Detection of Rubella-Specific Immunoglobulin G: Comparison of the Enzyme-Linked Immunosorbent Assay and an Automated Microparticle Enzyme Immunoassay (IMx)

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An automated microparticle enzyme immunoassay (IMx Rubella IgG Antibody Assay; Abbott Laboratories, North Chicago, Ill.) was compared with a conventional enzyme-linked immunosorbent assay (ELISA) for detection of rubella-specific immunoglobulin G (IgG) in 400 consecutive antenatal patients. There was complete agreement between the two tests in this population, which had a positive rate of 99% for rubella-specific IgG antibodies. The performance of the IMx was also evaluated at the cutoff zone by assaying 64 selected antenatal serum samples with low or negative rubella antibody titers as determined by ELISA. Overall, the IMx was found to be a specific, sensitive assay for the detection of rubella-specific IgG and is virtually fully automated for easy performance.

The accurate determination of rubella antibodies to screen pregnant women and women of childbearing age for past exposure to rubella virus or for the diagnosis of recent infection is of paramount importance. Hemagglutination inhibition (HI) has been the ‘‘gold standard’’ procedure for many years for such determination but has the disadvantages of being time-consuming and requiring the removal of nonspecific inhibitors and agglutinins, and standardization between laboratories can be difficult (2). Consequently, enzyme-linked immunosorbent assay (ELISA) methods with the advantage of no pretreatment of sera, with the potential for semiautomation, and with performance comparable to that of HI have largely replaced HI in many routine diagnostic laboratories (3).

A modification of the traditional solid-phase ELISA, a microparticle capture enzyme immunoassay (IMx Rubella IgG Antibody Assay [IMx]), has been developed by Abbott Laboratories as an automated immunoassay with a particulate solid phase of latex particles rather than the traditional microtiter wells. Immune complexes are detected by a fluorescent substrate.

We evaluated the performance of the IMx to determine the levels of rubella-specific immunoglobulin G (IgG) in 400 serum samples from antenatal women and compared it with a conventional ELISA method.

Serum samples were collected from 400 consecutive pregnant patients at their first antenatal visits to the Royal Women's Hospital, Carlton, Victoria, Australia, from 2 November to 23 November 1989. Routine screenings for syphilis, hepatitis B surface antigen, and rubella antibodies, as well as blood grouping, tests for erythrocyte antibodies, and hemograms, were performed. Since a high proportion of the study population had been previously vaccinated against rubella virus (in the schoolgirl vaccination program), selected serum samples with low titers of antibody or negative by ELISA were also assayed by IMx. This was to determine the performance of this new screening test at around the cutoff zone. Therefore, 64 serum samples which were collected from a population of 1,232 antenatal patients and had been assayed as negative or weakly positive by ELISA were retested by IMx. Where any discrepancies occurred HI was determined (5) by the Virology Department, Fairfield Infectious Diseases Hospital, and the State Reference Laboratory, Victoria, Australia.

Levels of IgG antibodies to rubella virus were determined both by a conventional ELISA and by the IMx. The ELISA is an in-house assay which has been used for the past 8 years and was performed as previously published but with the modifications of substituting poly-L-lysine (Sigma Chemical Co., St. Louis, Mo.) for albumin and using o-phenylenediamine dihydridorhochrome (Sigma) as a substrate (6). These two modifications did not change the sensitivity or specificity of the assay and were introduced to improve the ease of performance of the assay (unpublished data). For the IMx procedure, serum samples were loaded into supplied disposable reaction cells and then placed into the IMx carousel. The instrument then automatically performed all the steps required for analysis. Briefly, the assay utilized latex microparticels coated with rubella antigen, which were transferred to the reaction cells containing the diluted serum. Any rubella-specific IgG present in the serum reacted with rubella antigen present on the surface of the microparticles to form an immune complex. The reaction mixture was then transferred to an inert glass-fiber matrix to which the immune complexes bind irreversibly. The detection of the immune complexes was accomplished by addition of an alkaline phosphatase-labeled conjugate. The complex was then washed with buffer to remove any unbound conjugate, and a substrate (4-methylumbelliferyl phosphate) was added.

The rate of conversion of the substrate to a fluorescent product (methylumbelliferone) was measured fluorometrically. (The rate of fluorescent product generated on the matrix is proportional to the concentration of antibody in the test sample.) Positive and negative controls were included once per day. Rubella-specific IgG concentration in international units per ml was then determined from a stored calibration curve created by using known standards and point-to-point data reduction. Specimens found to contain <10 IU of IgG were considered negative, while those containing ≥10 IU were positive.

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measuring the rubella-specific IgG concentration for one serum sample which was run as 21 replicates in one run.

Of the 400 serum samples received for routine rubella antibody screening and tested by both methods in parallel, there was complete agreement (100%) between the two tests. Four (1%) serum samples were negative, and 396 (99%) were positive for rubella-specific IgG by both tests. The interassay coefficient of variation was 7.35% (mean = 9.98 IU) at the cutoff value of the assay when a World Health Organization standard (3 IU/ml) was used. When a linear regression analysis was performed with a World Health Organization standardized serum, the cutoff point of the ELISA was equivalent to 3 IU/ml. The corresponding E value (6) for the 10 IU/ml cutoff was 0.148.

Of the 64 serum samples which had previously been shown to be negative (22) or of low titer (42) (<0.240 enzyme immunoassay units) by ELISA, 22 were negative and 38 were positive by both methods; 4 detected as weakly positive by ELISA were detected as negative by IMx (all tested between 4 and 10 IU/ml). When these four serum samples were retested by HI, two were weakly positive at 1:20, while the remaining two were negative at <1:10. These results indicate a close correlation between the assays when very low levels of antibody were measured.

ELISA has proved to be a convenient, sensitive, and reproducible method for measurement of rubella antibodies (1). It is particularly adaptable to large-scale screenings of populations. By the use of a spectrophotometer, results can be read quantitatively with only one serum dilution, since the E values have been shown to be proportional to standard antibody levels (6). This has the advantage of reducing both the handling time in test performance and the volume of serum required for testing. The IMx assay is virtually a fully automated modification of the traditional ELISA. It requires only that the technician add serum to the cartridges, enter the identification numbers of the samples, and press "RUN" on the machine. On performance testing, the concordance of the IMx with our in-house ELISA was excellent: 100%. In low-level or negative-titer serum samples the IMx called two specimens negative which had been determined to be positive by ELISA and HI. Similar evaluation for Toxoplasma gondii antibody and for detection of rubella IgG and IgM by IMx have validated the procedure as fast, sensitive, and specific (4).

The IMx was capable of being interfaced with our computer system (ICL SYSTEM 25), allowing a shorter turnaround time for rubella testing and reporting. With the ELISA system, it took up to 5 days to issue a report as samples were batch tested. However, with IMx, because of smaller batches, shorter runs, and automatic data reduction, the time can be reduced to less than 2 days. With such excellent results and the time advantage the routine laboratory has now switched to use of the IMx for all rubella-screening tests. In addition, the stored calibration curve remained stable over the 5-month period during which it was used.

With regard to diagnosis of recent infection it would appear that the cutoff used for the IMx assay has more relevance to past exposure status than to the detection of very low levels of rubella-specific IgG. Since the package insert carries warnings that the kit has yet to be approved for diagnosis of recent infection, it would be prudent to run all paired serum samples that show apparent seroconversion by IMx by another sensitive assay to distinguish between primary infection and reinfection.

REFERENCES