, the proportion of false-negative ELISA results among HAI-positive serum samples was 5.3% (24 of 452). However, in studies where borderline specimens ($0.500 \leq \text{Rubazyme Index} \leq 1.499$) near the cut-off value were studied, this figure was 41.0% (103 of 251) (10).

Of interest were positive ELISA results for 4.6% (2 of 43) of the HAI-negative serum samples. This is similar to an earlier report of 4.3% (14 of 148) false-positive Rubazyme test results for borderline specimens (10). In contrast, Kleeman and colleagues, using a different commercial ELISA kit, found 39% (34 of 87) HAI-negative, ELISA-positive serum samples (5). They concluded that newer, more sensitive methods will detect low-level rubella antibodies which are undetected by HAI.

Agreement between PLA and HAI was high (99.2%), with no observed difference in sensitivity. These findings are consistent with those of Meegan and co-workers, who found 97.5% agreement, with a discrepancy in 8 of 276 serum samples, which were HAI negative and PLA positive (6). Like ELISA, the PLA method yielded 4.6% (2 of 43) negative results in HAI-positive serum samples. Unlike ELISA, there were only 0.4% (2 of 452) PLA-negative results among HAI-positive serum samples. This very low false-negative rate could be instrumental in avoiding unnecessary immunizations in women who are actually immune.

TABLE 1. Comparison of rubella immune status by HAI, ELISA, and PLA with 495 serum specimens

Procedure	HAI		PLA 1:10		% Agreement
	Immune	Non-immune	Immune	Non-immune	
ELISA					94.7
Immune	428	24			
Nonimmune	2	41			
PLA 1:10					99.2
Immune	450	2			
Nonimmune	2	41			
ELISA					94.7
Immune			428	24	
Nonimmune			2	41	

TABLE 2. Performance characteristics of PLA 1:10 and ELISA rubella tests compared with HAI standard

Characteristic	PLA 1:10	ELISA
Sensitivity ^a	99.6	94.7
Specificity ^b	95.3	95.3
PV+ immune ^c	99.6	99.5
PV- nonimmune ^d	95.3	63.1
Agreement with HAI standard ^e	99.2	94.7

^a Sensitivity = [true positives/(true positives + false negatives)] × 100.

^b Specificity = [true negatives/(true negatives + false positives)] × 100.

^c PV+ = [true positives/(true positives + false positives)] × 100.

^d PV- = [true negatives/(true negatives + false negatives)] × 100.

^e Agreement = [(true positives + true negatives)/(true positives + true negatives + false positives + false negatives)] × 100.

Both PLA and ELISA provide an adequate means of detecting rubella antibodies, with the reservation that Rubazyme yielded more false-negative results. The establishment of a range of indeterminate values of Rubazyme results might aid in solving this problem (10). Both methods have distinct technical advantages over HAI, such as no serum pretreatment and a much shorter time until reporting. The PLA method is especially simple and rapid, being readable after an 8-min incubation period.

Both the PLA and ELISA methods for detection of rubella antibodies have reached acceptance in clinical laboratories in a relatively short period of time. In the 1978-1980 College of American Pathologists survey, 45% of the laboratories testing for rubella reported using HAI (9). However, the 1982 College of American Pathologists survey showed that HAI was used for only 19% of rubella testing. ELISA was used for 13% (Rubazyme, 10%), PLA was used for 25%, passive hemagglutination was used for 29%, indirect fluorescent-antibody assays were used for 11%, and radioimmunoassay was used for 3%. This switch from HAI to other techniques has come about because of the speed, accuracy, reliability, and cost savings which the newer methods offer.

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