

Measles (*Measles 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 *measles virus*.

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1 PHLN Summary laboratory definition

1.1 Condition:

Measles

1.1.1 Definitive Criteria

- Isolation of *measles virus* from clinical material;
- Detection of *measles virus* RNA in clinical material;
- Detection of *measles virus* antigens in clinical material, and
- IgG seroconversion or four fold or greater rise.

1.1.2 Suggestive Criteria

- Detection of IgM antibody to *measles virus*

2 Introduction

Measles virus is a negative sense enveloped RNA virus that is a member of the *Morbillivirus* genus of the family *Paramyxoviridae*.

Measles is highly infectious by the respiratory route, typically infecting more than 90% of susceptible household contacts, and more than 50% of contacts in schools and day care centres. The clinical presentation is typically of a prodromal illness comprising cough, fever and conjunctivitis followed quickly by a pathognomonic enanthem on buccal mucosa: Koplik's spots, and then a few days later by a generalised maculopapular rash which typically moves down the body from the head to the extremities. Approximately 10% of cases are complicated, most commonly by otitis media, diarrhoea, and pneumonia. Acute encephalitis occurs 1 in 2000 people. For every ten children who develop

measles encephalitis one will die and up to four will develop permanent brain damage. Subacute sclerosing panencephalitis (SSPE) is a late complication of measles in about 1 in 25,000 cases. SSPE causes progressive brain degeneration and is always fatal.

Several other infectious diseases can mimic measles, and when measles is well controlled the majority of suspected cases have alternative aetiologies. The most common of these are *human herpes virus 6*, *rubella*, *enteroviruses*, *human parvovirus B19*, and *dengue viruses* in endemic regions. In cases of suspected measles which are rejected on the basis of serological testing, it is recommended that testing for at least *rubella* and *parvovirus* should be undertaken and other diseases if clinically indicated.

A measles-containing vaccine was first introduced in Australia in 1968. Initially the uptake of the vaccine was poor, however the vaccine had a dramatic impact on the number of measles cases by the early 1970s. Levels of immunisation coverage were not sufficient to prevent outbreaks in Australia, and a large nationwide epidemic of measles occurred in 1993 to 1994. In 1994 a two dose vaccination schedule was introduced, and in 1996 the NH&MRC developed national guidelines for the prevention and control of measles outbreaks in Australia. Due to the change in timing of the second dose of the MMR vaccine from 10-16 years to 4-5 years, it was necessary to offer all primary school children a second dose of the MMR vaccine. A one-off school-based measles control campaign conducted in 1998 vaccinated the majority of 1.8 million school children aged between five and 12 years. Subsequent surveys of immunity levels in the Australian community demonstrated protective levels of antibody in 89-94% of children aged 2-18 years.

The severe consequences of a measles infection, the highly infectious nature of the disease and the rapidity of its spread emphasised the need to both maintain high levels of vaccination coverage and to have the facilities for the rapid and accurate diagnosis of acute cases of measles available throughout Australia. IgM serology is the mainstay of measles laboratory diagnosis. Direct detection of *measles virus* by nucleic acid test (NAT), immunofluorescence or viral culture may aid diagnosis when persisting IgM from vaccination is a possibility. When any case is suspected every effort should be made to collect specimens for *measles virus* isolation or nucleic acid detection and positive material submitted for nucleic acid sequencing for molecular epidemiologic purposes.

3 Tests

3.1 Serological diagnosis

Serological diagnosis requires the identification of an IgM antibody specific for measles or demonstration of a rising titre of IgG antibodies. Optimally timed serum specimens may allow demonstration of both specific IgM and the appearance or strengthening of the IgG response.

Testing for these antibodies is readily available using enzyme immunoassay which is the most widely used technique. Current commercially available indirect IgM enzyme assays are comparable to the published evaluation of in-house IgM capture assay developed by CDC. This assay was positive in 80% of cases within 3 days of the onset of rash increasing to 100% between days 4 and 14 but declining thereafter (reviewed in reference 3).

Plaque reduction neutralisation may be used to demonstrate protective measles antibody, a titre of 120 correlating with immunity, or to demonstrate antibody rises between paired sera. However this reference technique is not widely available and too cumbersome for general diagnostic use.

3.1.1 Suitable specimens

Serum or plasma collected at the time of presentation with rash followed by a second specimen during convalescence at least 10-14 days later. EIA may be adapted to detect either IgG or IgM from oral fluid, or blood spots on filter paper, but these approaches don't offer significant logistic advantages in Australia and are little used here.

3.1.2 Test sensitivity

IgM less than 50% at four days after onset of rash increasing to 88-100% at 1-3 weeks post onset.

3.1.3 Test specificity

Variable 60-97%

3.1.4 Suitable acceptance criteria

Negative and positive controls within range

3.1.5 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.

3.1.6 Suitable 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.

3.1.7 Suitable external QC programme

Participation in the National Association of Testing Authorities (NATA)/ RCPA quality control programs.

3.1.8 Special considerations

In SSPE elevated levels of specific measles IgG (relative to plasma) may be found in CSF. IgM may also be detected.

3.2 Culture of Measles

3.2.1 Methods

A number of cell lines are suitable for sensitive culture of *measles virus* from clinical specimens including Vero cells, Primary Monkey Kidney (PMK), the EBV transformed marmoset B-lymphocyte cell line B95-8 (until recently the cell line recommended by WHO), and Vero SLAM which is a Vero cell line stably expressing the human signalling lymphocytic activation molecule (SLAM) a *measles virus* receptor. The latter cell line is now recommended by WHO for measles culture. Cultures should be maintained for 7-14 days after inoculation *measles virus* should grow in 2-14 days but blind passage of the cell cultures should be considered if the cultures failed to yield a positive result at the first attempt. *Measles virus* growing in cell culture may be detected by the presence of a characteristic

cytopathic effect but this may not be reliable. Cultures should be tested by either haemadsorption by PCR, or by staining of the cell culture using immunofluorescence or immunoperoxidase techniques. The latter are usually performed using a commercial monoclonal antibody against the M protein. Any *measles virus* that is isolated should be amplified by repeat passage then frozen for long-term storage and referred to a reference laboratory for further characterisation. Sequencing of at least one *measles virus* RNA isolate from every cluster is important for tracing lines of transmission and for molecular epidemiological purposes.

3.2.2 Suitable specimens

Nasopharyngeal aspirates, swabs and washes and throat swabs are acceptable for culture as are endotracheal aspirates, bronchoalveolar lavage fluid and lung biopsy tissue. Urine and heparinized blood are also suitable specimens. 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. Culturable virus may be present in respiratory secretions for up to one to two days after the onset of rash and in the urine for up to 10 days.

3.2.3 Test sensitivity

Approximately 50-60% even when specimens taken from acute cases are sent to experienced laboratories.

3.2.4 Test specificity

Should approach 100% (providing the culture is confirmed by staining). Sequencing is required to distinguish vaccine from wild type.

3.2.5 Predictive values

100% (when wild type measles is found).

3.2.6 Suitable acceptance criteria

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

3.2.7 Suitable internal controls

Cell cultures maintained at the same time with and without inoculation with control measles (vaccine strain) virus stocks. Controls should be stained in parallel with cells inoculated with clinical material.

3.2.8 Suitable 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.

3.2.9 Suitable external QC programme

Participation in the National Association of Testing Authorities (NATA)/RCPA quality assurance programs if available.

3.2.10 Special considerations

Any positive cell culture should be passaged to generate a stock of the viral isolate. *Measles virus* isolates should be referred to a reference laboratory for nucleic acid sequencing to enable strain identification and epidemiological investigation of outbreaks.

3.3 Nucleic Acid Diagnosis

Nucleic acid amplification procedures may target any viral suitable gene sequence but most often are designed to target the haemagglutinin or nucleocapsid genes. Positive PCR reactions may be identified provisionally by gel electrophoresis but additional steps should be undertaken to confirm the identity of the PCR product. Sequencing of amplified *measles virus* DNA from at least one case in each cluster should be performed for molecular epidemiological purposes.

3.3.1 Suitable specimens

Blood, urine, CSF, saliva and respiratory specimens, suitable for cell culture, are also suitable for the direct detection of *measles virus* RNA by nucleic acid amplification techniques such as PCR. Viral RNA may be detected in throat swabs, peripheral blood lymphocytes and urine for more than two weeks after rash onset, and in serum prior to the appearance of specific IgG. Specimens containing non-viable virus may also be suitable eg those delayed in transit.

3.3.2 Test sensitivity

High. Sensitivity varies between assays but may optimally be 100% of proven measles cases.

3.3.3 Test specificity

Should approach 100%. Great care should be taken to ensure clinical specimens are not contaminated during RNA extraction and PCR performance. Sequencing is required to distinguish vaccine from wild type.

3.3.4 Suitable 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 hybridisation or sequencing.

3.3.5 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. Each clinical sample should also be spiked with positive DNA to exclude inhibition.

3.3.6 Suitable test validation criteria

Auditors should have available evidence of:

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

3.3.7 Suitable external QC programme

Participation in the National Association of Testing Authorities (NATA)/RCPA quality assurance programs

3.3.8 Special considerations

Measles RNA detection by NAT on CSF may be of use in the diagnosis of acute measles encephalitis but its use in the diagnosis of sub-acute sclerosing pan-encephalitis is less well established.

3.4 Antigen detection

Rapid diagnosis of measles by antigen detection can be achieved using smears made from nasopharyngeal or respiratory secretions stained with a monoclonal antibody(s) directed against virus structural proteins. These are usually directed against the *Measles virus* N protein, the most abundant viral antigen in infected cells. Although cytological appearances of giant cells or intranuclear or intracytoplasmic eosinophilic inclusions may be suggestive of measles infection a more positive identification is recommended. Immunofluorescence results should be available on the same day as receipt of the specimen.

3.4.1 Suitable specimens

Nasopharyngeal or respiratory secretions

3.4.2 Test sensitivity

Antigen detection is of a similar sensitivity to cell culture (50-60%).

3.4.3 Test specificity

Not known (?90-95%).

3.4.4 Suitable acceptance criteria

- Absence of staining in negative control cells.
- Absence of staining of infected cells with control antisera.
- Staining of positive controls cells demonstrating intra-cellular inclusions.

3.4.5 Suitable internal controls

- Control slides containing infected and uninfected cells should be included in each run.
- When an indirect immunofluorescence assay is used a control serum of the same species as the primary antibody should be included in each run.

3.4.6 Suitable original test validation criteria

Auditors should have available evidence of:

- records of material received and transport and storage before use;
- records of control tissue preparation and testing; and
- records of reagents used in the staining.

3.4.7 Suitable external QC programme

Participation in the National Association of Testing Authorities (NATA) RCPA QAP quality control programs

4 Agreed typing & subtyping methods

In 1998, WHO established a uniform nomenclature for designating wildtype *measles viruses* and describing genotypes (WHO 1998). The WHO subtyping scheme is based on the sequence of 450 nucleotides that code for the COOH-terminal 150 amino acids of the nucleoprotein (N). Further characterisation of an isolate is obtained by sequencing the complete haemagglutinin gene (1845 nucleotides). WHO published guidelines for the designation of new genotypes in 2001 and in 2003 added a further 2 genotypes to the original 20 described in 1998. Periodic updates on the geographical distribution of genotypes are available via the internet on www.cdc.gov/ncidod/dvrd/revb/measles. This excellent website also provides links to other aspects of measles virology.

5 References

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4. WHO (1998) Expanded programme on immunization – standardization of the nomenclature for describing the genetic characteristics of wild-type measles viruses. Weekly Epidemiological Record 73:265-269 (<http://www.who.int/wer>)
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6. Hummel K, Lowe L, Bellini B, and Rota P Development of quantitative gene-specific real-time RT-PCR assays for the detection of measles virus in clinical specimens J Virol Methods 132 p166-173 (2006).