Time-resolved fluorometric immunoassay for rubella antibody—a useful method for serosurveillance studies

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Received 4 September 2001; accepted 5 November 2001

Abstract
Rubella antibody (IgG) has been measured in females reporting for antenatal screening using single radial haemolysis (SRH) and time-resolved fluorescence immunoassay (TRFIA). We have shown TRFIA to be a simple, specific, highly sensitive, quantitative assay for rubella IgG with a lower limit of detection of 0.2 IU/ml. Out of 506 sera tested by SRH, 18 (3.6%) had low levels of antibody (<15 IU/ml) compared to 83 (16.4%) tested by TRFIA and, of these, 32 (6.3%) had rubella antibody concentrations <10 IU/ml. The lowest level (3.1%) of rubella susceptibility (antibody levels <10 IU/ml) was found in females aged 25–29 and the highest level of susceptibility (23.5%) occurred in females aged 40 years and over. Geometric mean rubella antibody concentrations (IU/ml) were 26.8, 34.4, 34.8, 29.7, 27.5 and 20.0 for age groups <20, 20–24, 25–29, 30–34, 35–39 and ≥40 years, respectively. Our rubella vaccination policies have built up good levels of rubella immunity in women of childbearing age in our locality, and using TRFIA technology we can accurately monitor changes over time. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Time-resolved fluorescence immunoassay; Single radial haemolysis; Rubella antibody

1. Introduction
Sir Norman McAlister Gregg first associated the embryopathic consequences, manifested by cataract formation, resulting from infection of the mother by “German measles” [1]. It is now widely recognised that women who acquire rubella during the first 4 months of pregnancy run a significant risk of giving birth to infants with congenital malformation [2].

In the UK, rubella vaccine was introduced in 1970 for girls’ aged 11–14 years, and for seronegative women of childbearing age in 1974. From 1988, the selective rubella vaccination programme of females was augmented by the introduction of mass childhood immunisation, in the second year of life, with combined measles, mumps and rubella (MMR) vaccine [3]. Currently, a two-dose course of MMR vaccination is recommended; the first dose at 12–15 months and the second at approximately 4 years [4]. Coverage with MMR has ranged between 90 and 92% for children with second birthdays in the years 1991–1992 until 1997–1998 [5]; however, a reduction in coverage to 88% occurred from 1998 as a result of adverse publicity falsely attributing a connection between MMR immunisation and autism [6].

MMR vaccination in the UK has significantly reduced rubella infection in pregnant women [7]. Surveillance of rubella susceptibility in the antenatal population and the determination of age specific antibody prevalence is essential to monitor the success of the rubella immunisation programme [8]. Immunisation uptake can vary between regions and the generation of regional data is important for driving local vaccination initiatives. A pilot scheme to monitor the success of rubella immunisation in the Bristol area has been initiated and our first priority was to develop a quantitative, rubella IgG specific assay to replace the semi-quantitative single radial haemolysis (SRH) assay [9] used in our laboratory for rubella immunity screening. We have previously used time-resolved fluorescence immunoassay (TRFIA) for serosurveillance purposes [10] and this seemed an ideal technology to use for rubella immunity screening. Here we report the results of our evaluation of the TRFIA and our findings from the serosurveillance study.

2. Materials and methods

2.1. Sera tested

A total of 506 sera from females submitted to the Bristol Public Health Laboratory Virology Department for routine rubella immunity screening, during a 2 weeks period, in
January 2001 were used for this study. A total of 364 sera (71.9%) of the sera chosen were from pregnant women.

2.2. Single radial haemolysis (SRH)

The 1-day-old chick red blood cells (Tissue Culture Services, Botolph Claydon, UK) washed twice in dextrose gelatin veronal buffer (DGV) were prepared, using haematocrit, as an 8% suspension. For cells to be used in test plates reconstituted Virion/Serion rubella haemagglutinin antigen (Binding Site, Birmingham, UK) was added and for control plates DGV was added in equal volume, and both sets of cells were left to stand at room temperature for 30 min with gentle shaking. The cell suspensions were then washed twice in DGV and brought to 37°C whereupon they were added in appropriate volumes to agarose, held at 60°C, and after thorough mixing, they were poured into square petri dishes avoiding air bubbles. Three to 10-day-old plates were used for the SRH test and serum was heat treated at 60°C for 20 min to inactivate complement and any heat labile lipids. Sera were added (5 μl), using a micropipette, to holes cut in the agar of both test and control plates together with Virion/Serion rubella 15 IU control serum (Binding Site, UK) and laboratory internal quality control serum. The plates were left overnight at 4°C, warmed for 30 min at 37°C, and then flooded with diluted complement (Don Whitley Scientific Ltd., Shipley, UK). After 3 h incubation at 37°C superfluous complement was aspirated and zone sizes assessed. Any sample with a zone size greater than the 15 IU control was reported as immune and those with zone sizes less than, or equal to, the 15 IU control were deemed to have low levels of antibody and to be in need of vaccination.

2.3. Rubella time-resolved fluorimetric immunoassay (TRFIA)

Rubella antigen for SRH (Binding Site, UK) was reconstituted in 1.0 ml sterile distilled water and diluted 1:25 in sterile carbonate buffer, pH 9.6. DELFIA microtitration plates (Perkin-Elmer Life Sciences, Cambridge, UK) were coated with the antigen (100 μL per well) and left overnight at 4°C. Before use, the plates were washed four times with DELFIA wash buffer (Perkin-Elmer, UK) using a DELFIA plate washer (Perkin-Elmer, UK). Internal quality control and test sera were diluted 1:50 in DELFIA assay buffer (10 + 490 μL). The plates were left incubated, in a humid chamber, for 2 h at 37°C. The plates were then washed four times and europium labelled anti-human IgG conjugate (Perkin-Elmer, UK), diluted 1:500 in assay buffer, added 100 μL per well using a multichannel pipette. After a further incubation of 1 h at 37°C the plates were washed four times and DELFIA enhancement solution (Perkin-Elmer, UK) added at 100 μL per well and after 10 min rotating incubation at room temperature, the plates were read using a Victor multilabel reader (Perkin-Elmer, UK). Multiscal software, Version 2.5 (Wallac Oy, Finland) was used for curve fitting and interpolation of counts.

2.4. Rubalex latex agglutination test

Sera shown to have low rubella antibody levels by SRH and antibody levels less than 10 IU/ml by TRFIA were also tested by Rubalex latex agglutination assay (Orion Diagnostica, Espoo, Finland). The manufacturer’s instructions were followed and the stated cut-off for the test was 10 IU/ml.

3. Results

A simple, yet sensitive and specific, quantitative assay for rubella IgG has been developed. In Fig. 1, the dose response relationship for concentrations of First International Standard anti-rubella serum is shown with counts ranging from 20,000 cpm for a concentration of 0.07 IU/ml to 1,420,000 cpm for a concentration of 10 IU/ml. The analytical limit of detection for plates containing only assay buffer was in the region 500–1000 cpm. Out of 506 sera tested by SRH, 18 (3.6%) had low levels of antibody; however, when the same sera were tested by TRFIA, 83 (16.4%) were found to have rubella antibody concentrations less than 15 IU/ml and 32 (6.3%) had antibody concentrations less than 10 IU/ml. Rubalex latex agglutination assay was used to test the sera containing low levels of antibody. Four of the 18 sera shown by SRH to contain low levels of rubella antibody were Rubalex negative and 5 of the 32 sera shown by TRFIA to have rubella antibody concentrations less than 10 IU/ml were Rubalex negative. The four SRH tested, Rubalex negative, sera had a mean rubella antibody concentration of 0.11 IU/ml by TRFIA. The additional serum detected by TRFIA and shown to be Rubalex negative had a rubella antibody concentration of 7.7 IU/ml yet had been interpreted as immune by SRH on account of it producing a faint zone which cleared after overnight incubation at 4°C. The mean rubella antibody concentration of the 27 sera shown by TRFIA to contain less than 10 IU/ml and which tested Rubalex positive was 6.3 IU/ml. The mean rubella antibody concentration of the 27 sera shown by TRFIA to contain less than 10 IU/ml and which tested Rubalex positive was 6.9 IU/ml.

The distribution of geometric mean rubella antibody concentrations, determined by TRFIA, by age group is shown in Fig. 2. There were 51 women aged less than 20 years, 73 aged 20–24, 128 aged 25–29, 158 aged 30–34, 79 aged 35–39 and 17 aged 40 or above. In Table 1, the percentage of susceptibles versus immunes, by age group, for each of the methods and interpretative criteria used is shown. Using both SRH and TRFIA with a cut-off of 10 IU/ml, 3.9% of sera...
Fig. 1. Standard curve of rubella TRFIA.

Fig. 2. Distribution of rubella antibody by age group in 506 female sera from the Bristol area.

Table 1
Percentage of immune and susceptible sera for each age group as determined by the various methods and interpretation criteria

<table>
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<th>&lt;10 TRFIA</th>
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<td>Susceptible</td>
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<td>Susceptible</td>
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<td>3.9</td>
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<td>1.4</td>
<td>94.5</td>
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</tr>
<tr>
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<td>2.4</td>
<td>96.9</td>
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from teenagers and 23.5% of sera from those aged 40 years and over were susceptible. When a 15 IU/ml cut-off was used for TRFIA, 19.6% of teenagers and 35.3% of sera from those aged 40 and over were susceptible. Finally, the ages (and details) of the five women found to be Rubalex negative were as follows: 18 years (vaccination history unknown, pregnant), 22 years (not pregnant, vaccination history unknown), 24 years (one previous birth, pregnant, vaccination history unknown), 27 years (not vaccinated, pregnant) and 32 years (one previous birth, not vaccinated, pregnant).

4. Discussion

We have shown time-resolved fluorometric immunoassay to be highly sensitive and capable of detecting rubella antibody concentrations as low as 0.2 IU/ml. The application of time-resolved fluorescence technology to rubella antibody detection has previously been investigated by Meurman and co-workers [11] and Shankaran and co-workers [12] although using different assay techniques to the one used here. Meurman and co-workers found their TRFIA assay to have comparable sensitivity and specificity to radioimmunoassay. The enhanced sensitivity and specificity of rubella TRFIA compared to conventional immunoassay is due to the use of lanthanide chelates and time-resolved fluorescence measurement. Lanthanide chelates (e.g. europium) have been optimised to produce large Stokes’ shift (up to 270 nm) which is associated with a long fluorescence decay time (i.e. over 10 ns) and secondly, the long fluorescence decay time (i.e. over 1 μs) enables non-specific fluorescence (typical decay time less than 10 ns) to be excluded from the specific signal which is measured after 10 ns using the time resolution technology.

The enhanced sensitivity of the TRFIA assay is shown by the finding that 3.6% of sera tested by SRH had levels of rubella antibody less than 15 IU/ml compared to 16.4% of sera tested by TRFIA which had antibody concentrations less than 15 IU/ml. Many authorities [13,14] advocate that using 10 IU/ml as a cut-off for rubella susceptibility is more appropriate. When we applied a 10 IU/ml cut-off, 32 (6.3%) of sera tested by TRFIA had levels less than 10 IU/ml and five (0.98%) were Rubalex latex agglutination assay negative. Four of the Rubalex negative sera had rubella antibody concentrations ranging from 1.8 and 2.3 IU/ml and had produced no zone in SRH assay; however, the remaining Rubalex negative serum had been classified as immune by SRH and had a concentration of rubella antibody of 7.7 IU/ml by TRFIA. On further investigation this discrepant serum had produced a “soft” haemolytic zone characteristic of the presence of low avidity antibody [15]. Sera which had been identified as containing levels of rubella antibody less than 15 IU/ml by SRH and less than 10 IU/ml by TRFIA, but were Rubalex positive had a mean rubella antibody concentration of 6.6 IU/ml.

In a recent, major study [16] of rubella antibody levels in western Europe cut-offs of less than 4 IU/ml = low positive/equivocal, and greater than 10 IU/ml = positive (i.e. immune) have been used. For England and Wales, based on the results of this survey, the proportion of females of childbearing age estimated to be seronegative to rubella was 4% for females aged 15–39 years. Applying the same criteria to our results we have found 0.79% of women of childbearing age to be seronegative to rubella. This suggests that the rubella immunisation programme in the Bristol area has been highly successful and this assertion is further justified by our finding that the highest geometric mean rubella antibody level of 34.8 IU/ml was found in the 25–29 years age group. According to our records, women in the 25–29 years age group comprised the highest number of individuals who had received rubella vaccination.

Finally, building up population immunity to rubella is a long-term enterprise and this study has dealt only with women who have been submitted for antenatal screening. Males can act as a reservoir for transmission of rubella [17] and the impact of reduced MMR uptake in children in terms of future seronegativity in women of childbearing age will not be apparent for a good number of years. More important is the potential for increase in child susceptibles, which will prejudice our attempts to eradicate rubella in pregnant women. To monitor the success of our rubella immunisation programme we need to look at a representative selection of our local population and the development of the rubella TRFIA will enable the laboratory component of this task to be achieved. Furthermore, when monitoring changes of immunity over time it is important that a single standardised method is used to avoid variation introduced following changes in methodology.

Acknowledgements

We would like to thank Mrs. Elisabeth A North who was the Medical Laboratory Scientific Officer responsible for conducting the routine SRH and Rubalex latex agglutination assays at the time of the survey.

References


