# Serum Levels of Rubella Virus Antibodies Indicating Immunity: Response to Vaccination of Subjects with Low or Undetectable Antibody Concentrations

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**To define the concentration of anti–rubella virus (RV) antibodies discriminating nonimmune from immune persons and to characterize immune responses to rubella vaccination, serologic studies were performed after rubella vaccination in persons with low or undetectable antibody concentrations. Thirty-six subjects with primary immune responses had prevaccination anti-RV IgG concentrations <15 IU/mL by ELISA and negative results by radial hemolysis. Eighty-three subjects with secondary immune responses had mean IgG increases of 9 IU/mL within 2 weeks. Eight of them had initial IgG levels <15 IU/mL, and 2 were negative by radial hemolysis. Both groups attained similar antibody levels after 1–3 months. Secondary immune responses to rubella vaccination were delayed by >2 weeks and thus resembled the time course of primary immunization, but IgM responses and IgG avidity were distinct between subjects with primary or secondary immune responses. Thresholds for immunity <15 IU/mL entail the risk of withholding rubella vaccination from susceptible persons.**

Prevention of the congenital rubella syndrome rests on the efficient implementation of childhood vaccination programs and on the detection and vaccination of women of childbearing age who are susceptible to rubella [1]. Definition of a cutoff level for anti–rubella virus (RV) antibodies that reliably indicates previous exposure and immunization by RV, and thereby presumably immunity in terms of protection from intrauterine infection, is therefore important [1–3]. Hemagglutination inhibition and radial hemolysis are time-honored techniques with disadvantages such as nonspecific inhibition by serum components other than antibodies [4–6] and difficulties in providing suitable reagents. They are increasingly being replaced by various ELISAs or latex agglutination assays [1, 7–10] that may detect lower concentrations of anti-RV IgG antibodies than do older techniques [7]. This creates problems for standardization and uncertainties as to the antibody levels that indicate immunity [4, 5, 9–14]. The estimation of the persistence of anti-RV antibodies [15–19] and the surveillance of rubella vaccination strategies by age-stratified seroprevalence studies [20] also depend on the reliability of the techniques in detecting susceptible persons in need of vaccination.

The response to vaccination may give insight into the immunologic experience of a person irrespective of the current serum level of antibodies [21–24]. The production of high-avidity IgG within several days after reexposure to RV [25–27] and the lack of an IgM response are the expected hallmarks of secondary immune responses [8, 11, 12, 28–30]. However, rubella revaccination may induce only weak or transient responses [11, 21, 22]. The question ''are many women immunized against rubella unnecessarily?'' [12] remains unanswered, particularly for the allegedly more sensitive modern tests. We therefore studied the immune response in persons who had received rubella vaccine because of low or undetectable anti-RV antibodies, in order to establish the cutoff for an automated IgG ELISA and to compare its performance with radial hemolysis as well as to characterize the kinetics and vigor of primary and secondary responses.

## Materials and Methods

*Study population.* Between 30 July 1992 and 1 November 1995, consecutive testing of 5060 subjects for anti-RV IgG revealed 1015 with low or undetectable levels of IgG (i.e., <40 IU/mL). Of these, 501 were offered serologic testing if their physicians considered rubella vaccination to be indicated and if they were negative for anti-RV IgM. Determination of anti-RV IgG was offered at 1 week and 1–2 months after vaccination. We obtained information on rubella vaccination and serum samples from a total of 165 subjects (only 4 were men). Of the subjects, 139 had been tested in a pregnancy screening program, 19 were health care workers, and 7 were tested for other reasons. One hundred forty-seven first samples were taken within a mean of 7 days (SD, 2.6) and 119 samples within 37 days (SD, 9) after vaccination. A third sample was obtained from 11 subjects after 56–545 days. The group without follow-up contained 282 women and 54 men. The median age and anti-RV IgG concentration were similar in vaccinated women before vaccination and in women without follow-up (28.9 vs. 28.7 years and 20 IU/mL in both groups; $P > .05$, Mann-Whitney $U$ test). The distribution of IgG values was also comparable in women who were not included in the study and those who were followed up, with about one-third having <10 IU/mL in both groups. About two-thirds of the men had values <10 IU/mL, but most of them were not available for follow-up.

*Methods.* Serum was separated from blood cells within 2–14 h after venipuncture and tested for anti-RV IgG. The serum samples

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were maintained at 2–8°C for 2–10 days, until they were aliquoted and kept frozen at −20°C for further testing. Anti-RV IgG concentrations were measured by ELISA. During the recruitment period we used the semiautomated VIDAS Rubéole IgG Test version 1 (Vi1-G; bioMérieux, Marcy-l'Etoile, France), and results were reported as international units per milliliter. Anti-RV IgG concentrations from 20 to 40 IU/mL were interpreted as weak positive results. For final analysis, all available serum samples from vaccinated persons were subjected to batchwise testing using the VIDAS Rubéole IgG Test version 2 (Vi2-G), in which IgG concentrations <15 IU/mL were interpreted as negative according to the manufacturer's recommendations. Both versions of this assay are based on the indirect immunosorbent principle, with a solid phase coated by inactivated wild virus antigen and alkaline phosphatase–conjugated mouse monoclonal anti-human IgG antibodies and 4-methylumbelliferylphosphate as detecting reagents. In addition, anti-RV antibodies were measured by Hämolyse-Gel-Test für Röteln (RHG; Labor Dr. Koch + Dr. Merk, Ochsenhausen, Germany). This test is based on the complement-mediated lysis of RV hemagglutinin-sensitized baby chick erythrocytes embedded in agarose gel that has been penetrated by serum from punch holes. Hemolysis zone diameters of ⩾9 mm (corresponding to 20 IU/mL by ELISA or 1:32 by hemagglutination inhibition) were considered positive.

The avidity of anti-RV IgG was determined by the rubella IgG avidity test (Labsystems, Helsinki), which applies the elution principle using a washing step with urea to an indirect solid-phase ELISA with alkaline phosphatase–conjugated anti-human IgG [31, 32]. Results were analyzed by using an Excel-based Macintosh program that calculates the shift to the left of the dilution curves caused by the elution step.

Anti-RV IgM was detected by the AxSYM Rubella IgM assay (Abbott Laboratories, Abbott Park, IL), an automated ELISA that uses microparticles coated with purified RV (strain HPV-77), which bind on a glass fiber matrix after reaction with diluted serum, and alkaline phosphatase–conjugated anti-human IgM with 4-methylumbelliferylphosphate as detecting reagents; in this procedure, all sera were absorbed with the IMx rheumatoid factor neutralization reagent according to the manufacturer's instructions. As a second IgM test, we used the VIDAS Rubéole IgM Test (bioMérieux), an automated $\mu$-capture ELISA with inactivated wild virus antigen and alkaline phosphatase–labeled Fab′ fragments of a monoclonal anti–RV hemagglutinin antibody and 4-methylumbelliferylphosphate for detection.

Low IgG avidity (<15%) or intermediate IgG avidity (15%–25%) and positive IgM test results 1–3 months after rubella vaccination were classified as primary immune responses. High IgG avidity and negative IgM results at this time interval were considered to represent a secondary immune response; that is, these persons must previously have been exposed to RV antigens. From 46 of 165 subjects, there were no adequately timed samples for the definite characterization of the type of the immune response by these techniques.

Data were analyzed using the StatView 4.02 program (Abacus Concepts, Berkeley, CA) for paired or unpaired means comparisons, Mann-Whitney *U* tests, Wilcoxon signed rank tests, and box plots. Statistical significance was defined as $P < .05$.

## Results

Prevaccination anti-RV antibodies were negative by Vi2-G (figure 1A) and RHG (figure 1B) in 35 subjects who developed a primary immune response to rubella vaccination characterized by low anti-RV IgG avidity (figure 2A) and positive anti-RV IgM (figure 2B) after 1–3 months (test specificity, 100%). One person who responded with low-avidity IgG (9.6%) but had negative results with both IgM tests was arbitrarily considered to have a primary response. Anti-RV IgG concentrations measured by Vi2-G remained unchanged within 2 weeks after rubella vaccination of 29 persons with primary immune responses ($P = .16$; paired means comparison).

Secondary immune responses could be documented by high avidity of anti-RV IgG antibodies and negative specific IgM responses after 1–3 months in 83 persons (figure 2). Nine subjects with secondary immune responses to rubella vaccination by these criteria had prevaccination anti-RV antibody results below the cutoffs recommended by the manufacturers for Vi2-G and/or RHG, and a response to rubella vaccination was not evident within 1–2 weeks (table 1; figure 1). After 1–2 months, specific IgG concentrations increased >10-fold in 5 and to a lesser degree in the rest of them. With a cutoff at 7 mm for RHG, this test would be negative in only 2 persons and yet remain 100% specific.

The sensitivities of Vi2-G and RHG were 90.4% and 97.6%, respectively, in the selected group of 119 persons who could be tested appropriately for the type of immune response to rubella vaccination. If these results are representative of all 5060 persons tested during the study period, the sensitivity and predictive value of negative Vi2-G results can be estimated at ~98.5% and 75%, respectively. The seroprevalence of anti-RV IgG in young Swiss adults is 96% [20]. Thus, ~1% of them lack detectable RV antibodies despite previous immunization.

Anti-RV IgG concentrations increased slightly but significantly within 2 weeks in 74 subjects with secondary responses, from a mean value of 32.5 to 41.5 IU/mL (mean difference, 9 IU/mL; 95% confidence interval, 5–13 IU/mL; $P < .001$; paired means comparison) (figure 1A). A similar response was evident with anti-RV antibody determinations by RHG ($P = .75$ and $P = .01$ for primary and secondary responses, respectively; paired means comparisons) (figure 1B). A clearcut increase in anti-RV IgG levels was evident 1–3 months after vaccination in persons with both primary and secondary immune responses (median intervals, 34 and 37 days, respectively), reaching similar mean (median) values of 112 (87) and 127 (105) IU/mL, respectively (tied *P* value = .19; Mann-Whitney *U* test for difference between groups at 1–3 months). Subjects with secondary immune responses and prevaccination anti-RV IgG concentrations of <15 IU/mL ($n = 8$), 15–29 IU/mL ($n = 30$), and ⩾30 IU/mL ($n = 43$) showed a mean increase of 17.3-, 5.6-, and 3.4-fold, respectively ($P < .02$ for all groups, unpaired means comparison) (figure 3A). Seventeen of the secondary responses were <2-fold. Mean IgG increases in primary immune responses were 58-fold. A similar evolution of the responses was evident with RHG (figure 3B).

After a median observation interval of 152 days (range, 56–545), anti-RV IgG concentrations of 17 persons were

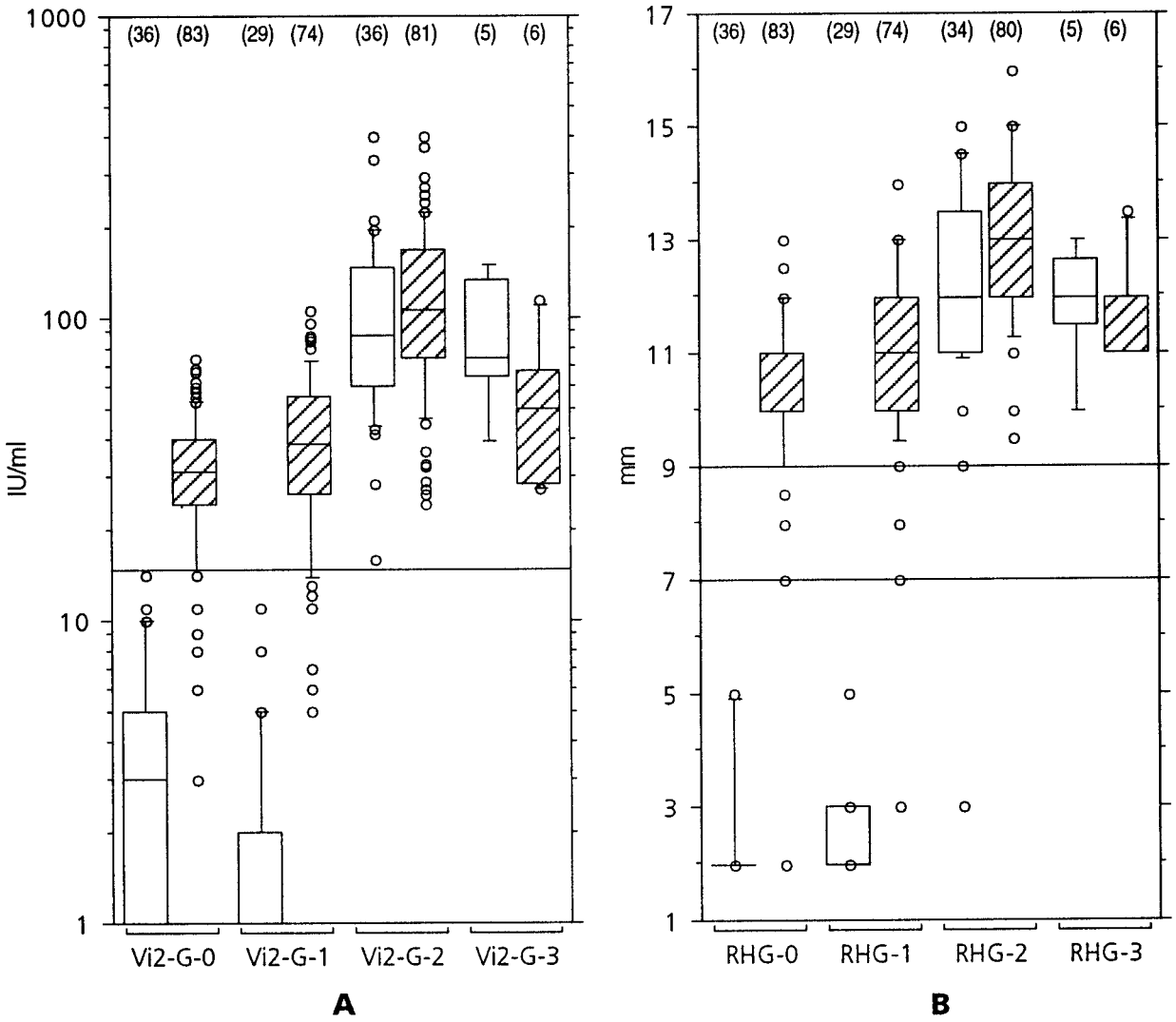


**Figure 1.** **A**, Anti-RV IgG antibodies measured by ELISA before (Vi2-G-0) and 1–2 weeks (Vi2-G-1), 1–3 months (Vi2-G-2), or up to median of 152 days (Vi2-G-3) after rubella vaccination in persons responding with IgM and low-avidity IgG antibodies 1–3 months after vaccination (primary response, open boxes) compared with those producing no IgM and high-avidity IgG (secondary response, hatched boxes). **B**, Similar presentation for anti-RV antibodies measured by radial hemolysis (RHG) before and after rubella vaccination. Box plots show median and 50% of values within boxes and 90% within bars. Solid lines indicate cutoff values for positive results and equivocal range for Vi2-G and RHG, respectively. Numbers in parentheses indicate how many samples were available for corresponding tests and subgroups.

between 27 and 150 IU/mL (median, 67 IU/mL). Eleven persons with prevaccination results ⩾15 IU/mL had slightly lower values than the 6 with negative prevaccination results (medians, 66 and 74 IU/mL, respectively; $P \geqslant .48$, Mann-Whitney $U$ test). This is also reflected in 11 of these 17 subjects who could be grouped according to their immune response (figures 1A, 3).

No vaccination side effects were reported.

## Discussion

Antibodies produced after exposure to wild type RV usually confer lifelong protection from reinfection [17, 33]. Successful rubella vaccination has a high protective efficacy, in particular for viremic infections [1, 2, 22, 28, 34]. However, in the absence of wild virus exposure, protective vaccine-induced immunity may wane [35, 36]. A few cases of reinfection during pregnancy, with transmission of the virus to the fetus and the emergence of congenital rubella syndrome, have been described in women with well-documented immune responses to vaccine or wild virus before conception [37, 38]. In spite of this, any level of detectable antibody to RV is generally considered presumptive evidence of protective immunity [1–3], especially with tests that correlate with neutralizing antibodies [39, 40]. Although the presence of anti-RV antibodies detectable at any level does not completely rule out the possibility of viremic infections by wild type RV and transmission to the fetus [37,

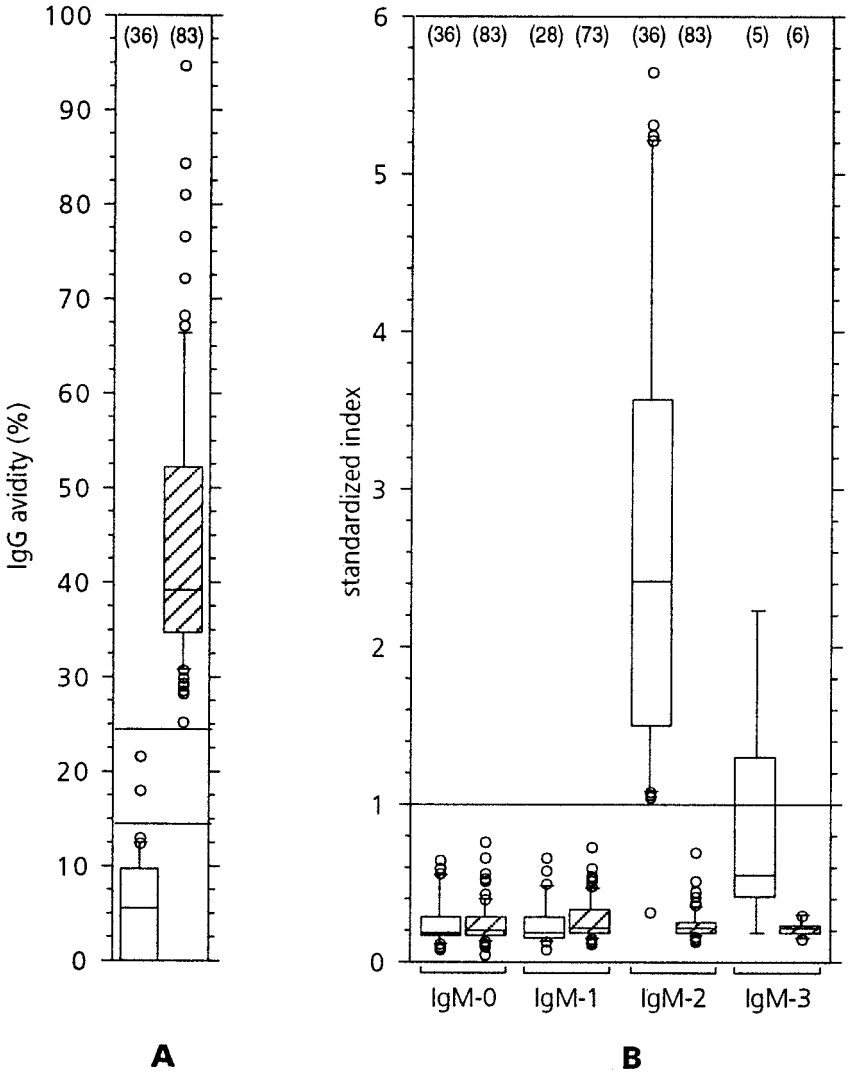


**Figure 2.** **A**, Avidity of anti-RV IgG antibodies 1–3 months after rubella vaccination in persons with primary or secondary immune responses to vaccination. 2 serum samples with equivocal results (between horizontal lines) were anti-RV IgM–positive. **B**, Anti-RV IgM responses before (IgM-0), and 1–2 weeks (IgM-1), 1–3 months (IgM-2), or up to median of 152 days (IgM-3) after rubella vaccination, grouped according to type of immune response to vaccine. Standardized index = fluorescence value of sample/fluorescence value of cutoff. Values >1 (horizontal line) are positive. For explanation of box plots see legend to figure 1. Nos. of samples available for testing in different subgroups are shown in parentheses.

38], the demonstration of the lack of previous exposure and immune response to wild type or attenuated vaccine virus is of preeminent importance. However, standardization of anti-RV antibody concentrations for a variety of different techniques is difficult to achieve, particularly at low levels [4, 8, 14]. This may jeopardize the recognition of susceptible persons who need vaccination in order to curtail transmission of RV into and within the female population of childbearing age. We approached this problem by studying the immune response to rubella vaccination.

The evolution of the avidity of anti-RV IgG and the production of specific IgM antibodies within 3 months after rubella vaccination clearly distinguished groups with primary and secondary immune responses. Primary immune responses evolved as expected, with an IgM response and IgG antibodies of low avidity appearing within 4 weeks.

Secondary immune responses to vaccines are expected to show a rapid increase of IgG production [41–43] and no IgM response [11, 22, 23]. Reinfections by wild type virus, however, have the potential to induce IgM antibodies, but usually at low levels [22, 44–46]. In our study, secondary immune responses to rubella vaccination were unexpectedly characterized by a quantitatively negligible, albeit statistically significant, increase in anti-RV IgG concentrations within 2 weeks and by peak levels after 1–3 months that were not significantly higher than those in primary responders. Such delayed and attenuated secondary antibody responses have previously been described in a few subjects after intranasal RV challenge [22, 46] or RV revaccination [21, 24]. As we do not know the immunization history of most of our study participants, the potential contribution of the type of primary immunization (by wild type or vaccine virus) to this sluggish secondary response remains undefined and will be impossible to ascertain in a population with ongoing wild virus circulation [47, 48]. The presence of neutralizing antibodies in spite of very low or undetectable antibody levels measured by other

**Table 1.** False-negative or equivocal anti-RV antibodies in 9 female patients with secondary immune responses to rubella vaccination characterized by high IgG avidity 1–3 months thereafter and negative IgM tests throughout the observation period.

| | Before vaccination | | | After vaccination | | | |
|---|---|---|---|---|---|---|---|
| Patient | Interval (days) | Vi2-G (IU/mL) | RHG (mm) | Interval (days) | Vi2-G (IU/mL) | RHG (mm) | Comment |
| 1 | −6 | 3 | 2 | 15 | 5 | 7 | Pharyngitis |
| | | | | 56 | 219 | 16 | |
| 2 | −42 | 6 | 2 | NA | NA | NA | Pregnancy screening |
| | | | | 48 | 120 | 13 | |
| 3 | −168 | 6 | 7 | 7 | 6 | 8 | Pregnancy screening |
| | | | | 35 | 24 | 11.5 | |
| 4 | −113 | 8 | 7 | 9 | 7 | 3 | Pregnancy screening |
| | | | | 41 | 32 | 10 | |
| 5 | −2 | 9 | 8 | 7 | 13 | 8 | NA |
| | | | | 28 | 93 | 13 | |
| 6 | −7 | 11 | 9 | 4 | 11 | 10 | Pregnancy screening |
| | | | | 36 | 45 | 12 | |
| 7 | −234 | 14 | 8.5 | 6 | 18 | 10.5 | Pregnancy screening |
| | | | | 41 | 26 | 9.5 | |
| 8 | −28 | 14 | 9 | 4 | 12 | 10.5 | Health care worker |
| | | | | 32 | 294 | 15 | |
| 9 | −11 | 15 | 8.5 | 5 | 12 | 9 | 210 IU/mL IgG after vaccination 15 |
| | | | | 30 | 367 | 13 | years ago, pregnancy screening |

NOTE. NA, not available. Vi2-G, VIDAS Rubéole IgG Test version 2; RHG, Hämolyse-Gel-Test für Röteln.

techniques [11] may inhibit the replication of vaccine virus and thereby delay or abort the secondary immune response [21, 22, 46] by preventing the production of sufficient amounts of immunogenic material. The inverse relation between the increase of anti-RV IgG concentrations attained within 3 months after vaccination and prevaccination values of IgG is in agreement with this interpretation.

Some persons fail to respond to repeated RV vaccinations [40], and patients with congenital RV infection may be tolerant to RV epitopes [40, 49–52]. In addition, antibody responses to vaccination in previously immunized subjects tend to be transient (this study and [11, 21, 22]). Therefore, boosting previously immunized persons with low anti-RV antibody concentrations may be ineffective, and additional vaccine doses after childhood vaccination should mainly be targeted at the unimmunized. In contrast, reinfection by wild type RV may overcome the neutralizing capacity of antibodies and induce antibody levels above those attainable by revaccination [28].

Both a modern automated ELISA and a radial hemolysis test for the determination of rubella immunity had excellent specificity in identifying persons who have previously mounted an immune response to RV. Thus, the cutoffs recommended for these tests avoid false-positive results and ensure that vaccination can be targeted at all susceptible persons, especially if the indication to vaccinate is extended into a safety margin of weakly positive results (e.g., up to 25 IU/mL). Lowering the cutoff value would compromise specificity, yet it would improve the predictive value of positive results only slightly, as the majority of false-negative results could not be avoided. After revaccination, rates of adverse reactions (particularly joint-related complaints in females) have been lower than after primary vaccination [53]. We therefore decline to withhold vaccination from potentially susceptible persons and offer boosting to persons with low antibody levels. By the same token, this approach avoids overestimating vaccine efficacy in population studies for the surveillance of mass vaccination programs.

A well-calibrated ELISA for the detection of anti-RV IgG has the potential to be as reliable as established techniques such as radial hemolysis, which clearly separates susceptible from immune subjects, even at a lower cutoff than the one recommended by the manufacturer of RHG. The sequential use of the two tests in case of a negative ELISA could reduce the number of false-negative results and still provide the ease and rapidity of an automated test for most cases. The results obtained with the automated ELISA used in this study are not necessarily transferable to other assays, even if they are based on similar test principles. Some recently developed automated ELISAs for the detection and quantitation of anti-RV IgG may give rise to false-positive results in a large proportion of subjects mounting a primary immune response to vaccination, particularly when using a cutoff value below 15 IU/mL (data not shown). Therefore, for low levels of antibodies, the correct calibration of every assay should be ascertained using serum panels that have been characterized according to biologic criteria instead of relying exclusively on standard serum preparations. In defining the cutoff values, priority should be given to the avoidance of false-positive results.

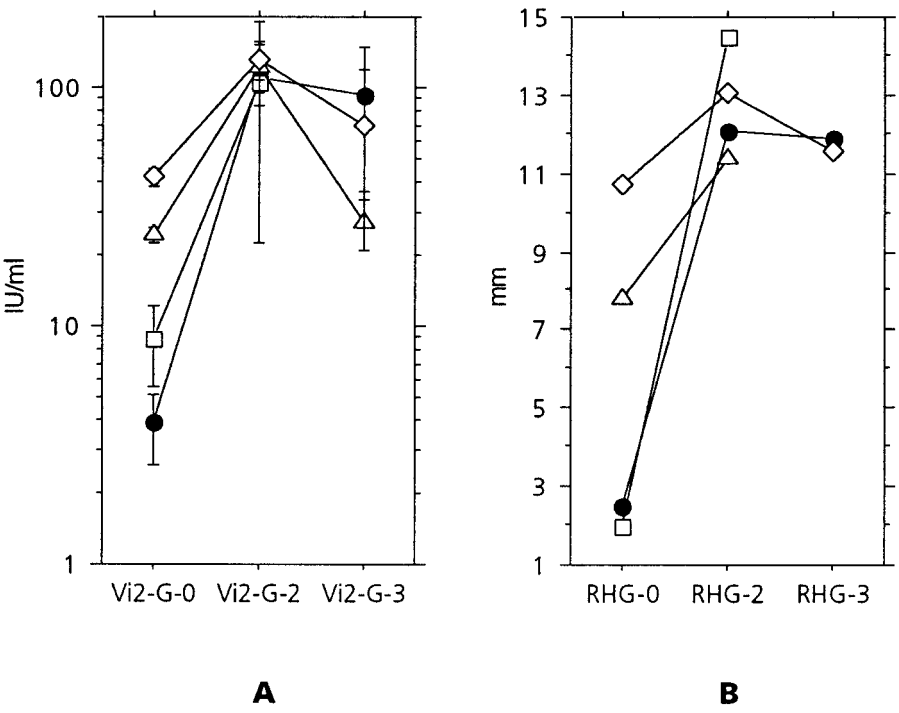


**Figure 3.** **A**, Mean values and 95% confidence intervals of anti-RV IgG concentrations before and 1–3 months and >2 months after rubella vaccination. Results are grouped by primary and secondary immune responses and by prevaccination anti-RV IgG concentrations (Vi2-G-0). ●, primary immune responses ($n$ = 36); □, secondary immune responses, Vi2-G-0 <15 IU/mL ($n$ = 8); △, secondary immune responses, Vi2-G-0 15–29 IU/mL ($n$ = 30); ◇, secondary immune responses, Vi2-G-0 ⩾30 IU/mL ($n$ = 43). **B**, Similar presentation for radial hemolysis (RHG), grouped by primary and secondary immune responses and by prevaccination results (RHG-0). ●, primary immune responses ($n$ = 34); □, secondary immune responses, RHG-0 <7 mm ($n$ = 2); △, secondary immune responses, RHG-0 7 to <9 mm ($n$ = 5); ◇, secondary immune responses, RHG-0 ⩾9 mm ($n$ = 73).

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**References**

1. Centers for Disease Control and Prevention. Rubella prevention: recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR Morb Mortal Wkly Rep **1990**;39(RR-15):1–18.
2. Davis WJ, Larson HE, Simsarian JP, Parkman PD, Meyer HM. A study of rubella immunity and resistance to infection. JAMA **1971**;215:600–8.
3. Orenstein WA, Herrmann KL, Holmgreen P, et al. Prevalence of rubella antibodies in Massachusetts schoolchildren. Am J Epidemiol **1986**;124:290–8.
4. Public Health Laboratory Service. Laboratory diagnosis of rubella. Public Health Lab Microbiol Digest **1988**;5:49–51.
5. Bradstreet CM, Kirkwood B, Pattison JR, Tobin JO'H. The derivation of a minimum immune titre of rubella haemagglutination-inhibition (HI) antibody. A Public Health Laboratory Service collaborative survey. J Hyg Camb **1978**;81:383–8.
6. Chernesky MA, Mahony JB. Rubella virus. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology. 6th ed. Washington, DC: American Society for Microbiology, **1995**:968–73.
7. Kleeman KT, Kiefer DJ, Halbert SP. Rubella antibodies detected by several commercial immunoassays in hemagglutination inhibition-negative sera. J Clin Microbiol **1983**;18:1131–7.
8. Storch GA, Myers N. Latex-agglutination test for rubella antibody: validity of positive results assessed by response to immunization and comparison with other tests. J Infect Dis **1984**;149:459–64.
9. Grangeot-Keros L, Pustowoit B, Hobman T. Evaluation of Cobas core rubella IgG EIA recomb, a new enzyme immunoassay based on recombinant rubella-like particles. J Clin Microbiol **1995**;33:2392–4.
10. Herrmann KL. Available rubella serologic tests. Rev Infect Dis **1985**;7(suppl):S108–12.
11. Serdula MK, Halstead SB, Wiebenga NH, Herrmann KL. Serological response to rubella revaccination. JAMA **1984**;251:1974–7.
12. Mortimer PP, Edwards JMB, Porter AD, Tedder RS, Mace JE, Hutchinson A. Are many women immunized against rubella unnecessarily? J Hyg Cambr **1981**;87:131–8.
13. Skendzel LP, Edson DC. Evaluation of enzyme immunosorbent rubella assays. Arch Pathol Lab Med **1985**;109:391–3.
14. Steece RS, Talley MS, Skeels MR, Lanier GA. Problems in determining immune status in borderline specimens in an enzyme immunoassay for rubella immunoglobulin G antibody. J Clin Microbiol **1984**;19:923–5.
15. Hillary IB, Griffith AH. Persistence of rubella antibodies 15 years after subcutaneous administration of Wistar 27/3 strain live attenuated rubella virus vaccine. Vaccine **1984**;2:274–6.
16. Chu SY, Bernier RH, Stewart JA, et al. Rubella antibody persistence after immunization: sixteen-year follow-up in the Hawaiian Islands. JAMA **1988**;259:3133–6.
17. Rossier E, Phipps PH, Weber JM, Meurman OH. Persistence of humoral and cell-mediated immunity to rubella virus in cloistered nuns and in schoolteachers. J Infect Dis **1981**;144:137–41.
18. O'Shea S, Best JM, Banatvala JE, Marshall WC, Dudgeon JA. Persistence of rubella antibody 8–18 years after vaccination. Br Med J **1984**;288:1043.
19. Robinson RG, Dudenhoeffer FE, Holroyd HJ, Baker LR, Bernstein DI, Cherry JD. Rubella immunity in older children, teenagers, and young adults: a comparison of immunity in those previously immunized with those unimmunized. J Pediatr **1982**;101:188–91.
20. Matter L, Germann D, Bally F, Schopfer K. Age-stratified seroprevalence of measles, mumps and rubella (MMR) virus infections in Switzerland

after the introduction of MMR mass vaccination. Eur J Epidemiol **1997**; 13:61–6.
21. Grangeot-Keros L, Badur S, Pons JC, Duthu S, Pillot J. Use of in vivo challenge to assess rubella immunity determined by haemagglutination inhibition and latex agglutination. Res Virol **1989**;140:437–42.
22. O'Shea S, Best JM, Banatvala JE. Viremia, virus excretion, and antibody responses after challenge in volunteers with low levels of antibody to rubella virus. J Infect Dis **1983**;148:639–47.
23. Preblud SR, Nieburg PI, Brandling-Bennett AD, Hinman AR, Herrmann KL. Rubella vaccination [letter]. Am J Dis Child **1979**;133:1202–3.
24. Böttiger M. Immunity to rubella before and after vaccination against measles, mumps and rubella (MMR) at 12 years of age of the first generation offered MMR vaccination in Sweden at 18 months. Vaccine **1995**;13: 1759–62.
25. Thomas HIJ, Morgan-Capner P. Rubella-specific IgG subclass avidity ELISA and its role in the differentiation between primary rubella and rubella reinfection. Epidemiol Infect **1988**;101:591–8.
26. Rousseau S, Hedman K. Rubella infection and reinfection distinguished by avidity of IgG. Lancet **1988**;1:1108–9.
27. Balfour HH, Amren DP. Rubella vaccination. Am J Dis Child **1979**;133: 1202–3.
28. Horstmann DM, Liebhaber H, Le Bouvier GL, Rosenberg DA, Halstead SB. Rubella: reinfection of vaccinated and naturally immune persons exposed in an epidemic. N Engl J Med **1970**;283:771–8.
29. Balfour HH, Groth KE, Edelman CK. Rubella-viraemia and antibody responses after rubella vaccination and reimmunisation. Lancet **1981**;1: 1078–80.
30. Butler AB, Scott RMN, Schydlower M, Lampe RM, Schwab JA, Muelenaer AA. The immunoglobulin response to reimmunization with rubella vaccine. J Pediatr **1981**;99:531–4.
31. Hedman K, Hietala J, Tilikainen A, et al. Maturation of immunoglobulin G avidity after rubella vaccination studied by an enzyme linked immunosorbent assay (avidity-ELISA) and by hemolysis typing. J Med Virol **1989**;27:293–8.
32. Polanec J, Seppälä I, Rousseau S, Hedman K. Evaluation of protein-denaturing immunoassays for avidity of immunoglobulin G to rubella virus. J Clin Lab Anal **1994**;8:16–21.
33. Stehr-Green PA, Cochi SL, Preblud SR, Orenstein WA. Evidence against increasing rubella seronegativity among adolescent girls [letter]. Am J Public Health **1990**;80:88.
34. Plotkin SA, Farquhar JD, Ogra PL. Immunologic properties of RA 27/3 rubella virus vaccine: a comparison with strains presently licensed in the United States. JAMA **1973**;225:585–90.
35. Brody JA. The infectiousness of rubella and the possibility of reinfection. Am J Public Health **1966**;56:1082–7.
36. Cusi MG, Valensin PE, Cellesi C. Possibility of reinfection after immunisation with RA27/3 live attenuated rubella virus. Arch Virol **1993**;129: 337–40.
37. Best JM, Banatvala JE, Morgan-Capner P, Miller E. Fetal infection after maternal reinfection with rubella: criteria for defining reinfection. Br Med J **1989**;299:773–5.
38. Morgan-Capner P. Does rubella reinfection matter? In: Mortimer PP, ed. Public health virology, 12 reports. London: Public Health Laboratory Service, **1986**:50–62.
39. Schluederberg A, Horstmann DM, Andiman WA, Randolph MF. Neutralizing and hemagglutination-inhibiting antibodies to rubella virus as indicators of protective immunity in vaccinees and naturally immune individuals. J Infect Dis **1978**;138:877–83.
40. Mitchell LA, Zhang T, Ho M, et al. Characterization of rubella virus–specific antibody responses by using a new synthetic peptide–based enzyme-linked immunosorbent assay. J Clin Microbiol **1992**;30: 1841–7.
41. Loutan L, Bovier P, Althaus B, Glück R. Inactivated virosome hepatitis A vaccine. Lancet **1994**;343:322–4.
42. Shouval D, Ashur Y, Adler R, et al. Single and booster dose responses to an inactivated hepatitis A virus. Vaccine **1993**;11:S9–14.
43. Peltola H, Eskola J, Kayhty H, Takala AK, Mäkelä PH. Clinical comparison of the *Haemophilus influenzae* type B polysaccharide–diphtheria toxoid and the oligosaccharide-CRM197 protein vaccines in infancy. Arch Pediatr Adolesc Med **1994**;148:620–5.
44. Forsgren M, Sörén L. Subclinical rubella reinfection in vaccinated women with rubella-specific IgM response during pregnancy and transmission of virus to the fetus. Scand J Infect Dis **1985**;17:337–41.
45. Morgan-Capner P, Hambling MH, Coleman TJ, et al. Detection of rubella-specific IgM in subclinical rubella reinfection in pregnancy. Lancet **1985**;1:244–6.
46. Schiff GM, Young BC, Stefanovic GM, et al. Challenge with rubella virus after loss of detectable vaccine-induced antibody. Rev Infect Dis **1985**; 7(suppl 1):S157–63.
47. Matter L, Hohl P, Abelin Th, Schopfer K. Rötelnepidemiologie in Rekrutenschulen. Schweiz Med Wochenschr **1992**;122:1606–13.
48. Matter L, Bally F, Germann D, Schopfer K. The incidence of rubella virus infections in Switzerland after the introduction of the MMR mass vaccination programme. Eur J Epidemiol **1995**;11:305–10.
49. Cooper LZ, Florman AL, Ziring PR, Krugmann S. Loss of rubella hemagglutination inhibition antibody in congenital rubella—failure of seronegative children with congenital rubella to respond to HPV-77 rubella vaccine. Am J Dis Child **1971**;122:397–403.
50. Katow S, Sugiura A. Antibody response to individual rubella virus proteins in congenital and other rubella virus infections. J Clin Microbiol **1985**; 21:449–51.
51. Mauracher CA, Mitchell LA, Tingle AJ. Selective tolerance to the E1-protein of rubella virus in congenital rubella syndrome. J Immunol **1993**; 151:2041–9.
52. Hardy JB, Sever JL, Gilkeson MR. Declining antibody titers in children with congenital rubella. J Pediatr **1969**;75:213–20.
53. Seager C, Moriarity J, Ngai A, Staehle BO, Nalin DR. Low incidence of adverse experiences after measles or measles-rubella mass revaccination at a college campus. Vaccine **1994**;12:1018–20.