Evaluation of a commercial rubella IgM assay for use on oral fluid samples for diagnosis and surveillance of congenital rubella syndrome and postnatal rubella


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Abstract

Background: Clinical diagnosis (surveillance) of rubella is unreliable and laboratory confirmation is essential. Detection of virus specific IgM in serum is the most commonly used method. However, the use of serum necessitates the drawing of blood, either through venipuncture or finger/heel prick, which can be difficult in young babies. Oral fluid samples have proved useful as an alternative, less invasive sample for virus specific IgM detection however until recently no commercial rubella IgM tests were available, restricting the usefulness of this approach.

Objectives: To evaluate the performance of the Microimmune Rubella IgM capture EIA using oral fluid samples from outbreaks as well as in cases of suspected congenital rubella syndrome (CRS).

Study design: Paired serum and oral fluids were collected from cases during a rubella outbreak in three provinces in Turkey. Matched serum and oral fluid samples were collected from children with suspected CRS in an active surveillance programme at the Aravind Eye Hospital in South India. Serum samples were collected as part of the measles surveillance programme in Ethiopia.

Results: On serum samples the sensitivity and specificity of the Microimmune Rubella IgM capture EIA compared to Behring Enzygnost rubella IgM test was 96.9% (62/64; 95% CI 94.2–100%) and 100% (53/53; 95% CI 93.2–100%). On oral fluids compared to matched Behring results on serum the sensitivity was 95.5% (42/44; 95% CI 84.5–99.4%). The sensitivity and specificity of Microimmune Rubella IgM capture EIA on oral fluids from suspected CRS cases compared to serum results using Behring Enzygnost IgM assay was 100% (95% CI 84.5–100%) and 100% (95% CI 95.8–100.0%) respectively.

Conclusion: Microimmune Rubella IgM capture EIA has adequate performance for diagnosis and surveillance of rubella in outbreak using either serum or oral fluid specimens.

Keywords: Rubella; Oral fluid; IgM

1. Introduction

Rubella is generally a mild rash fever disease acquired in childhood, but when it occurs in pregnant women during the first trimester of pregnancy it frequently leads to infection of the foetus and a high risk of...
congenital abnormalities—congenital rubella syndrome (CRS; McAlister Gregg, 1941; Miller et al., 1982).

An effective live attenuated vaccine for rubella has been available for more than 30 years and has been used effectively to prevent CRS in large populations either as part of universal vaccination programmes or targeted at susceptible women (Banatvala and Brown, 2004). However, a large burden of CRS remains in both developing and developed countries and the World Health Organisation recommends inclusion of rubella vaccine into immunization programmes.

The clinical diagnosis of rubella may not be possible in all cases, since the clinical signs are transient and can be confused with measles (Banatvala and Brown, 2004) and as a consequence laboratory confirmation of infection is essential to provide accurate surveillance data to support the diagnosis.

The detection of rubella and specific IgM antibodies in serum samples from CRS cases and following acute postnatal infection is the common method of laboratory diagnosis and a range of commercial tests are available. In recent years oral fluid (OF) has been used as an alternative sample to search for detecting antibodies. In the UK an in-house IgM test has been used to confirm rubella infection using OF samples for several years (Perry et al., 1993; Ramsay et al., 1998). OF is particularly suitable for diagnosis of rubella in childhood. Specific studies have shown that rubella is detectable in OF from CRS cases (Eckstein et al., 1996). The lack of a commercial assay that performs accurately on OF samples has limited wider use of this approach.

This report describes an evaluation of the performance of a commercial IgM assay for detection of rubella specific IgM in serum and OF samples from CRS and postnatal rubella cases.

2. Materials and methods

2.1. Rubella antibody detection

Microimmune Rubella IgM capture EIA (Microimmune Limited, UK), Enzygnost anti-rubella virus IgM, anti-rubella IgG and IgG avidity (Dade Behring, Marburg, Germany), were performed and results interpreted according to manufacturer’s instructions.

The performance of the Microimmune Rubella IgM capture EIA (MI) was evaluated on the following panels of sera.

A: 73 sera collected as part of an active measles surveillance programme during 2003 and 2004 in various districts throughout Ethiopia and previously tested by a Behring Enzygnost indirect rubella IgM EIA (75% of the sera were collected within ten days of rash onset); B: 20 sera received for routine measles surveillance in the Virus Reference Department, Health Protection Agency, Colindale, UK and tested positive for measles IgM by Behring EIA; C: 18 sera positive for rheumatoid factor by a latex agglutination test (RapiTex RF, Dade Behring) and D: 18 sera that formed part of the Accupanel (Quest Biomedical, UK) and consisted of five specimens each for measles, varicella zoster, rubella and three for mumps. Only three sera in the panel were virus specific IgM positive and at least one was RF positive.

The performance of Microimmune rubella IgM EIA on OF specimens was evaluated using 55 paired serum/blood spot and OFs samples collected from four regions in Turkey between May and June 2003 during a rubella outbreak and were mostly taken between 10 and 14 days post onset of rash. Matched serum or blood-spot specimens from these subjects were tested in Behring rubella IgM.

One hundred eleven matched serum and OF samples were available from suspected CRS cases. These were collected during an active population based study at the Aravind Eye Hospital, Madurai, South India. Cases were classified using the WHO recommended case definition for CRS (WHO, 1999) and laboratory confirmed based on the detection of serum rubella IgM. The serum samples were evaluated by Behring, Human, Denka and Radim EIAs for rubella IgM and by Behring anti-rubella IgG and OF samples by Microimmune rubella IgM.

3. Results

3.1. Evaluation of Microimmune IgM capture EIA on serum samples

Results for the evaluation of MI rubella IgM capture EIA on serum samples are shown in Tables 1 and 2.

Of the two MI negative and Behring IgM EIA positive subjects, one was a 5-year-old boy and the sample was taken four days after onset of symptoms. The IgG in the serum...
from this subject was of high avidity (69%) suggesting that the case was unlikely to be an acute rubella infection. IgG was not detected in the second subject, an 8-year-old female, and this may be a false negative in the MI test.

Of the nine subjects giving equivocal results in the Dade Behring assay, seven were negative in the MI test. For four of these subjects IgG avidity results were available and all four had high IgG avidity (>72%). Two further samples were from infants under seven months of age. Two subjects giving an equivocal result in the Dade Behring assay and positive result in the MI test were from 3-year-old and 7-year-old males and sera were collected 8 and 1 day post onset of symptoms. IgG was not detected in serum from the seven year old and IgG avidity was not determined in the serum from the 3-year-old.

Overall, the sensitivity of the MI rubella IgM capture EIA on serum samples compared to the Dade Behring assay was 96.9% (95% CI 94.2–100%). The positive predictive value for the MI test was 100% (95% CI 94.2–100.0%).

Excluding the two RF sera that gave equivocal results (Table 2) in the MI, the specificity of the test was 100% (53/53; 95% CI 93.2–100.0%).

3.2. Evaluation of MI rubella IgM capture EIA on OFs

Serum or blood spots taken from outbreaks in four provinces in Turkey were tested by Behring rubella IgM test and the matched OFs from these subjects tested by MI rubella IgM capture EIA. Concordant results were obtained on 92.7% (51/55) samples tested for rubella IgM in OF compared to serum/blood spot results (Table 3). Clinical details were insufficient to interpret the discordant result in this cohort. The sensitivity of the MI test compared to the matched serum results using Behring test was 95.5% (42/44; 95% CI 84.5–99.4%).

3.3. Evaluation of MI test on suspected congenital rubella samples

One hundred eleven matched serum-OF pairs were tested by MI rubella IgM capture EIA (Table 4). The serum samples from this set were previously tested by Behring, Denka, Human and Radim and at least three tests gave concordant results. There was agreement for 107/111 (96.4%) of serum specimens using the MI rubella IgM EIA compared to consensus serum results obtained with the other tests. Of the 111 subjects tested, 22 were rubella IgM positive in the serum test and matched OFs from these subjects were all positive in the MI rubella IgM test. Of the 89 rubella IgM and IgG sero-negative subjects, 4 matched OF samples gave repeatable equivocal results. If the four equivocal results are treated as negative (as per manufacturer’s instructions), the sensitivity and specificity of the MI OF test compared to serum IgM results was 100% (95% CI 84.5–100%) and 100% (95% CI 95.8–100.0%), respectively.

4. Discussion

Detection of rubella IgM is the key diagnostic test underpinning rubella surveillance programmes. Two recent evaluations of commercial rubella IgM assays based on testing serum samples reported the range of sensitivities and specificities of between 74.1–76.8% and 93.9–96.1% (for the best four of seven assays; Tipples et al., 2004) and 84.2–96.5% and 96.8–99.9% (for three automated assays Dimech et al., 2005) respectively. Sensitivity was strongly influenced by timing of collection. The evaluation by Tipples et al., included serum specimens from diseases with rash-like illness similar in presentation to rubella, whereas the evaluation by Dimech et al. was an evaluation on known rubella positive serum specimens, seroconversion panels and blood donors. The MI assay was evaluated on sera predominantly from acute rubella cases, 75% of which were collected within 10 days of rash onset, measles cases and on sera known to be rheumatoid factor positive. The sensitivity and specificity of the MI test compared to the Behring test was high (96 and 100%, respectively).

The MI rubella IgM test also gave concordant results with three other commercial tests (Behring, Radim and Denka Seiken) on serum samples collected from infants with suspected CRS.

Sensitive tests for rubella IgM based on OF testing have until recently been restricted to laboratories that could carry out radioimmunoassay. These tests have been described to perform accurately in both postnatally acquired rubella (Perry et al., 1993) and CRS (Eckstein et al., 1996). In this study we have shown good performance of the MI rubella IgM test in outbreak situations in Turkey where serum or blood spots and OFs were available, in Ethiopia where serum was collected as part of a measles surveillance programme and in South India where serum and OF specimens were collected during an active population based survey of congenital rubella. The
availability of a commercial ELISA suitable for use on OFs opens up the prospect of a wider role for OF testing in rubella and measles surveillance programmes worldwide.

The sensitivity of the MI test on OF compared to results obtained on matched serum from the same subjects, in both postnatally acquired rubella and in suspected CRS cases, was high. There was complete concordance of results between the MI OF test result compared to results obtained with Behring, Radim and Denka Seiken tests on matched serum in the suspected CRS cases. Samples were not available during the first week of illness but these promising results indicate the need for more comprehensive evaluations of timing of specimens in a wider range of clinical settings.

The use of OF specimens for rubella IgM testing, particularly in mild rash-fever disease in childhood, leads to more complete specimen collection (Nokes et al., 1998). The use of OF for surveillance of measles, mumps and rubella in the UK over a ten year period has demonstrated greater compliance from subjects when OF is used compared to blood. Thus, provided tests are sensitive and specific, OF sample is preferable to blood for carrying out sero-surveillance. In addition rubella virus can be detected in OFs from clinical cases (Jin et al., 2002). Although we have demonstrated that OF can be used to confirm CRS cases, for rubella infection with long-term clinical implications, such as infection in pregnancy or congenital rubella cases, confirmatory testing based on serum samples is recommended to ensure appropriate clinical guidance.

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References