AMPATHCHAT no. 86

RUBELLA LABORATORY DIAGNOSIS

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KEY MESSAGES

- Testing for protective immune response by means of rubella IgG is advisable prior to a planned pregnancy.
- Rubella IgM testing during pregnancy should ideally be limited to cases where a clinical suspicion of rubella is present, or where a possible exposure occurred, owing to a low positive predictive value in asymptomatic patients, particularly in low prevalence settings.
- Rubella IgM remains the first-line test to diagnose clinically apparent rubella infection. Confirmation can be done by means of rubella PCR on a throat swab or urine, owing to the short viraemic period of the virus.

INTRODUCTION

Rubella virus causes the classic childhood exanthem sometimes referred to as German measles. Although this vaccine-preventable illness generally causes mild symptoms, particularly in children, primary rubella during pregnancy may have devastating consequences.

Transmission is via large particle aerosol inhalation. Infected individuals are usually contagious from seven days prior to the onset of symptoms, to seven days after. The average incubation period is between 14 and 18 days following exposure.

Clinical illness presents with fever, a fine maculopapular rash, usually starting on the face and spreading to the trunk, as well as postauricular and posterior cervical lymphadenopathy. Approximately 70% of adolescents or adults with primary rubella infection will also experience polyarthralgia or arthritis, while between 25 and 50% may be entirely asymptomatic.

CONGENITAL INFECTION

Congenital infection may occur when rubella is transmitted from an infected mother to the foetus via the placenta. The risk of transmission to the foetus varies depending on the stage of the pregnancy, and whether the infection is a primary infection versus a reinfection.

Primary rubella infection during the first trimester carries a risk to the foetus of 80% to 90%, with a particularly high risk of congenital defects owing to disruption of organogenesis. The risk of foetal transmission decreases to around 25% late in the second trimester, with the risk increasing thereafter to 35% at weeks 27 to 30, up to 100% after week 36 of pregnancy. It is important to note that while the risk of foetal transmission is high towards the end of the third trimester, the risk of notable congenital defects falls mostly in maternal infections prior to week 17 of gestation. Primary rubella infection after week 20 of pregnancy carries almost no risk of congenital rubella syndrome (CRS), but intrauterine growth retardation may occur as a result of infection in the third trimester. The risk of transmission to the foetus is significantly lower following a maternal reinfection, and is estimated to be approximately 5% to 8% if the maternal reinfection occurs in the first 16 weeks of pregnancy. No cases of congenital rubella syndrome have been reported in reinfections after 12 weeks of gestation.

The classic triad of clinical features of CRS includes cataracts, sensorineural deafness and congenital heart defects (typically peripheral pulmonary artery stenosis, patent ductus arteriosus, or ventricular septal defects), but may also present with additional clinical features such as congenital glaucoma, purpura, splenomegaly, microcephaly and developmental delays.

RUBELLA VACCINATION AND CHANGING EPIDEMIOLOGY

Since the introduction of rubella vaccines in 1969, the incidence of rubella has decreased in countries with good vaccine coverage, and has led to the successful elimination of rubella in some countries. Rubella vaccines are live attenuated vaccines with a high effectiveness of 99.3% for the RA27/3 strain, which is the most common strain.

It is contraindicated to administer rubella vaccination in pregnancy, and women should be advised to avoid pregnancy in the 28 days following the vaccination. Although these precautions are advocated, congenital rubella syndrome has never been documented following inadvertent rubella vaccination in a pregnant woman.

At present, rubella vaccines are only available in South Africa as a combined formulation with measles and mumps vaccines (or MMR), or alternatively the MMRV vaccination, which includes varicella zoster virus vaccine. Although rubella vaccines are not currently administered as public sector childhood vaccinations according to the South African Expanded Programme on Immunisation (SA-EPI), rubella vaccination is available in the private health sector in South Africa, administered according to the measles vaccination schedule (usually at six and 12 months of age). The possible introduction of rubella vaccination in the South African public sector is still under consideration, particularly in light of a goal set by the World Health Organization (WHO) to target rubella elimination. A paradoxical increased risk of CRS may occur if the coverage of rubella immunisation among infants and children is insufficient, yet enough to reduce likelihood of exposure to wild type virus during childhood, leading to an increase in susceptibility to primary rubella infection among women of childbearing age. For this reason, the introduction of routine rubella vaccination should only be considered in settings where sufficient coverage of at least 80% is achieved for routine measles vaccination, as per WHO recommendations, to ensure sufficient interruption of rubella transmission in order to remove the risk of rubella exposure for pregnant women.

LABORATORY DIAGNOSIS

Laboratory methods for the diagnosis of rubella include both antibody and molecular test methods.

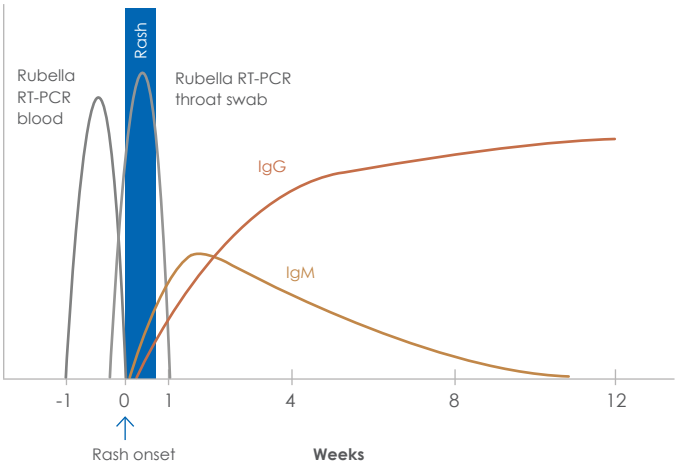

FIGURE 1: RUBELLA VIRUS TEST WINDOW OF DETECTION

Antibody tests are considered the first-line diagnostic and screening assay for the diagnosis of suspected rubella virus infection. Rubella IgM antibodies are mostly detectable by the fourth day following the onset of rash, and usually disappear after three months, whereas IgG antibodies remain positive lifelong. Antibody tests can detect both naturally acquired and vaccine-derived antibody rubella IgM and IgG responses, but are unable to distinguish between these.

Detectable rubella vaccine-derived immune responses may differ from those following natural infection, possibly due to antigenic differences. Post-vaccination rubella IgM response may persist for longer than three months, and may even remain detectable for years following vaccination. In addition to this, maturation of the post-vaccination IgG response may differ, potentially leading to IgG avidity remaining at lower levels than what is found following rubella infection. This may complicate the interpretation of rubella serology results during pregnancy, particularly if no baseline rubella testing was performed prior to pregnancy in patients who received rubella vaccination. Owing to the complexity of result interpretation during pregnancy, it is advisable for women of childbearing age to have a rubella IgG test done to determine their immune status prior to falling pregnant (in the case of post-vaccination testing, preferably six to eight weeks following rubella vaccination).

Furthermore, it should be kept in mind that the positive predictive value of rubella IgM will be low if the prevalence of rubella infection is low. In settings where rubella has been eliminated, positive rubella IgM results are all likely to be false positive, unless exposure to a rubella case had occurred. Cross reactive antibody responses may cause false positive IgM results, such as rheumatoid factor, parvovirus IgM or EBV IgM antibodies, and heterophile antibodies, as well as alloantibodies during pregnancy. For these reasons, positive rubella IgM antibody results must always be regarded with caution in scenarios where there is no clear clinical disease. These pitfalls are frequently encountered in the use of rubella IgM testing in routine screening in asymptomatic pregnant women.

Molecular testing in the form of a rubella virus PCR is available to confirm rubella virus infection in certain settings. The viraemic period is short, and usually becomes undetectable by the time of rash onset, as such rubella PCR on blood samples has limited utility. Throat swabs are usually the recommended specimen type to confirm a current rubella virus infection by means of rubella PCR, as rubella RNA is usually detectable from two days prior to the onset of rash to four days after.

Table 1 outlines the rubella assays that are available at Ampath Laboratories and the clinical utility thereof.

TABLE 1: DIAGNOSTIC TESTS FOR RUBELLA VIRUS AT AMPATH LABORATORIES

Test name	Specimen type	Clinical indications
Antibody tests		
Rubella IgM Mnemonic: RUBM* TAT: 24–48 hours ***Also available in combination with rubella IgG: Mnemonic: RUB***	Serum (SST tube)	Rubella suspected clinically, or to aid in the diagnosis of CRS. **NOTE:** IgM results may still be negative on samples taken less than five days after the onset of the rash, repeat testing should be considered.
Rubella IgG Mnemonic: RUBG** TAT: 24–48 hours ***This test is also included in the antenatal profiles: Mnemonics: ANT – excluding HIV Or ANTHIV – including HIV***	Serum (SST tube)	To determine immune status (especially before or during pregnancy) following either natural infection or vaccination. An IgG level of >10 IU/ml indicates immunity to rubella. May also assist in the diagnosis of suspected rubella infection, either when tested in combination with IgM, or in serial testing to demonstrate seroconversion or a fourfold rise in IgG titer between the acute and convalescent phase samples after 14 days.
Rubella IgG avidity Mnemonic: RUBAV TAT: Batched twice weekly	Serum (SST tube)	Indicated to determine whether primary rubella infection occurred recently or in the more distant past (at least 10 weeks prior). Typically indicated in scenarios where maternal rubella IgM and IgG are both positive, to establish the likelihood of a recent primary infection versus reinfection. Avidity refers to the strength of binding between the antibody and the antigen, which increases with maturation of the immune response. Low avidity IgG (low binding strength) is usually associated with recent primary infections, while high avidity antibodies indicate that the antibodies have matured and are associated with a primary infection in the distant past.
Rubella IgG Western Blot (line immunoassay) Mnemonic: RUBGWB TAT: Performed as needed on weekdays only	Serum (SST tube)	Can be applied to assist in narrowing down the time of infection, in addition to the rubella IgG avidity assay. IgG antibodies against rubella virus envelope protein E1 develop four to six days following the onset of infection, whereas IgG antibodies against E2 can only be detected at least three months after the onset of primary infection. The presence of IgG antibodies against E2 indicates a high IgG avidity and therefore a primary rubella infection is unlikely to have occurred in the three months prior to sample collection. The absence of IgG antibodies against E2 may indicate a recent rubella infection or vaccination, but a late infection stage cannot be ruled out as the formation of antibodies against E2 may sometimes be delayed, reduced or absent.
Molecular tests		
Rubella virus PCR Mnemonic: RUBPCR	Plasma, urine, CSF, throat swab, amniotic fluid, tissue	Recommended sample type for suspected current rubella virus infection is a throat swab (alternatively urine). **NOTE:** Owing to the limited window of detection, a negative PCR does not exclude a recent infection in the absence of a specific history of exposure, or appearance of a rash.
NOTE: Rubella IgM will no longer be included routinely in the antenatal screen profile from 1 October 2023. The rubella IgG will remain part of the antenatal screen. Rubella IgM can be ordered separately if clinically indicated.		

DIAGNOSTIC APPROACH TO CONGENITAL RUBELLA INFECTION (CRS)

The diagnosis of congenital rubella infection may either be established by means of prenatal or postnatal testing.

Prenatal testing for CRS can be considered where a maternal rubella infection has been confirmed, as outlined in Figure 2. As previously discussed, the risk of transmission to the foetus will depend on whether the maternal infection is a primary infection or reinfection, as well as the gestational age, therefore maternal infection does not necessarily imply foetal infection. It should also be kept in mind that foetal ultrasound may not detect congenital rubella syndrome, owing to the nature of malformations.

While there is no defined gold standard method to establish a prenatal diagnosis of foetal rubella infection, rubella PCR testing on amniotic fluid is the most common method used. The optimal timing of amniocentesis is at 20 weeks of gestation (if more than six weeks after maternal infection with rubella). Recent studies have also suggested that PCR testing of chorionic villus samples collected as early as 10 to 12 weeks of gestational age may allow for earlier detection of foetal infection, however, it should be noted that maternal tissue may contaminate the sample, which may possibly lead to false positive results. Alternatively, rubella IgM may be considered on foetal blood, although these results may be false negative until later in the course of the pregnancy. It is recommended that patients be counselled extensively about the limitations of prenatal testing, as well as the risks involved in the required procedures in order to obtain relevant specimens. Consultation with a foetal-maternal specialist is strongly recommended. It is also important to allow at least a 6-week time frame between the maternal infection and the collection of specimens, to ensure optimal sensitivity to detect foetal infection.

A postnatal diagnosis of CRS can be established by means of the detection of rubella IgM in an infant below three months of age. It is recommended that further confirmatory testing be performed in infants with positive rubella IgM results, by means of a rubella PCR on either a nasopharyngeal swab or a urine sample. Children with CRS may shed rubella virus in their saliva and urine for months or even years. IgG antibodies that remain stable or increase in the first seven to 11 months of life (before rubella vaccination is given) may further aid in the diagnosis. Laboratory confirmation should be sought in infants born with anomalies that may be in keeping with CRS, regardless of a history of maternal rubella infection or a known exposure during the course of the pregnancy. It is also important to perform postnatal testing in any infants with an antenatal exposure history even in the absence of any clinical signs of CRS, to ensure that confirmed cases can be monitored for possible neurological or hearing impairments.

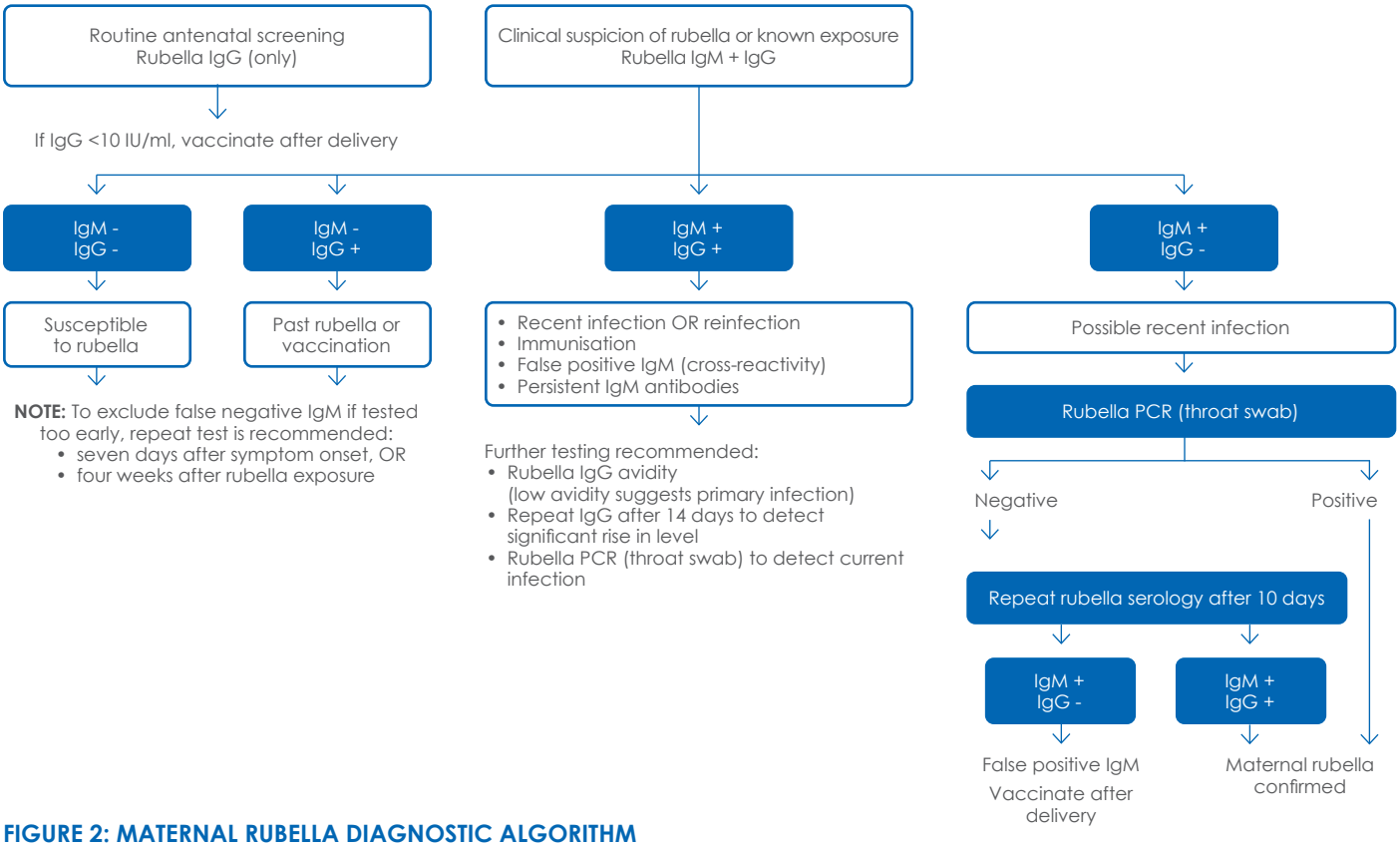

FIGURE 2: MATERNAL RUBELLA DIAGNOSTIC ALGORITHM

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REFERENCES AVAILABLE ON REQUEST