

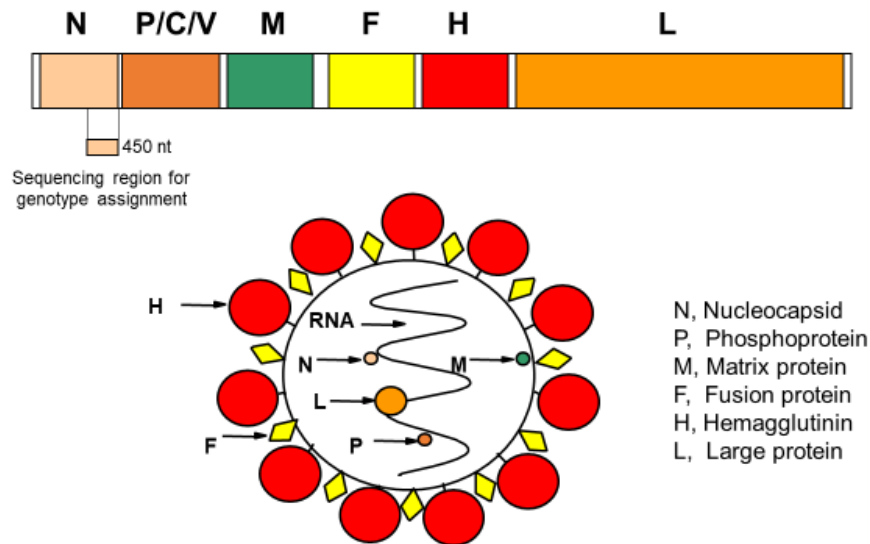
Manual for the Laboratory-based Surveillance of Measles, Rubella, and Congenital Rubella Syndrome

Chapter 1

1.1 The structure and biology of measles virus

Measles virus is a paramyxovirus belonging to the genus *Morbillivirus*. It is a pleomorphic virus ranging in diameter from 100 to 300 nm. Within the morbilliviruses, measles is most closely related to the rinderpest virus group, and more distantly related to the canine distemper virus group [1]. Two envelope glycoproteins are important in pathogenesis. These are the F (fusion) protein, which is responsible for fusion of virus and host cell membranes, viral penetration, and haemolysis, and the H (haemagglutinin) protein, which is responsible for binding of virus to cells. The measles genome consists of six genes, each encoding a single structural protein. One of these genes, the phosphoprotein (P) gene, also encodes two non-structural proteins (V and C). Refer to **Figure 1.1, Structure of measles virus**.

Figure 1.1 Structure of measles virus



The measles genome consists of 15,894 nucleotides although some variation in genome length has been described [2]. Measles virus has a single, non-segmented, negative-sense RNA genome with a

linear arrangement of genes that are separated by an intergenic trinucleotide, GAA. Each gene contains a single open reading frame (except P), transcriptional start and stop signals, and a polyadenylation signal. The encapsidated genomic RNA is termed the nucleocapsid (NC) and serves as a template for transcription and replication. The nucleoprotein (N) mRNA is transcribed first and N is the most abundant of the viral proteins.

Measles virus, as an RNA virus, has the potential for generation of variants during replication due to the lack of proof-reading capacity of RNA-dependent polymerases. This has implications for the molecular epidemiological interpretation of nucleotide differences that may be observed from viruses collected during outbreaks. Although there is only one serotype of measles, there are distinct genetic lineages of wild-type measles viruses based on the nucleotide sequence of the 450 nucleotides that code for the carboxyl-terminal region of the nucleoprotein.

As of 2015, there are 24 genotypes of measles virus with reference strains recognized by WHO. Only six of the genotypes have been detected since 2011 [3]. Most importantly, the genetic variation does not appear to be biologically significant, as all genotypes are neutralized by measles vaccine-induced antibodies [4]. More information on the molecular epidemiology, genetic characteristics of measles virus and measles genotypes is provided in chapter 7.

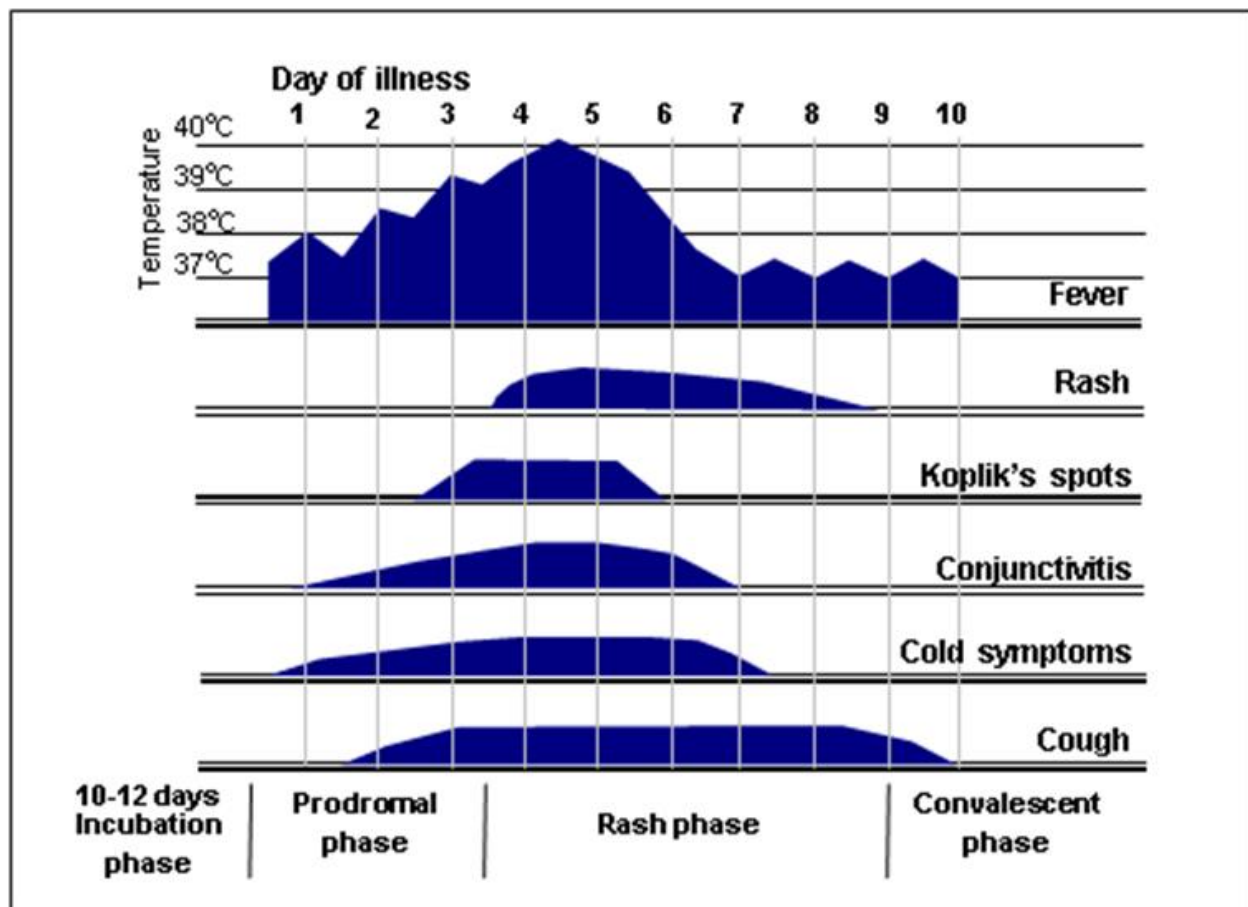
Measles virus is viable for less than 2 hours at ambient temperatures on surfaces and objects, while the aerosolized virus remains infectious for 30 minutes or more. It is heat-labile and is inactivated after 30 minutes at 56°C. However, the virus appears to survive freeze-drying relatively well and, when freeze-dried with a protein stabilizer, can survive storage for decades at -70°C. The virus is inactivated by solvents, such as ether and chloroform, by acids (pH less than 5), alkalis (pH greater than 10), and by UV and visible light. It is also susceptible to many disinfectants, including 1% sodium hypochlorite, 70% alcohol and formalin.

1.2 The clinical description and complications of measles

Measles is an illness characterized by generalized maculopapular rash lasting 3 or more days with a temperature of 38.3°C or higher, and typical symptoms include one or more of the “3 c’s”: cough, coryza, or conjunctivitis. Clinically, the diagnosis of measles is supported by the appearance of

irregular red lesions with bluish white centres in the buccal mucosa (Koplik spots) that appear 1-2 days before the onset of rash [5]. Otitis media is a common respiratory complication. The rash, which appears about 14 days after exposure, progresses from the head to the trunk and out to the extremities. Refer to **Figure 1.2, Clinical features of primary measles infection – time course from onset of illness.**

Figure 1.2. Clinical features of primary measles infection – time course from onset of illness



The non-specific nature of the prodromal signs and the existence of mild cases, however, makes clinical signs and symptoms unreliable as the sole diagnostic criteria of measles disease. Other viral illnesses that may produce a rash suggestive of a measles infection include rubella, dengue, enteroviruses, coxsackie virus, parvovirus B19, zika, and human herpesvirus 6 (HHV6). A rash

resembling that of measles may also accompany bacterial infections such as toxic shock syndrome, certain medical conditions such as Kawasaki's disease, or as a consequence of allergic reactions to treatment with antibiotics. Despite the existence of alternative etiologies for rash and fever, it is critical that measles infection is considered in the differential diagnosis. As the incidence of measles declines, medical practitioners may overlook the possibility of measles, particularly when such cases occur outside the context of an outbreak.

Many factors can contribute to the severity of measles in developing countries including poor nutrition, exposure to high doses of virus in crowded conditions, and an early age at which infants are exposed to the community at large. Death resulting from measles infection is usually attributed to the immunosuppression associated with measles infection that can lead to secondary bacterial and viral infections resulting in life-threatening pneumonia [6]. Diarrhoea is a complication of measles that can be particularly severe among malnourished young children in developing countries. Measles-specific immune suppression begins with the onset of clinical disease before the rash appears, and continues for several weeks after apparent recovery. Patients with defects in cell-mediated immunity often suffer severe progressive measles infections and have a significantly increased risk of death. Immunocompromised individuals may develop giant cell pneumonia as well as measles inclusion body encephalitis.

Measles can have serious complications such as deafness and blindness among immune-competent individuals. In addition, severe neurological sequelae can result from acute encephalomyelitis, which occurs in about 1 in 1,000 cases. Symptoms appear within 1-2 weeks after rash onset and can result in death (5-30%) or permanent impairments among about 30% of the survivors [1].

Measles infection is the cause of subacute sclerosing panencephalitis (SSPE), a progressive neurodegenerative disease that manifests 4-10 years after acute infection with wild-type measles virus and is invariably fatal. Although SSPE is considered a rare complication of measles, revised estimates of the rate of SSPE have been reported using denominators derived from defined outbreaks or populations. These studies have calculated rates of SSPE that are much higher than the earlier estimates, approximately 10 cases of SSPE per 100,000 cases of measles compared to 10 per million cases. The risk of SSPE is higher among children who contracted measles at <5 years of age and the

likelihood of developing SSPE may be highest when measles infection occurs at less than 1 year of age [7,8,9].

1.3 Infection, immune response and laboratory diagnosis of measles

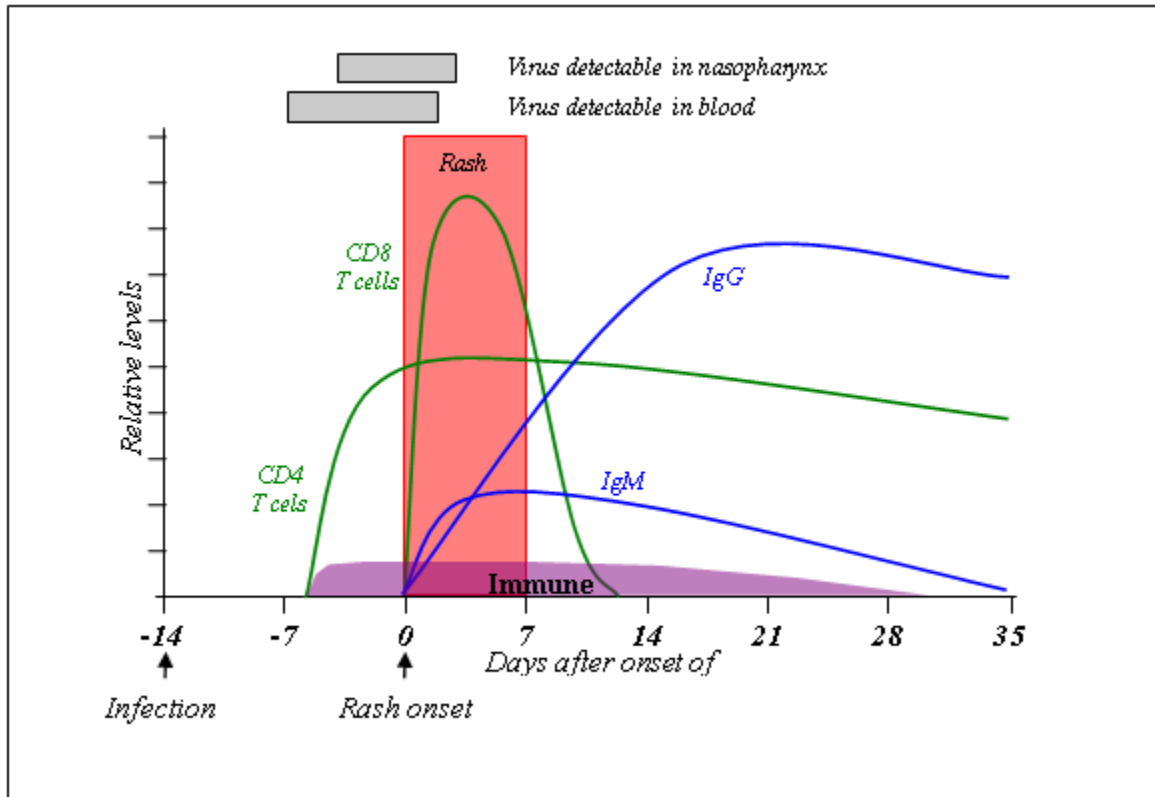
Measles virus is highly infectious to humans, causing a self-limiting febrile illness characterized by a maculopapular rash. The H protein of wild-type measles virus binds to the cellular receptor, the signalling lymphocyte activation molecule (SLAM, also known as CD150). Studies in animal models indicate that the initial target cells are alveolar macrophages and dendritic cells which are infected via this receptor [4]. The virus is transported to draining lymphoid tissues, seeding a systemic infection with preferential tropism to B and T-lymphocytes. The incubation period is estimated to last 10 to 14 days, and is associated with leukopenia.

Viral shedding begins in the prodromal phase prior to rash onset. Following viremia mediated by infected lymphocytes, the respiratory epithelium is infected basolaterally via a second receptor, nectin 4, producing a large amount of progeny viruses in the respiratory tract [4]. Transmission of virus occurs through respiratory secretions. Rash is generally observed from 3 to 5 days following onset of fever. Individuals are considered to be infectious from approximately four days before rash to four days after rash onset. Total uncomplicated disease course is 17-21 days from first sign of fever.

Infection of the respiratory tract can give rise to croup, bronchiolitis and pneumonia. Generalised damage to the respiratory tract causes the loss of cilia and predisposes to secondary bacterial infections, such as pneumonia and otitis media. Immune reactions to the virus in the endothelial cells of dermal capillaries cause the measles rash and the measles enanthem (Koplik spots), while interaction between virus-infected cells and local cellular immune factors is thought to be involved in measles encephalitis.

Both IgM and IgG antibodies are produced during the primary immune response and measles-specific IgM can be detected in the serum as early as the first day of rash onset, while IgG is usually detectable a few days after the IgM appears. Refer to **Figure 1.3, Immune response in acute measles infection**.

Figure 1.3. Immune response in acute measles infection



Using sensitive EIAs to detect IgM, 90% of measles cases can be expected to have positive results for measles-specific IgM at ≥ 3 days post rash onset [10]. The IgM antibody levels peak about 7–10 days after the onset of rash and then decline rapidly. IgM may be undetectable after 6–8 weeks. Production of IgG antibody gradually increases, with levels peaking within about 4 weeks following rash onset and persisting long after infection. Serum IgA and secretory IgA antibodies are also produced.

When immune individuals are re-exposed to measles virus, circulating measles-specific antibodies generally provide protection from clinical disease and a rise in neutralizing antibody concentration may be measured if pre-exposure blood is available. It is sometimes possible to detect virus-specific IgM in post-exposure serum specimens among individuals that remain asymptomatic [11,12]. Cellular immunity, consisting of cytotoxic T-cells and possibly natural killer cells, plays a prominent role in immunity and recovery from acute infection. However, a fully symptomatic reinfection with measles has been demonstrated by the presence of high avidity measles IgG antibody among laboratory-

confirmed measles cases [13,14]. The serologic confirmation of measles reinfections can be challenging. Symptoms may be mild, and/or the clinical progression may differ from that seen among primary cases of measles. Health-care workers may be particularly at risk of reinfection, due to a high force of infection when measles cases present in a hospital or clinical setting [14,15]. A more detailed discussion of measles reinfection cases and the approaches for laboratory confirmation for suspected reinfection cases are provided in chapter 8.

Detection of measles-specific IgM antibody from serum or oral fluid specimens containing gingival crevicular fluid, (chapter 4) and the detection of measles RNA by RT-PCR (chapter 6) are standard methods that are used among laboratories in the Global Measles and Rubella Laboratory Network (GMRLN) to confirm suspected acute cases of measles [16]. However, as measles vaccine also elicits IgM production, recent vaccine history must be considered when evaluating suspected cases by these standard methods for case confirmation. In addition, low prevalence of measles in a population greatly reduces the positive predictive value of IgM detected by EIA [17].

1.4 Epidemiologic features of measles

Despite a dramatic decrease in measles incidence since 2000, epidemics occur in areas with low vaccination coverage or pockets of susceptible groups. Measles is highly contagious and is transmitted from person to person by infectious aerosols, large respiratory droplets or direct contact with nasal or throat secretions from an infected person. The basic reproduction number, R_0 , is the average number of secondary cases that are generated from a primary case in a population that is completely susceptible. Measles has an R_0 of 12-18, the highest of any infectious communicable disease [18]. In the absence of measles vaccination, in a population with homogeneous contact patterns, nearly everyone will become infected.

The current measles vaccine, a live-attenuated strain of measles, is safe and highly effective at providing protection against measles. Measles vaccine is often administered as measles-rubella (MR) or measles-mumps-rubella (MMR) vaccine. Measles continues to disproportionally affect children in developing countries, where it is one of the leading causes of under-five mortality. Currently the WHO recommends administration of the first dose of measles-containing vaccine (MCV) at nine

months of age where there is a high risk of mortality among infants due to ongoing transmission and at 12 months of age in areas with low rates of measles transmission [19].

After a single dose of MCV at 8-9 months of age, 89.6% of children develop protective immunity to measles. However, since maternal antibody can interfere with the response to vaccination, a higher rate of seroconversion is achieved if vaccination can be delayed until most maternal antibody has waned. Following administration of MCV at 11-12 months of age, 99% of children seroconvert [19]. To achieve the 95% coverage required to interrupt transmission of measles virus, a two-dose vaccination strategy is recommended. The second dose of MCV may be delivered as part of routine immunization services or as school entry requirements. In many countries with gaps in their routine immunisation programme, supplemental immunization activities (SIAs) are important to achieve high vaccination coverage.

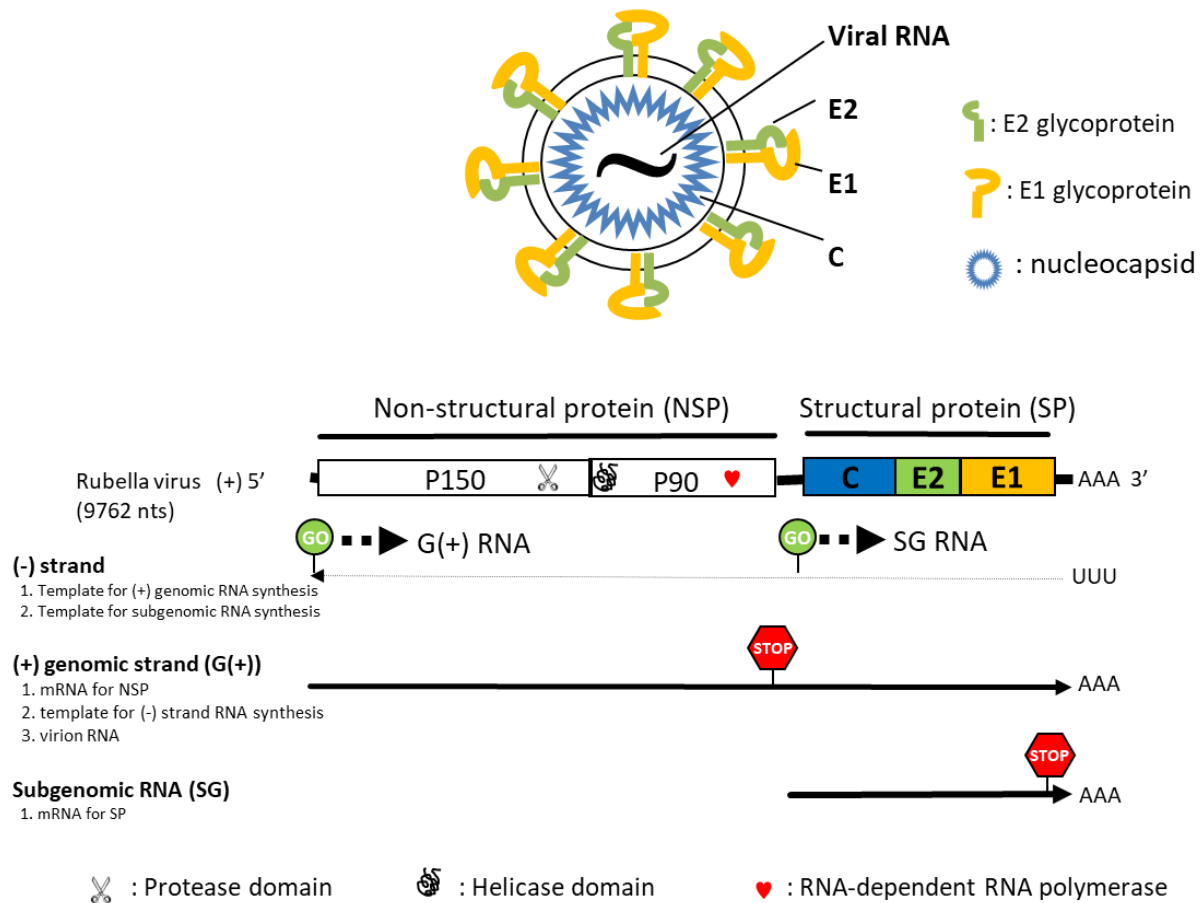
Vaccination programmes have greatly reduced the worldwide incidence of measles, including the successful elimination of endemic measles in the region of the Americas. However, measles outbreaks can still occur in countries with high vaccination coverage. Prolonged outbreaks may indicate that an immunity gap exists in the population involved. After elimination has been achieved, continued introduction of measles will inevitably lead to small outbreaks. Most cases occur among individuals who were never vaccinated, those who failed to seroconvert following vaccination, or those persons who had a suboptimal response and lack full protection. Because pockets of susceptibility may exist in some communities, the maintenance of adequate herd immunity to contain outbreaks is critical to sustain elimination [17].

Part B. Rubella

1.5 The structure and biology of rubella virus

Rubella virus is an RNA virus, the only member of the *Rubivirus* genus within the family *Togaviridae*. The rubella virus is roughly spherical with a diameter of 60–70 nm. It is composed of a pleomorphic nucleocapsid containing a single-stranded, positive-sense RNA genome with 9,762 nucleotides. The virus contains three structural proteins, two in the envelope (E1 and E2) and one in the core (capsid or C protein) surrounding the RNA. Refer to **Figure 1.4, Structure of rubella virus**.

Figure 1.4 Structure of rubella virus



The cellular receptor for rubella has not yet been identified. The envelope proteins, E1 and E2, are glycoproteins that exist as heterodimers that project from the virus to form 6 to 8 nm surface spikes [20]. E1 appears to be the dominant surface molecule and is associated with neutralizing and antigenic epitopes. There is only one serotype of the virus. As is true for the measles virus, the host range of rubella virus is limited to humans.

Genetic characterisation of rubella viruses has identified two distinct genetic groups (clades) of rubella viruses. The two clades, clade 1 and clade 2, differ by 8-10% at the nucleotide level. As of

2015, there are 10 genotypes (including 1 provisional genotype) described for clade 1 and 3 genotypes are recognized in clade 2 based on phylogenetic analysis of 739 nucleotides within the E1 coding region [21]. The data available from the genetic analysis of circulating rubella viruses are limited due to collection of fewer specimens for molecular characterisation compared to measles. In addition, rubella sequences from many regions of the world are either underrepresented in the sequence database for rubella or remain unavailable. It is possible that more genotypes may be identified as surveillance improves. More information on the molecular epidemiology, genetic characteristics of rubella virus and rubella genotypes is provided in chapter 7.

The rubella virus is somewhat more stable than measles virus at ambient temperatures. The virus is heat-labile and can be inactivated after 30 minutes at 56°C. When stabilised with protein it can be repeatedly frozen (at -60°C or below) and thawed without loss of titre. Infectivity is rapidly lost at -20°C. Lipid solvents, weak acids and alkalis, and UV light inactivate the rubella virus. It is also susceptible to a wide range of disinfectants and is inactivated by 1% sodium hypochlorite, 70% ethanol and formaldehyde.

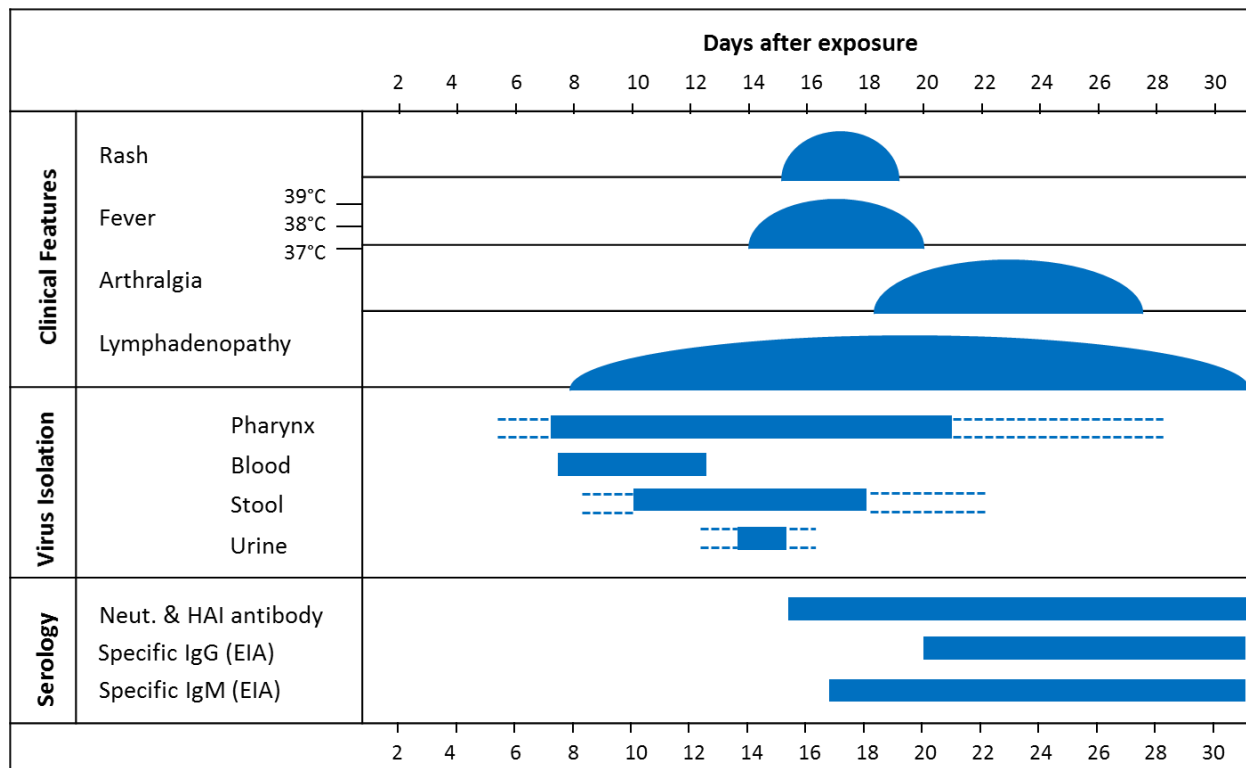
1.6 The clinical description of rubella and congenital rubella syndrome

The clinical diagnosis of rubella (postnatal rubella, German measles) is unreliable because there are many other causes of rash that mimic rubella infection. In addition, many cases go unrecognized or index cases may be missed since up to 50% of rubella infections are subclinical. While rubella virus causes a mild febrile rash illness in children, maternal infection with rubella can have serious consequences for the developing foetus. During the first 11 weeks of gestation, there is a very high risk (90%) that the child will be born with congenital rubella syndrome (CRS) [22].

The average incubation period for rubella is 14 days but can range from 12–23 days. In adolescents and adults, a short prodromal phase (1–5 days) occurs before the rash appears in. In children, prodromal symptoms are rare, and a rash is usually the first manifestation. The prodrome consists of a low-grade fever, malaise and mild conjunctivitis. Other symptoms may include headache, anorexia, coryza, sore throat, and cough. Enlargement of the lymph nodes (lymphadenopathy) occurs from 5–10 days before the onset of the rash. At approximately 14–17 days after infection, a maculopapular rash

(a pink skin rash of discrete spots) develops. The rash starts on the face and neck, progresses down the trunk to the extremities and lasts about 3 days. The rash is much fainter than that seen in measles and is occasionally pruritic. Refer to **Figure 1.5, The relationship between clinical signs, virus isolation and serological markers of postnatal rubella infection.**

Figure 1.5 The relationship between clinical signs, virus isolation, and serological markers of postnatal rubella infection



Although these symptoms are not specific to rubella, lymphadenopathy may be more pronounced and last longer (several weeks) with rubella than with other exanthematic diseases, such as measles. Joint pain and temporary arthritis, which are uncommon in children, occurs in approximately 70% of adults. Other complications of rubella are thrombocytopenia (1 in 3,000 cases) and post infectious encephalitis, which occurs in about 1 in 6,000 cases of rubella [23,24].

A rubella infection during the first trimester of pregnancy can cause miscarriage, stillbirth, or the birth of a child with CRS. Common manifestations of CRS are ocular defects including cataracts,

deafness, congenital heart disease, and developmental delay. The list of possible defects is extensive since the rubella virus can affect any of the organs of the developing foetus. The severity and nature of these defects depend on the gestational age of the foetus at the time of infection. Infants with CRS usually present with more than one sign or symptom consistent with congenital rubella infection. However, infants may present with a single manifestation of CRS, deafness or hearing impairment being the most common defect [23,24].

When maternal infection occurs after 18 weeks of gestation, the risk of CRS is much lower. However, the newborn may still have a congenital rubella infection (CRI). A congenital rubella infection (CRI) applies to all infants confirmed with rubella infection, with or without CRS [20]. If manifestations of CRS are not present, the infant is diagnosed as having CRI only. However, some effects of foetal rubella infection may not manifest for several years.

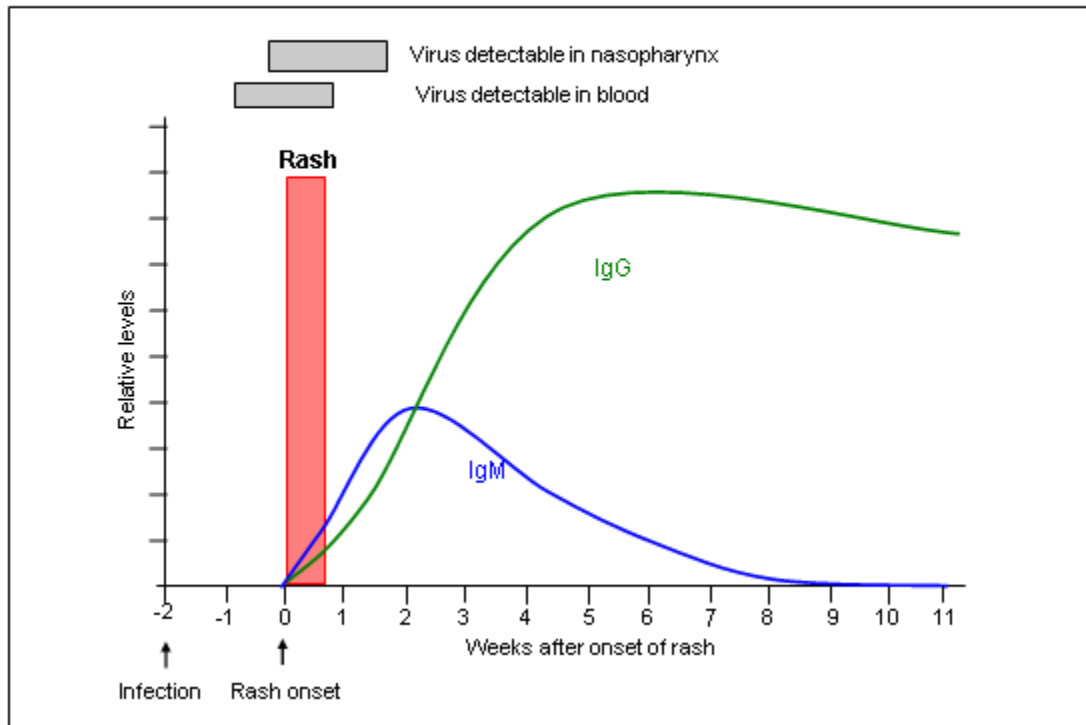
Additional information regarding rubella and CRS surveillance can be accessed online, including the document, *Introducing rubella vaccine into national immunization programmes: a step-by-step guide* [25].

1.7 Infection, immune response and laboratory diagnosis of rubella and CRS

After the rubella virus infects the nasopharynx, it multiplies in the lining of the respiratory tract and in local lymph nodes before passing into the bloodstream. Viraemia begins 5–7 days after infection, spreading throughout the rest of the body, including the skin. As with measles, the rash is immunologically mediated and coincides with the development of rubella-specific antibodies. Virus can be isolated from the nasopharynx from up to 1 week before, and for up to 2 weeks after the onset of rash.

Humoral and cell-mediated immunity develop following a rubella infection. Rubella-specific IgG and IgM antibodies are observed about 14–18 days after rubella infection, at about the time when the rash appears. Refer to **Figure 1.6, Immune response in postnatal rubella infection**.

Figure 1.6 Immune response in postnatal rubella infection



Rubella IgM antibodies wane quickly and are usually undetectable after 2 months, whereas rubella IgG antibodies persist. A rubella-specific, cell-mediated lymphocyte response begins 1 week after the humoral response and persists for a lifetime. Although natural rubella infection generally confers lifelong immunity, rare cases of serologically confirmed reinfections after earlier infection (or immunization) have been reported. There have also been cases of CRS following reinfection in pregnant women with natural or vaccine-induced immunity, but this is extremely rare. Although maternal rubella antibodies provide protection against rubella for the first months of life, vaccination of infants prior to the waning of maternal antibody can result in primary vaccination failure or an attenuated immune response.

Laboratory confirmation of a primary infection of rubella is generally performed by detection of rubella-specific IgM by EIA. Although IgM is sometimes detectable at rash onset, by 5 days after rash >90% of cases are IgM positive [22]. However, false-positive rubella IgM results are known to occur, particularly among persons infected with parvovirus B19. Blood collected from pregnant women for antibody screening purposes may be inappropriately tested for rubella IgM. Positive results obtained under these circumstances will require additional testing. An approved or certified laboratory will make use of the most appropriate method(s) to confirm suspected cases of rubella according to the details of the suspected case including the epidemiologic setting and vaccine history. Refer to chapter 4 for additional information on serologic testing.

The detection of rubella-specific RNA by RT-PCR can be used to complement serologic testing to confirm suspected cases. The use of RT-PCR to confirm rubella infection is described in chapter 6. Although virus isolation confirms rubella infection, propagation of rubella virus in cell culture is a labour-intensive and time-consuming method. Moreover, rubella virus does not generally produce cytopathic effect (CPE) in cell culture and must be confirmed by virus detection methods such as immunohistochemistry, immunofluorescence or RT-PCR. However, the ability to produce virus isolates is important for providing ample sources of RNA for the molecular characterization of viruses and for the collection of novel strains for virus banks (chapter 7).

If rubella infection occurs in a pregnant woman after 20 weeks of pregnancy, the infant may be infected with rubella and yet not develop the signs and symptoms of CRS. An infant born without clinical signs of CRS but with laboratory confirmation of rubella must be monitored since virus can be shed for up to 1 year. A laboratory confirmed CRS case is defined as a clinically confirmed CRS case that has a positive blood test for rubella-specific IgM. Although IgM antibodies may persist for up to 1 year, about 50% of CRS cases are IgM negative at 6 months of age. Because negative IgM results may be obtained from CRS cases tested shortly after birth, the IgM assays should be repeated at 1 month of age.

For infants over 6 months of age, a negative IgM should not be relied upon to rule out CRS. In such cases, serial IgG testing should be included. A sustained level of IgG antibody over several months confirms CRS. The recommendations for sample collection for laboratory confirmation of suspected

CRS cases is provided in chapter 3, section 3.6. Laboratory confirmation may also be performed through detection of rubella virus RNA from a suitable specimen (i.e., throat swab, urine). Specific chapters in this manual address the collection of samples, antibody testing and RT-PCR for confirmation of CRS cases.

The publication, *WHO-recommended standards for surveillance of selected vaccine-preventable diseases* [26], includes guidance and definitions for suspected, clinically confirmed, and laboratory confirmed CRS cases. Regional surveillance field guides are also available which include specific activities and technical information for the investigation, laboratory testing, and classification of suspected cases of CRS and CRI.

1.8 Epidemiologic features of rubella and CRS

Rubella has a worldwide distribution except in countries where the disease has been eliminated. It usually occurs in a seasonal pattern (i.e. in temperate zones during the late winter and spring), with epidemics every five to nine years. However, the extent and periodicity of rubella epidemics is highly variable in both developed and developing countries.

Rubella is spread through contact with respiratory secretions of an infected person. This may result from airborne droplet spread, direct contact with an infected person or indirect contact with freshly infected articles. Rubella is moderately contagious, primarily just before and just after the appearance of the rash, but transmission of virus can occur from 1 week before the onset of the rash and up to 1 week after the appearance of rash. Infants with CRS shed large quantities of rubella virus in their pharyngeal secretions and in urine, and can serve as a source of transmission. In closed institutions, such as in military barracks and child day-care centres, all exposed and susceptible individuals may become infected. The R_0 for rubella is 6-7, less than half that for measles. To interrupt transmission of rubella, a herd immunity threshold of approximately 83-85% is required [18].

An estimate of worldwide CRS incidence in 2010 was 105,000 cases (95% CI: 54,000-158,000) [27]. The majority of these cases occur in developing countries that have not yet introduced rubella vaccine. Many countries have not yet included CRS in their communicable disease surveillance systems and not all countries with well-established CRS surveillance have provided complete data to regional

offices or to WHO/UNICEF. Therefore, until more countries establish an effective CRS surveillance and reporting system, a more precise estimate of the burden of rubella cannot be provided.

The existing, internationally-licensed rubella vaccines, single or in combination with vaccines against measles (and mumps) have proved to be highly efficacious in the prevention of rubella infection and CRS in different parts of the world. Seroconversion following a single dose is $\geq 95\%$. Through immunization programmes, endemic transmission of rubella virus has been successfully eliminated in the western hemisphere and several European countries. Rubella-containing vaccine is usually administered in a combination vaccine with measles-containing vaccine, following the schedule recommended for measles [26,28].

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