Reliable serological testing in measles and other vaccine-preventable diseases

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Introduction

Vaccine-preventable diseases continue to take their toll worldwide despite the availability of safe and inexpensive vaccines. Outbreaks of infections with measles virus, in particular, are increasing in frequency in many parts of the world and have led to many deaths, especially in small children. Serological tests are an indispensable tool to support diagnosis of acute infections and to establish past infections or the immune status in individuals. Other vaccine-preventable diseases for which serological diagnostics play an important role include mumps, rubella, varicella, pertussis, tetanus and diphtheria.

Measles

The measles virus belongs to the genus Morbillivirus within the family Paramyxoviridae. The virus causes an acute febrile illness, which occurs mainly in childhood and is very contagious. Nowadays, the disease generally occurs less frequently than in previous generations due to vaccination. In recent years, however, outbreaks of measles have been increasingly observed worldwide (Figure 1), in part due to stagnating vaccination rates. For example, in the European Region the infection rate increased more than three-fold between 2017 and 2018 from 25,869 to 83,540 cases, according to the World Health Organisation. Measles represents one of the most common causes of death in infants worldwide. In the WHO European Region, 74 deaths from measles were recorded in 2018.

Persons with acute infections exhibit a wide range of clinical symptoms. Characteristic is a mild self-limiting infection. Severe cases can, however, be fatal or cause severe disability. The incubation period for measles is typically about 10 days. The illness manifests with flu-like symptoms, encompassing fever, malaise, catarrh of the upper respiratory tract, cough, congestion and conjunctivitis. Soon afterwards the measles rash, a typical exanthema, appears first near the ears, then on the forehead, face and over the rest of the body.

Complications from measles include secondary bacterial pneumonia, otitis media, encephalitis, myocarditis, miscarriage and subacute sclerosing panencephalitis (SSPE). The latter is a progressive, generally fatal brain disorder caused by chronic measles virus infection. It occurs about 7 to 10 years after the infection and generally kills within three years from the onset of symptoms. Patients suffer from behavioural changes, cognitive deterioration, visual problems, and advanced neurological symptoms, which lead to severe physical and mental impairment and eventually death.

Vaccination

Due to the complications arising from measles virus infections, vaccination of infants is recommended by public health authorities. For example, the Robert-Koch Institute in Germany recommends two immunisation shots at ages 11 to 14 months and 15 to 23 months. The measles vaccine is often given together with mumps and rubella vaccines. Immunity is generally lifelong but can wane in individuals over 60. Moreover, antibody levels in post-vaccine sera are eight to 10 times lower than in convalescent sera. Consequently, a booster immunisation is recommended in persons over 60 to prevent severe life-threatening disease.

Serology

Serological testing is used to confirm the clinical diagnosis, alongside other methods such as direct detection of the virus. In primary infections, antibodies of class IgM develop soon after the onset of symptoms. According to the MiQ Immunological Methods for the Detection

of Infectious Diseases (INSTAND e.V.), 70 per cent of patients exhibit IgM antibodies within three days of the appearance of the rash. The detection of IgM antibodies is considered an indicator of an acute infection. IgG antibodies are produced slightly later, soaring within two to four weeks. A significant increase in the IgG titer in two successive samples demonstrates an acute infection. A fresh infection can also be confirmed by means of an IgG avidity test. The presence of high-avidity IgG antibodies indicates an infection or vaccination that has occurred in the recent past. Breakthrough infections following earlier vaccination are characterised by moderate- to high-avidity antibodies and high neutralising antibody titers shortly after reinfection. IgM may be negative in these cases.

IgG antibodies are also suitable for determining the immune status, since they remain detectable throughout the lifetime of the individual. It should be noted, however, that no difference can be serologically made between wild virus infection and vaccination. Therefore, knowledge of a patient's medical history regarding illness and vaccination is paramount. New research has also shown that the immune protection from measles virus involves a complex interaction between cellular and soluble components of the immune system. Care should, therefore, be taken in the interpretation of results, as the immune response varies greatly between individuals. In general, an IgG concentration of over 200 mIU/ml is considered to be sufficiently protective according to the Robert Koch Institute.

Measles myelitis or encephalitis can be verified by analysing IgG antibodies against the virus in the cerebrospinal fluid (CSF). The involvement of the central nervous system in the disease is established by calculating the quotient of specific antibodies in CSF and serum compared to the quotient of total antibodies in CSF and serum, also known as the antibody specificity index.

Antibody detection methods

Specific antibodies against measles virus can be measured in patient samples by immunological methods such as ELISA or chemiluminescence immunoassay. In ELISA, the use of viral lysate as the detection substrate enables highly sensitive detection of IgM antibodies in early infections. In the EUROIMMUN Anti-Measles Virus ELISA (IgM), for example, an inactivated lysate of cells infected with the measles virus strain Edmonston is used for the antibody detection. Viral lysates may, however, also occasionally induce unspecific reactions. A higher specificity can be achieved by using recombinant protein from measles virus for the antibody detection. The Anti-Measles Virus NP ELISA (IgM) is based on a recombinant nucleoprotein from measles virus, which provides more specific detection compared to lysate-based systems (Table 1).

Lysate-based ELISAs are preferable for determination of IgG antibodies. The EUROIMMUN Anti-Measles Virus ELISA (IgG) provides reliable measurement of IgG antibodies in serum or plasma and is suitable for both diagnostics and immune status determination. The ELISA is calibrated against the 3rd International Standard serum NIBSC 97/648. An IgG avidity test is also available for determination of the antibody avidity. The lysate-based ELISA for CSF diagnostics enables reliable detection of intrathecal antibody synthesis.

The performance of the ELISAs has been verified in various studies. In quality assessment schemes with pre-characterised samples, the Anti-Measles Virus NP ELISA (IgM) and the Anti-Measles Virus ELISA (IgG) achieved 100 per cent agreement with the target results. The lysate-based Anti-Measles Virus ELISA (IgM) yielded 99 per cent agreement, excluding one borderline result.

Further vaccine-preventable diseases

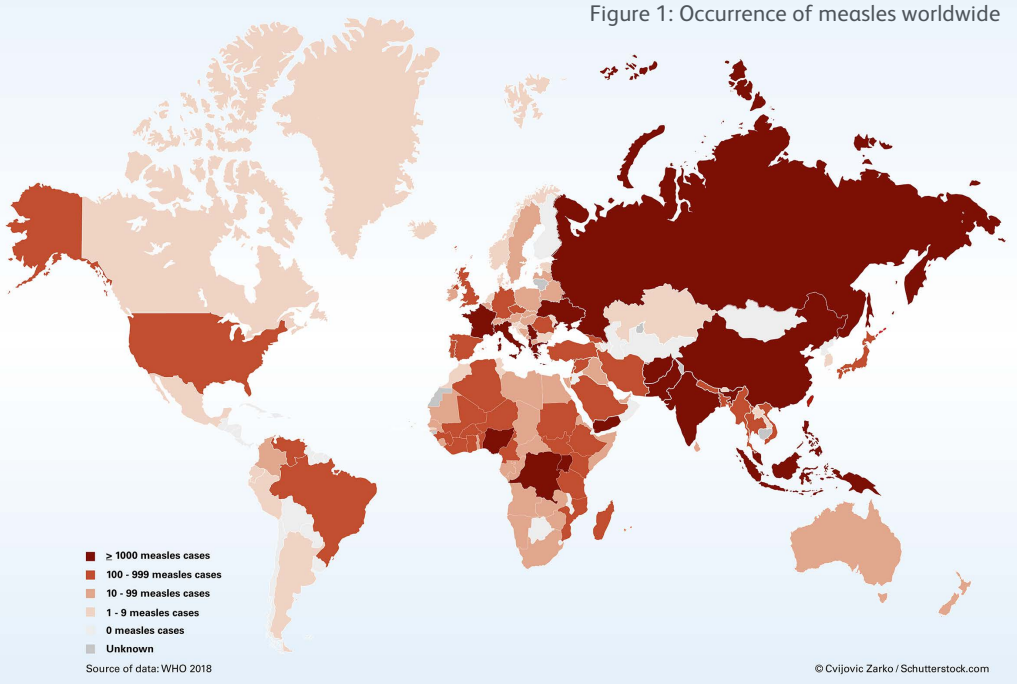
Serological analyses are also important in diagnostics and immunity surveillance of numerous other vaccine-preventable diseases. For example, detection of specific antibodies against rubella virus or varicella zoster virus (VZV) supports diagnosis of the respective infections and determination of the immune status. For detection of IgM antibodies against these viruses in acute

Measles myelitis or encephalitis can be verified by analysing IgG antibodies against the virus in the CSF.

TABLE 1: Comparison of nucleoprotein-based and lysate-based IgM ELISAs

Panel	n	EUROIMMUN ELISA (positive and borderline results)	
		Anti-Measles Virus NP ELISA (IgM)	Anti-Measles Virus ELISA (IgM)
Patients with acute measles virus infection	16	16	16
Patients with other infectious diseases	112	2	6
Blood donors	500	1	3

Figure 1: Occurrence of measles worldwide



diagnostics, purified viral glycoproteins are preferably used as the ELISA substrate in order to minimise unspecific reactions. Existing immunity to rubella virus or VZV can be established by measuring IgG antibodies using viral lysate-based ELISAs calibrated according to international reference sera.

Acute infections with mumps virus can be confirmed by determining specific IgM antibodies using ELISA based on viral lysate from either the wild type strain Enders or the currently circulating G5 strain. Immunity to mumps virus following vaccination cannot be conclusively verified serologically due to the lack of an international reference serum. However, seroconversion can be demonstrated by determining IgG antibodies against mumps virus. A special ELISA combining native antigens from the wild type strain Enders and the vaccination strain Jeryl Lynn provides an increased serological detection rate following vaccination, especially in children.

Serology also supports diagnostics of infections with Bordetella pertussis, the causative agent of whooping cough. Different reference laboratories recommend using only immunoassays which are based on species-specific pertussis toxin (PT) and which enable quantification in international units (IU/ml). The EUROIMMUN Anti-Bordetella PT ELISA detects specific antibodies against B. pertussis following infection or vaccination and enables exclusion of a B. parapertussis infection. Detection of IgG anti-PT antibodies at concentrations greater than 100 IU/ml indicates with high certainty a recent B. pertussis infection or vaccination. However, differentiation between infection and vaccination is not possible serologically. Moreover, the measurable antibody concentrations do not allow for any conclusions to be drawn regarding existing protection or immunity after vaccination.

For tetanus and diphtheria, ELISAs based on the toxoids from the corresponding bacteria, Clostridium tetani or Corynebacterium diphtheriae respectively, enable determination of the immune status and vaccination controls. The ELISA results are evaluated according to international reference preparations.

Perspectives

Health authorities worldwide are striving to eliminate a range of infectious diseases through comprehensive vaccination programmes. Nevertheless, outbreaks of vaccine-preventable diseases continue to occur worldwide. For highly contagious diseases such as measles, reliable identification and confirmation of cases is critical for early recognition of outbreaks, analysis of ongoing transmission, and estimation of the incidence. This enables among other things the development of vaccination strategies suited to local situations. Assessment of the immune status of individuals after vaccination or previous infection is important for identifying those with no or insufficient cover. These persons can consequently be offered additional vaccine shots to boost their immunity. This is beneficial both to protect the individuals and to bolster the herd immunity of communities.

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