Rubella (*Rubella virus*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *rubella virus*.

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1 PHLN Summary laboratory definition

1.1 Condition:

Rubella (Postnatal)

1.1.1 Definitive Criteria

- Isolation of *rubella virus* from clinical material;
- Detection of *rubella virus* RNA in clinical material; and
- Rubella-specific IgG seroconversion or four fold or greater rise

1.1.2 Suggestive Criteria

- Detection of rubella-specific IgM antibody

1.1.3 Guide for Use

- Detection of rubella-specific IgM antibody is suggestive of recent infection providing there has been a compatible clinical illness and no recent vaccination for rubella. Serological confirmation by rubella-specific IgG or detection of virus (nucleic acid testing or culture) is preferred.

1.2 Condition:

Rubella (Congenital)

1.2.1 Definitive Criteria

- Isolation of *rubella virus* from clinical material;
- Detection of *rubella virus* RNA in clinical material;

- Detection of rubella-specific IgM in infant serum

1.2.2 Suggestive Criteria

- High / rising rubella-specific IgG level in first year of life

2 Introduction

Rubella virus is a positive sense enveloped RNA virus that is the only member of the *Rubivirus* genus of the *Togaviridae* family.

Rubella was distinguished from other rash illness only in the late 19th century and the virus was isolated in 1962. It infects only humans and is transmitted by the respiratory droplet route; congenitally infected infants may shed the virus for months (in respiratory secretions and urine) and can also infect carers.

Many cases of postnatal infection are thought to be subclinical. Clinical illness follows a 12-23 day incubation period and feature acute onset and a generalised maculopapular rash, fever and lymphadenopathy (especially of postauricular and suboccipital nodes). Arthritis and arthralgia are seen especially in adult women.

Complications are not common but include arthritis, haemorrhage (1 in 3000 cases) and encephalitis (1 in 5000).

Infections in pregnant women can lead to fetal death, prematurity and congenital defects including cardiac defects, microcephaly and deafness. Infection after the 20th week of gestation is not likely to have fetal effects.

Re-infections with rubella can occur despite natural and vaccine-acquired immunity.

Rubella is difficult to diagnose clinically. Infections that could cause similar pictures include *human herpes virus 6, enteroviruses, human parvovirus B19*, mild measles, infectious mononucleosis and toxoplasmosis.[1, 2]

A rubella vaccine has been used in Australia since 1970. The majority (>97%) of women of child-bearing age would be expected to be rubella immune, however given the structure of past immunisation programs, men in the same age range have higher levels of non-immunity (~10%[3] Migrant women may have lower immunisation rates.[4]

Between 2004 and 2008, 193 cases of rubella were notified in Australia with 4 congenital infection cases.[5] However, recent reported congenital infections may represent imported cases and Australia may be close to the elimination of locally acquired rubella.[6]

Given the often non-specific clinical picture of rubella, laboratory diagnosis should be attempted in suspected cases (testing for some of the differential diagnoses as mentioned above eg *measles virus and parvovirus B19* should also be considered).

The potentially severe consequences of congenital rubella emphasise the need for continued high vaccine coverage and also for sensitive and specific diagnostic capabilities. However, in a low prevalence setting such as Australia, serological testing is likely to have low predictive value. Attempts to confirm the diagnosis by viral culture or nucleic acid testing (NAT) should be attempted.

3 Tests

3.1 Clinical specimens

3.1.1 Culture or Nucleic acid tests (NAT):

- Nasopharyngeal aspirate / swab
- Throat swab
- Urine
- Oral fluid (saliva)
- Amniotic fluid
- Blood (EDTA – minimum 0.5ml)
- Plasma (from EDTA blood - for PCR – minimum 0.5ml)

Swabs should be cotton, rayon or dacron-tipped, plastic-coated or aluminium shafted swabs. They should be placed into viral transport media and transported at 4°C or frozen at -70°C. Other samples should also be transported at 4° C or frozen at –70°C.

3.1.2 Serology

- Serum (preferred - minimum 0.5ml)
- Plasma (if specimen validated for test used - minimum 0.5ml)

Oral fluid / saliva and dried blood spots reported but not generally used in Australia for routine testing

3.1.3 Postnatal infection:

Culturable virus may be present in respiratory secretions in 90% on day of rash and in 50% 4 days after rash onset.

Rubella-specific IgM antibodies are present at the onset of the rash in ~50% and in over 90% >5 days after rash onset. Rubella-specific IgG response may be seen in most from 7-10 days after rash onset.[1]

Test Strategy:

- Rubella-specific IgM and IgM (acute and convalescent sera)
- Culture / NAT - appropriate specimens (collected as soon as possible after rash onset) if feasible.
- Pregnant women – in addition - NAT on an appropriately timed collection of amniotic fluid can be considered in cases of suspected / confirmed rubella.

3.1.4 Congenital infection:

Culturable virus is present (respiratory secretions, urine) in almost all at / soon after birth with 50% detection at 3 months of age. Rubella-specific IgM is present in most at / soon after birth (50% detectable at 6 months of age)[1].

Test Strategy:

- Rubella-specific IgM in first 6 months (and up to 12 months) of life.
- Culture or NAT of respiratory secretions and urine in first 12 months of life.
- Testing of rubella specific IgG levels to determine high / rising levels in the first year of life if required.

3.2 Isolation of *Rubella Virus*

3.2.1 Suitable specimens

See 3.1

Cell Culture Techniques
Vero cells are used in viral culture. After 1 week of culture, an aliquot of the medium in the initial culture should be inoculated into a fresh cell line. Two passages are required to deem a culture negative.
As there is no typical cytopathic effect in first passage culture, viral detection must be performed by monoclonal antibody detection of viral proteins (eg by IFA) or by NAT using RT-PCR for viral RNA. The WHO has published viral culture protocols using Vero/SLAM cells.7

3.2.2 Test sensitivity

Variable – variables include time in illness of specimen collection, type of specimen, specimen handling etc. A report on rubella testing using throat swabs collected mostly within 2 days of rash onset from apparent (postnatal) clinical cases gave a culture sensitivity of 77%.8

3.2.3 Test specificity

Should approach 100% (confirm by IFA or PCR).

3.2.4 Predictive values and relevant populations

Should approach 100%.

Positive results would be expected to be highly predictive of current or recent rubella infection (including subclinical infections), or congenital infection in tested individuals.

Rubella virus may be shed following vaccination (detectable by viral cultures of throat swabs for greater than 28 days)[9] and in recently immunised individuals positive results may represent shedding of vaccine virus rather than acquired infection.

3.2.5 Suitable test acceptance criteria

IFA
Staining by peroxidase/immunofluoresence within cells inoculated with positive control material, absence of staining in the negative control. Staining should be read independently by two laboratory staff.

Nucleic Acid Testing
Suitable results of negative and positive controls.

3.2.6 Suitable internal controls

Cell cultures maintained at the same time with and without inoculation with control *rubella virus*.

3.2.7 Suitable original test validation criteria

Auditors should have available evidence of:

- records of inocula;
- records of time specimen stored in the laboratory before inoculation;
- evidence of regular mycoplasma testing of cell lines;
- evidence of regular contamination testing of cell lines; and
- positive and negative control data from each run.

3.2.8 Test reporting

Result report may include method used and comment on significance of result. Advice about further testing or sample collection testing may be included.

3.2.9 Suitable external Quality Assurance Program(s) (QAP)

Participation in a program such as that conducted by RCPA Quality Assurance Programs Pty Ltd is essential.

3.2.10 Special considerations

Any positive cell culture should be passaged to generate a stock of the viral isolate. If indicated, *rubella virus* isolates or RNA should be referred to a reference laboratory** for nucleic acid sequencing for genotyping to assist epidemiological investigation of outbreaks.

**[Rubella genotyping appears not to be available in Australia – query specify an overseas reference laboratory]

3.3 Nucleic Acid Testing (NAT) of *Rubella Virus* RNA

3.3.1 Suitable and unsuitable specimens

See 3.1

3.3.2 Test details

Primers targeting the rubella E1 and NS1 genes have been reported.[10], [11] Gel-based or real time formats have been used. Amplicon specificity should be determined by probe hybridisation (eg by gel staining or probes in real time formats) or sequencing.

3.3.3 Test sensitivity

Not conclusively determined – expected to be greater than viral culture. A sensitivity of 91% reported by RT-PCR followed by Southern hybridisation on early throat swab specimens from clinical cases[8].

A study12 has reported the sensitivity of PCR (nested RT-PCR format) on amniotic fluid in determining congenital infection in pregnant women with primary rubella infection as between 83-95% (fetal blood rubella-specific IgM as comparator). Testing 6 weeks after maternal infection (seroconversion) and at

least at 22 weeks gestation was recommended – lower sensitivity seen if tested earlier; 95% if tested by these criteria.

3.3.4 Test specificity

Should approach 100%. Appropriate work flow practices and use of controls should reduce risk of and allow for detection of PCR contamination. Primers should not detect human nucleic acid sequences.[11]

3.3.5 Predictive values and relevant populations

With measures to reduce false positive results in place (see 2.3.4), positive NAT results would be expected to be highly predictive of current or recent rubella infection (including subclinical infections), or congenital infection in tested individuals.

Rubella virus may be shed following vaccination (detectable by viral cultures of throat swabs for greater than 28 days)[9] and in recently immunised individuals positive PCR results may represent shedding of vaccine virus rather than acquired infection.

3.3.6 Suitable test acceptance criteria:

- Absence of detectable contamination in the PCR.
- Successful detection of positive control material.
- Absence of inhibition in the clinical material.
- Confirmation of the identity of the PCR product by probe hybridisation or sequencing.

3.3.7 Suitable internal controls

Positive and negative virus control material should be included in the RNA extraction stage and all subsequent amplification steps. Adequate negative controls should be included to exclude PCR contamination. Appropriate controls to detect PCR inhibition should also be included.

3.3.8 Suitable original test validation criteria

Auditors should have available evidence of:

- records of inocula; and
- records of time specimen stored in the laboratory before inoculation.

3.3.9 Test reporting

NAT results are often reported as being ‘DETECTED’ or ‘NOT DETECTED’.

Appropriate comments may include an interpretation of result and comments regarding expected sensitivity and specificity of results.

3.3.10 Suitable external Quality Assurance Program(s) (QAP)

Participation in a program such as that conducted by RCPA Quality Assurance Programs Pty Ltd is essential.

3.4 Serological Diagnosis of *Rubella Virus* Infection

Serological diagnosis requires the identification of an IgM antibody specific for rubella or demonstration of a rising (at least four-fold) titre of rubella-specific IgG antibodies.

Testing for these antibodies is readily available using commercial kit enzyme immunoassays. IgM capture or indirect formats are used in rubella-specific IgM detection

Previously used / specialised testing, such as haemagglutination inhibition (HAI), sucrose density fractionation for IgM and IgG avidity, is not widely available.
Rubella-specific IgM antibodies are present at the onset of the rash in ~50% and in over 90% >5 days after rash onset. Rubella-specific IgG response may be seen in most from 7-10 days after rash onset.

In congenital infection, rubella-specific IgM is present in most at / soon after birth with subsequent fall-off in detection (50% detectable at 6 months of age). Rubella-specific IgG may be high or continue to rise in the second 6 months of life.[1]

3.4.1 Suitable and unsuitable specimens

Serum or plasma (minimum 0.5ml volume) collected at the time of presentation with rash followed by a second specimen during convalescence at least 10-14 days later. Salivary antibody testing for either rubella-specific IgG or IgM is possible but is not readily available within Australia.

3.4.2 Test details

Approved commercial assays (including manual and automated methods) for rubella antibody testing should be performed according to manufacturers' instructions.

3.4.3 Test sensitivity

In a comparison of 7 EIAs, sensitivity of IgM testing on convalescent sera (>10 days post rash) was between 94-98%.[13]

3.4.4 Test specificity

In the above comparison sensitivity was between 85-97%. Specificity of >99% has been reported.[14] Rubella-specific IgM may be detectable for at least 3 months following infection.[15]

3.4.5 Predictive values and relevant populations

Likely to be low in sporadic cases. In a highly immunised country such as Australia, a positive rubella-specific IgM antibody results has a low positive predictive value but may be confirmed by a rising rubella-specific IgG titre. Confirmation by viral isolation or detection of antigen or nucleic acid is preferred.

3.4.6 Suitable test acceptance criteria

Negative and positive controls within range.

3.4.7 Suitable internal controls

Positive and negative serum controls should be included in all runs. Consideration should be given to the inclusion of a low positive control in each run.

3.4.8 Suitable original test validation criteria

Auditors should have available evidence of:

- records of serum arrival and storage conditions;
- records of test kit storage conditions; and
- records of QC monitoring of test kit performance.

3.4.9 Test reporting

Rubella-specific IgM results can be reported as 'Positive', Negative' or 'Equivocal' as consistent with manufacturer's recommendation. Rubella-specific IgG is reported in IU/ml.

Interpretative comments should be provided with advice to test convalescent sera if indicated.

3.4.10 Suitable external Quality Assurance Program(s) (QAP)

Participation in a program such as that conducted by RCPA Quality Assurance Programs Pty Ltd is essential.

3.5 Serological Determination of Rubella Immunity

3.5.1 Utility

Measurement of rubella-specific IgG titre is widely used to establish immune status. It is used in antenatal serology screening and also in testing other high risk populations. It allows for determination of those likely to be at risk of infection and affords the opportunity of subsequent immunisation.

3.5.2 Test methods

Historically, measurement was by hemagglutination inhibition and a titre of 8 was considered protective based on *rubella virus* exposure studies.[16]

Testing is now generally by commercial immunoassay (with the intention to correlate with the above HAI titre). ELISA calibration is against a WHO international standard with results reported in IU/ml. 10IU/ml has been a commonly used cut-off for immunity.[15]

A comparison of 8 commercial rubella-specific IgG immunoassays with HAI as standard[17] found their sensitivities between 96% and 99.6% and specificities 85.4%-95.8% (equivocal results classed as negative). However the actual result in IU/ml varied with method used – some assays gave comparable results but others did not. Hence the IU/ml results by different methods should not be assumed to be comparable.

Proprietary assays should be conducted and reported in accordance with the manufacturers protocols. Any variation from the manufacturers protocols should be validated according to National Pathology

Accreditation Advisory Council requirements (see Requirements for the Development and Use of In-House In Vitro Diagnostic Devices (IVDS) (2007 Edition)).

3.5.3 Suitable test acceptance criteria

Negative and positive controls within range.

3.5.4 Suitable internal controls

Positive and negative serum controls should be included in all runs. Consideration should be given to the inclusion of a low positive control in each run.

3.5.5 Suitable original test validation criteria

Auditors should have available evidence of:

- records of serum arrival and storage conditions;
- records of test kit storage conditions; and
- records of QC monitoring of test kit performance.

3.5.6 Test reporting

Rubella-specific IgG is reported in IU/ml. Interpretative comment regarding immune status for results should be included using IU/ml cut-offs appropriate for the test used.

3.5.7 Suitable external Quality Assurance Program(s) (QAP)

Participation in a program such as that conducted by RCPA Quality Assurance Programs Pty Ltd is essential.

4 Typing and subtyping methods

4.1 Typing (Subtyping) Method

4.1.1 Utility

Determining *rubella virus* type has the potential to contribute to control and elimination of rubella transmission. It can allow for tracking transmission and determining changes in virus(es) present and interruption of virus transmission in a region. In the situation where elimination of local transmission of rubella is a goal the ability to genotype any incident cases may be important.[6]

4.1.2 Method

The WHO has published methods for the typing and nomenclature of *rubella viruses*.[18], [19] Sequencing of a 739 nucleotide portion of the E1 gene (nts 8731 to 9469) is recommended for phylogenetic analysis. Positive clinical specimens and viral isolates should be referred for genotyping where possible. (*Not avail in Aust*)

4.2 SNOMED CT concepts

- Rubella (Clinical finding) SNOMED CT ID 36653000
- Rubella virus (Organism) SNOMED CT ID 5210005
- Rubella IgG antibody (Procedure) SNOMED CT 313670007
- Rubella IgM antibody (Procedure) SNOMED CT 313479002

5 References

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