Evaluation of Three Immunoassays Used for Detection of Anti-Rubella Virus Immunoglobulin M Antibodies

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Received 1 April 2005/Returned for modification 4 May 2005/Accepted 13 June 2005

Three automated assays (Abbott AxSYM, Bayer ADVIA Centaur, and bioMerieux VIDAS) used for the detection of rubella virus-specific immunoglobulin M were evaluated. A total of 57 samples from individuals with evidence of infection with rubella virus were used to estimate sensitivity, and 220 samples from blood donors and individuals attending an antenatal clinic who had no evidence of recent infection were used to estimate specificity. Seroconversion panels comprising an additional 31 samples from four individuals were used to determine clinical sensitivity. Samples containing potentially cross-reacting substances were also tested. The sensitivities of the three assays ranged from 84.2 to 96.5%, and the specificities ranged from 96.8 to 99.9%. The Abbott AxSYM assay detected more reactive samples than the other two assays when a panel of 57 positive samples was tested. Bayer ADVIA Centaur detected more reactive samples in the seroconversion panels than the other two assays. All three assays evaluated reported a reactive result in 1 or more of the 48 samples containing potentially cross-reacting analytes. The assays demonstrated comparable performance in testing of a well-characterized panel of samples.

Infections with rubella virus (RV) are usually mild, often presenting with a maculopapular rash of the head and trunk, lymphadenopathy, a fleeting fever, and arthritis (11, 30). Studies have shown that 20 to 50% of infections are subclinical. However, serious sequelae result from maternal infection during the first trimester of pregnancy, with a 90% risk of fetal damage if the infection occurs in the first 2 months of pregnancy and a 50% risk if infection occurs in the third month (7, 11, 15). The teratogenic effects of infection in utero include ocular defects (cataracts, retinopathy, and glaucoma), partial or complete cochlear deafness, and mental retardation associated with microcephaly or encephalitis (7, 15, 30). The effects of congenital rubella syndrome are lifelong. In adulthood, affected individuals have been reported to have increased levels of diabetes, osteoporosis, and thyroid disorders (9).

Typically infection occurs via the respiratory route, with viral replication occurring in the nasopharynx. The incubation period is 2 to 3 weeks, with viremia occurring during the second week. Symptoms are usually apparent at the time of the viremia and for several days after the rash appears. Rubella virus can be isolated from nasopharyngeal samples for as long as 2 weeks after the rash (6, 30). The antibody response also coincides with the viremia (30). RV-specific immunoglobulin M (RV IgM) is detectable after the incubation period, usually at the time of the maculopapular rash. RV IgM usually declines to undetectable levels after approximately 8 weeks. RV IgG levels rise more slowly, reach a peak several weeks after symptoms disappear, and persist for life (30). As the RV IgG response matures, the avidity of the antibody reaction to RV increases (25, 26).

Diagnosis of primary RV infection in adults can be difficult (1, 6). The virus is present in the nasopharynx for a limited period, and symptoms are mild. Infected individuals may present to their clinician too late for viral detection. Detection of RV genomic sequences by nucleic acid tests (NAT) has been described previously (2, 3, 20). NAT are labor intensive and require considerable expertise; therefore, they are not generally available, especially in resource-limited countries where RV infection is most common. Seroconversion or a significant rise in the titer of RV IgG is good evidence of a recent RV infection and may still be detected even if the individual presents after the symptoms have subsided (30). If the RV IgG level is high at presentation and a rise in titer cannot be demonstrated, the presence of RV IgM is usually used to determine acute infection. Therefore, an accurate RV IgM assay is critical to the diagnosis of acute RV infection (23).

RV IgM assays used in diagnostic pathology laboratories are usually in the form of immunoassays, many of which have been evaluated in the past (5, 8, 13, 16, 27, 29). Originally immunoassays were in microtiter plate format using an enzyme-based color detection method, but more recently they have been made available on autoanalyzers that use a range of detection methods including chemiluminescence and immunofluorescence. There are few published evaluations of the performance of these newer assays. In the present study the analytical and clinical sensitivities and specificities of three automated immunoassays used to detect RV IgM were evaluated.

MATERIALS AND METHODS

Samples used in assay evaluation. (i) Sample storage. Serum or plasma samples were collected and stored at −20°C. Samples were stored in this manner for...
as long as 5 years; however, the majority of samples were less than 1 year old. Before testing they were thawed and aliquoted into single-use vials that were refrozen and stored at −20°C until use. The samples underwent no more than three freeze-thaw cycles. Thawed samples were held at 4°C for as long as 3 weeks or until use and then discarded.

(ii) Negative samples. A total of 220 negative samples were tested to determine specificity. Plasma samples were obtained from blood donors (n = 202), and serum samples were obtained from individuals presenting to antenatal clinics (n = 18). The negative status was assigned because the individuals had no clinical history of recent RV infection. Of the 18 antenatal samples, 11 were from prevaccination samples from individuals who had low levels of RV IgG when tested by the bioMerieux Vidas RUB IgG assay (bioMerieux, Marcy-l’Etoile, France). Four were from individuals who had been vaccinated more than 8 weeks prior to sample collection. The other three samples were obtained from individuals who had no detectable RV IgG upon routine testing but were found to be seropositive after the first blood sample was taken.

(iii) Positive samples. A total of 57 positive serum samples were tested to estimate sensitivity. Of these, four were from individuals who had recently been vaccinated. The other 53 samples were obtained from individuals who had been diagnosed clinically as having an acute RV infection; of these, 11 also had a demonstrated RV IgG seroconversion. A positive status was assigned to samples on the basis of a clinical history of recent exposure to RV, signs or symptoms of acute RV infection, and/or evidence of a seroconversion or rising titer of RV IgG.

(iv) Seroconversion panels. Serial samples were taken from individuals with clinical evidence of a recent RV infection. A total of 31 bleeds from four individuals were tested. The National Serology Reference Laboratory, Australia, obtained three of the seroconversion panels. The first sample for each of these panels was obtained at the time when symptoms were reported. Subsequent samples in these panels were obtained over at least a 2-month period. The fourth panel was a commercial seroconversion panel (identification number PTR901; Boston BioMedica Inc., West Bridgewater, MA). The initial samples of this panel were obtained prior to the development of symptoms and prior to detection of an RV IgM response. Additional testing for RV IgM was performed on these samples using a variety of assay systems.

(v) Sera with potential cross-reactivity. A panel of serum samples (n = 48) that contained antibodies or antigens that could potentially cross-react in RV IgM immunoassays was also tested. The majority of samples were obtained from individuals who had clinical or serological evidence of acute infections with Toxoplasma gondii (n = 9), Epstein-Barr virus (n = 7), influenza virus (n = 4), chlamydia (n = 3), cytomegalovirus (n = 3), or other pathogenic microorganisms (n = 6). Another three samples were falsely reactive in an alternate RV IgM immunoassay (Abbott IMx Rubella IgM assay; Abbott Laboratories, North Chicago, Ill.). Samples from individuals with autoimmune disease (n = 8) and samples reactive for hepatitis B surface antigen (n = 5) were also tested. The results of the potentially cross-reacting panel sample were not included in the assessment of the specificity of the assays.

Tests. All samples were tested with three automated immunoassays: the Abbott AxSYM Rubella IgM assay (Abbott Laboratories, Abbott Park, Ill.), the Bayer ADVIA Centaur Rubella M assay (Bayer HealthCare, Tarrytown, NY), and the bioMerieux Vidas RUB IgM assay (bioMerieux, Marcy-l’Etoile, France). These assays are referred to below as AxSYM, ADVIA, and Vidas, respectively. Testing was carried out according to manufacturers’ instructions. All samples were tested in singlicate.

The AxSYM and Vidas instruments have been described elsewhere (16, 22). The AxSYM is a fully automated enzyme immunoassay that uses rubella virus-coated microparticles as the solid phase. Samples are pretreated with rheumatoid factor neutralizing antibodies and incubated with the microparticles. Bound RV IgM is detected with anti-human IgM conjugate and 4-methylumbelliferyl phosphate as a substrate. The resulting fluorescence is read by the AxSYM optical assembly. The Vidas uses a solid-phase receptor coated with rubella virus as the reaction vessel. It is also a fluorescence-based enzyme immunoassay that uses an anti-human IgM conjugate and 4-methylumbelliferyl phosphate as a substrate. The ADVIA has not previously been described fully. Briefly, 20 μl of serum or plasma was prediluted with 335 μl of specimen diluent. A 20-μl aliquot of the diluted sample was incubated for 18 min with 250 μl of a paramagnetic-particle suspension coated with anti-human IgM. The IgM present in the sample was bound to the beads, unbound material was removed by washing, and 150 μl of acidinium ester-labeled RV was added. After a further 18-min incubation, a wash step removed the unbound substrate. Addition of acid and base promoted a chemiluminescent reaction if acidinium ester-labeled RV was bound to the beads. The amount of RV IgM present in the sample was proportional to the light generated by the chemiluminescent reaction.

Each assay system reports the test results as negative, equivocal, or positive. The equivocal ranges for AxSYM, ADVIA, and Vidas were 0.600 to 0.799, 0.80 to 0.99, and 0.80 to 1.20 index units, respectively.

Analyses. All samples were assigned a positive or negative status prior to testing. The analytical sensitivity and specificity of each assay was determined by expressing the results obtained as a ratio of samples with appropriately assigned positive or negative status. Both the sensitivity and specificity were calculated twice, interpreting equivocal results first as negative, then as positive. The seroconversion panel test results were used to determine the clinical sensitivities of the assays. The potentially cross-reacting samples were used to determine each assay’s robustness.

RESULTS

Sensitivity. The sensitivities of the assays ranged from 91.2 to 96.5% when the equivocal results were analyzed as positive samples and from 84.2 to 94.7% when the equivocal results were analyzed as negative samples (Table 1). Of the 57 positive samples, a total of 10 had a negative or equivocal result by one or more assay. Of these 10 samples, AxSYM reported 2 samples as negative and 1 as equivocal, ADVIA reported 3 samples as negative and 2 as equivocal, and Vidas reported 5 as negative and 4 as equivocal. Seven of these 10 samples had a negative or equivocal result by more than one assay. No significant difference in the estimated sensitivity was observed between the three assays.

Specificity. The specificity of each assay was estimated (Table 2). The specificity of the assays ranged from 97.3 to 100% when equivocal results were considered negative and from 96.8 to 99.5% when equivocal results were considered positive. All except one of the positive or equivocal results were obtained from the 202 blood donor samples. The AxSYM and ADVIA both reported a positive result for the same two blood donor samples. All other positive results reported for the 220 negative samples were limited to one assay only. There was no significant difference in the estimated specificity between the three assays.

| Assay     | No. reactive | No. nonreactive | No. equivocal | % Sensitivity (95% confidence interval), with equivocal results considered: |
|-----------|--------------|----------------|---------------|---------------------------------------------------------------------------------|
| AxSYM     | 54           | 2              | 1             | Negative: 94.7 (84.4–98.6), Positive: 96.5 (86.8–99.4)                           |
| ADVIA     | 52           | 3              | 2             | Negative: 91.2 (80.0–96.7), Positive: 94.7 (84.4–98.6)                          |
| Vidas     | 48           | 5              | 4             | Negative: 84.2 (71.6–92.1), Positive: 91.2 (80.0–96.7)                          |
Seroconversion panels. The results of testing of the seroconversion panels are shown in Table 3. Results for three samples from panel 1 and one sample from panel 2 are not shown because they were the same as those for the last sample shown in the respective series. ADVIA reported a reactive result for one or more samples after the AxSYM results were equivocal or nonreactive for all three noncommercial panels and after VIDAS results were equivocal or nonreactive for two of the three noncommercial panels. All assays reported the same samples as positive in the commercial seroconversion panel except for sample 4, which produced a positive result by AxSYM, and sample 9, which was reported as equivocal by VIDAS.

Cross-reactivity. Of the 48 samples with a potential to yield falsely reactive results, AxSYM, ADVIA and VIDAS reported 1, 3, and 3 samples as positive, respectively. Details are provided in Table 4.

**DISCUSSION**

The diagnosis of acute rubella virus infection relies on accurate serology results, because clinical symptoms may not be pathognomonic. Seroconversion to rubella virus-specific IgG is considered evidence of acute infection. Symptoms are often mild or subclinical, so infected individuals often present to the clinician after the IgG levels have risen. Even if a sample is collected prior to a rise in the IgG titer, another is required at least 10 days after the initial sample. Thus, basing diagnosis on a demonstration of seroconversion delays the diagnosis. A positive RV IgM result may also indicate a recent infection, since RV IgM is usually detectable at the onset of symptoms and remains detectable for approximately 8 weeks.

Testing for RV IgM and interpreting results from RV IgM assays can be problematic (1, 12). The sensitivities and specificities of assays differ (4, 8, 13, 16, 27, 29). The assays may fail to detect low levels of circulating antibody. Results may depend on how much time has elapsed between infection and sample collection. RV IgM may be detectable for more than 1 year following infection or vaccination and may occasionally be detected after asymptomatic reinfection (12, 19, 26). IgM antibodies generated in rheumatoid arthritis or polyclonal IgM produced by a nonrecompensated strain of the bacteria can cross-react with the rubella virus.
responses after infection with other agents can cause false-positive results in RV IgM assays due to cross-reactivity (17, 18, 24). It is therefore important that the limitations of the assays are well understood by laboratory personnel in order to avoid the misinterpretation of results.

In developed countries, vaccination programs have reduced the rates of rubella virus infection. Live attenuated rubella virus vaccines were licensed in 1969 (7, 21). Vaccination programs have reduced the notification rates for rubella virus infection in Australia from more than 20 per 100,000 in 1992 to 7.2 per 100,000 in 1997 (23). In the United Kingdom, the number of babies suspected of having congenital rubella virus infection fell from an annual average of about 800 (48 live births and 742 terminations) in 1971 through 1975 to 13 (4 live births and 9 terminations) in 1991 through 1995 (28). Similar success rates have been reported in the United States and Scandinavia (20).

The effectiveness of vaccination programs in developed countries has further complicated the interpretation of RV IgM assay results. Acquired rubella virus infection in these countries is rare and is most often associated with unvaccinated males and immigrants (10, 20, 28). Therefore, a reactive RV IgM assay result is more likely to be a false positive.

The performance of three commercially available automated immunoassays for the detection of RV IgM was assessed. The sensitivities of the assays were estimated using well-characterized clinical samples. The sensitivities and specificities of the assays were comparable by use of these panels. The AxSYM assay reported the lowest false-negative and VIDAS the fewest false-positive results. It is noteworthy that equivocal results were reported for both negative and positive samples. In two of the four seroconversion panels, ADVIA reported a positive result for one or more bleed between AxSYM and VIDAS reported equivocal or negative results. This may indicate that this assay has higher analytical sensitivity.

In other studies, the sensitivities of the assays evaluated ranged from 84.2 to 96.5%. These findings are similar to those reported for evaluations of other RV IgM assays not included in this study (range, 83.8 to 100%) (8, 13, 16). The specificity of AxSYM was reported to be 100% (8) and that of VIDAS 94.6% (16). A recent study (27) used two populations of positive sera: acute-phase samples collected on or prior to 10 days post-rash onset and convalescent-phase sera collected at least 10 days after the onset of the rash. AxSYM had a sensitivity of 57.5% for the acute-phase samples, 98.8% for the convalescent-phase samples, and an overall sensitivity of 78.9%.

Other studies of RV IgM assays not included in this evaluation have reported specificities ranging from 88.7 to 100% (8, 13, 14, 16), compared with a range of 96.8 to 100% in the present study. The specificity of AxSYM was reported as 99.2% (8) and that of VIDAS as 90.1% (16) in previous studies, compared with 97.3% and 100%, respectively, in the present study.

False-positive results caused by cross-reactivity were demonstrated for each of the assays. This phenomenon has been well described and must be considered in interpreting viral IgM serology results (17, 18, 24). In countries with established vaccination programs, the incidence of acute wild-type rubella virus infection is extremely low. Therefore, a positive reaction in an RV IgM assay is more likely to be due to cross-reactivity of a polyclonal IgM response elicited by infections with other infectious agents or an autoimmune antibody (27). Further investigations to confirm that the reactivity is due to acute rubella virus infection are essential. If appropriate samples can be obtained during the viremic stage, detection of RV by NAT would confirm infection. However, a negative NAT would not exclude RV infection. Other investigations may include eliciting a complete clinical and contact history of the patient, RV IgG serology including avidity assays, and testing of paired samples for IgG and IgM responses against other viruses known to cause cross-reactivity, primarily Epstein-Barr virus, cytomegalovirus, and parvovirus B19. However, the absence of reactivity against these viruses does not confirm an acute rubella virus infection.

ACKNOWLEDGMENTS

We thank Susan J. Best for expert advice and editing skills and Mayne Health, Livery Pathology, for contributing many samples to this study.

This study was funded by the Australian Government, Department of Health and Ageing, Diagnostics and Technology Branch, as part of “Quality Assurance Systems for Laboratories: Generalizing Their Use For Improving Pathology Services in Australia.”

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