

Title: MUMPS LABORATORY CASE DEFINITION

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1 PHLN SUMMARY LABORATORY DEFINITION

1.1 Condition: Mumps

1.1.1 Definitive criteria

- Isolation of mumps virus from clinical material;
- Detection of mumps virus RNA in clinical material; or
- IgG seroconversion or significant rise in IgG level (e.g. four-fold or greater rise in titre)
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1.1.2 Suggestive Criteria

- Detection of IgM antibody to mumps

2 INTRODUCTION

Mumps virus is a non-segmented, negative sense, enveloped RNA virus that is a member of the Rubulavirus genus of the family Paramyxoviridae. Mumps virus strains can be classified into 12 genotypes, from A to N (excluding E and M), based on the sequence diversity of the SH gene that encodes the small hydrophobic protein.

Mumps is a highly contagious infection with an R_0 of 10–12 in a susceptible population. Prior to the introduction of routine mumps vaccination, 95% of adults had serological evidence of infection, and regular seasonal outbreaks occurred every 2-5 years, mainly affecting children¹. Transmission is by respiratory secretions, direct contact with saliva, contaminated fomites and possibly urine. The mean incubation period is 18 days (range 14–25) days². The virus can be isolated from saliva of infected people 7 days before onset of parotitis and the disease is most contagious 1 to 2 days before the onset of symptoms. Infected individuals may remain infectious for up to nine days after onset³.

Approximately 30% of infections are asymptomatic. Clinically apparent infections are often preceded by a short prodrome of low grade fever, anorexia, malaise and headache. Over 95% of symptomatic cases will have the pathognomonic features of bilateral, swollen, painful salivary glands, most often involving the parotid glands. Epididymo-orchitis may occur in 15–30% of adult males, however it is rare in pre-pubertal boys. Mumps infection may result in a number of neurological complications, including meningitis (1–10% of mumps infections), encephalitis (less than 0.1%), that may result in hearing loss in up to 4% of cases, and permanent sensorineural hearing loss (1 in 20 000 cases). Oophoritis, mastitis, pancreatitis, thyroiditis, myocarditis, migratory polyarthropathy and spontaneous abortion have all been reported following mumps infection⁴.

Mumps is now an uncommon infection within Australia, particularly following the introduction of vaccination in 1981. Annual reported cases declined in Australia to a low of 60 in 2002, but have risen thereafter¹. Several large outbreaks of disease have occurred in developed countries during the past decade, predominately involving young adults. Many of these cases were in partially or fully vaccinated individuals. In Australia, serological data, notification rates and hospitalization rates have identified the birth cohort of late 1978-1982 as most susceptible¹.

Mumps vaccination has been delivered in combination as measles-mumps vaccine from 1983, and from 1989 as measles-mumps-rubella vaccine. Mumps vaccines are live attenuated viruses which can vary in effectiveness and degree of attenuation. Vaccines currently available in Australia all contain the Jeryl-Lynn strain. Post-licensure studies of two doses of MMR (Jeryl-Lynn strain)⁵ found a median vaccine effectiveness (VE) estimate of 88% (79-95%). A report on a mumps epidemic in the Netherlands found a VE of 82% for preventing hospitalisation, 76% for preventing all complications and 74% for preventing orchitis.

3 TESTS

3.1 Serological diagnosis

Serological diagnosis of mumps infection requires the identification of specific IgM antibody or demonstration of a rising titre of IgG antibodies. However, in vaccinated populations such as Australia, most cases occur in vaccinated individuals and IgM detection is of limited value (see below).

The optimal time of serum collection is 7–10 days following symptom onset. IgM antibodies are present in approximately 70% of individuals at the onset of illness (when glands are swollen)¹⁰, and may be present for several weeks or months following the illness, declining within four to eight weeks. Importantly, an IgM ELISA may give a false negative result if collected before day four of clinical presentation.

IgM-ELISA has been shown to be superior to IgM immunofluorescence, haemagglutination inhibition and complement fixation tests in diagnosing recent infection. A comparison of five commercial IgM-ELISAs during a recent outbreak in Scotland found a sensitivity of 24- 51% for samples collected in the first ten days after onset of symptoms⁶. Specimens collected after ten days had the highest positivity rates, with sensitivity of 80–100%. Specificity was around 82% for most assays. Equivocal or positive results were obtained in sera from patients with parainfluenza, Epstein-Barr virus and Parvovirus B19.

IgM is often not detectable in previously infected or immunised individuals. During an outbreak in Nova Scotia the sensitivity of IgM was found to be 23% in cases with a history of mumps vaccination⁷. Similarly in Western Australia, Speers et al⁸ found fewer cases of mumps were diagnosed by serology than PCR among fully vaccinated individuals compared with the unvaccinated group.

Where an appropriately timed IgM test is negative, infection may be confirmed by IgG seroconversion or four-fold rise in titre between acute and convalescent samples.

Recent vaccination with mumps vaccine or MMR can elicit a mumps IgM antibody response.

In individuals who are initially seronegative, IgG seroconversion or a four-fold rise in IgG titre will confirm infection. The utility of this test in an outbreak setting is limited however, particularly in fully or partially vaccinated individuals and its absence should not be used to rule out mumps. As ability of mumps IgG and IgM serology to confirm mumps among vaccinated patients is limited, serological tests among vaccinated patients should be interpreted cautiously and confirmed by RT-PCR tests at the beginning of a mumps outbreak.

Virus neutralisation is the most specific serological test determining infection and immunity. At low concentrations of antibody it can also be more sensitive due to the lower initial dilutions of serum used. There is no neutralising antibody titre established as surrogate marker for immunity, and as a result a “protective” IgG titre cannot be determined.

3.1.1 Suitable specimens

Serum or plasma collected at the time of presentation followed by a second specimen during convalescence at least 10 days later.

3.1.2 Test sensitivity

IgM antibody:

For specimens tested on or prior to day 4 of onset of symptoms: less than 20-50%

For specimens tested after day 4 of onset of symptoms: between 26%-100%, depending on vaccination status of patient

IgG antibody: Sensitivity of 76%.⁹

3.1.3 Test specificity

IgM: Overall 80-95%. Specificity is higher in vaccinated patients.

IgG: Specificity of 83%⁹

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 quality assurance (QA) monitoring of test kit performance.

3.1.7 Suitable external QA programme

Participation in a suitably accredited external QA program.

3.1.8 Special considerations

Mumps IgM can be detected in oral fluid in up to 56% patients with a clinical presentation suggestive of mumps infection¹⁵. IgM and IgG may be detected in CSF of patients with mumps meningitis or encephalitis.

3.2 Culture

3.2.1 Methods

A broad range of cell lines are suitable for sensitive culture of mumps virus from clinical specimens. These include Primary Monkey Kidney (PMK), Caco-2 (human colorectal adenocarcinoma epithelium cells), human embryonic kidney (HEK) and continuous cells lines such as HeLa and Vero (African green monkey kidney). The specimens should be collected within the first week of onset of symptoms, and the cultures should be maintained for 7-14 days after inoculation.

Characteristic cytopathic effect (CPE) should develop between 3–7 days following symptom onset, and consists of cell rounding and syncytium formation. The appearance may appear as non-specific granularity with progressive degeneration. As the CPE may be non-specific and some strains of mumps are non-cytopathic, the presence of virus should be confirmed by hæmadsorption with guinea pig red cells, immunofluorescence, immunocytochemical staining or RT-PCR.

3.2.2 Suitable specimens

CSF, oral fluid, parotid duct (buccal) swabs, urine or seminal fluid collected in the first week of the illness. Buccal swabs should be collected after 30 seconds of parotid gland massage using rayon or dacron-tipped swabs with 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.

3.2.3 Test sensitivity

Approximately 70-75% in acute mumps infection.

3.2.4 Test specificity

Close to 100% if CPE confirmed with an immunostaining, HA or NAT.

3.2.5 Predictive values

Close to 100%

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 mumps 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 QA programme

Not available

3.2.10 Special considerations

Any positive cell culture should be passaged to generate a stock of the viral isolate.

3.3 Nucleic Acid Detection

Nucleic acid amplification test is the most sensitive assay to diagnose early mumps infection. Any suitable viral gene sequence can be targeted, however most published studies target the small hydrophobic (SH), fusion (F) or nucleoprotein (N) gene. RT-PCR targeting the F and N genes have greater sensitivity than the SH gene^{10, 14} and are preferred for detection. The SH region is the most variable region of the mumps genome and the product can be used to genotype mumps strains. Positive PCR reactions should be confirmed using gel electrophoresis or another method.

3.3.1 Suitable specimens

Oral fluid, parotid gland (buccal) swabs, urine and CSF specimens are suitable for the direct detection of mumps virus RNA by nucleic acid amplification techniques such as PCR. Viral RNA may be detected in saliva for more than five days prior to and nine days after onset of symptoms, and viruria may persist for up to two weeks post onset.

3.3.2 Test sensitivity

Buccal swabs collected within the first two-three days after onset are the preferred sample for detection of mumps RNA, with a sensitivity of 57-83%, depending on gene target^{7, 10, 11}. Detection rates do not appear to be influenced by vaccination status early in the illness¹⁰. Beyond two to three days after onset positivity rates fall significantly, however buccal swabs may remain positive for up to 7 days after symptom onset. RT-PCR on urine is generally less sensitive with an overall sensitivity of 42%^{7, 14}. An inhibition rate of 5% has been reported. The sensitivity of PCR on CSF from cases of mumps neurological disease may be higher (70-90%)^{12,13,14}.

3.3.3 Test specificity

Should approach 100%.

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 a specific method such as 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.

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 QA programme*

Participation in a suitably accredited external QA program.

4 **AGREED TYPING AND SUBTYPING METHODS**

The molecular epidemiology of mumps virus is characterised by the co-existence of ten or more distinct genotypes designated A-N, based on the nucleotide sequence of the SH gene. Nucleotide sequencing and phylogenetic analysis of mumps virus have been used to characterise outbreaks geographically. It has been suggested that genotypes B, C, D and H have a higher potential for neurovirulence than genotype A.

4.1 **SNOMED concepts**

SNOMED CT concept	Code
Mumps virus (organism)	50384007
Mumps (clinical finding)	36989005
Mumps virus antigen assay (procedure)	122355008
Mumps virus RNA assay (procedure)	122377007
Mumps virus culture (procedure)	122273009
Mumps IgG level (procedure)	315155005
Mumps IgM level (procedure)	315156006

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