Microbiological Diagnosis of Mumps

Josep Costa*

Microbiology Service, Hospital Clínic, University of Barcelona, CIBEREHD Spain

Abstract: Mumps virus infection is normally diagnosed by serologic testing.

Identification of mumps-specific IgM antibodies in serum or plasma by enzyme immunoassay analysis (EIA) in samples taken during the acute phase is the most-widely used serologic test for the diagnosis of mumps, due to its simplicity, high sensitivity and specificity.

When the infection cannot be confirmed by serologic tests, the diagnosis can be made by direct methods such as isolation of the virus in cell cultures or detection of the viral genome by molecular analysis

This work describes these three methods: serologic testing, cell culture and gene amplification.

Keywords: Mumps virus, serologic tests, cell culture, gene amplification, sensitivity, specificity.

INTRODUCTION

Although the main manifestation of mumps virus infection is inflammation of the parotid gland, mumps is a systemic infection that affects many organs and tissues. The mumps virus is acquired by inhalation and initially infects the cells of the upper respiratory tract, where local replication occurs. Around 7-10 days after the infection, the virus disseminates to the spleen and peripheral lymph nodes and at approximately 15 days can be detected transitorily in blood. After a mean of 18 days, systemic disease occurs, affecting the salivary glands, genitals, pancreas, kidneys, central nervous system, etc. Fig. (**1**) shows the chronology of mumps virus infection.

Serologic Diagnosis

Mumps virus infection is normally diagnosed by serologic tests. The simplest procedure is the identification of mumps-specific IgM antibodies in sera or plasma by enzyme immunoassay analysis (EIA) [1] in samples obtained during the acute phase, preferably at around seven days after onset of symptoms.

The time of sampling is critical for the diagnosis of mumps virus infection. In primary infection, IgM antibodies are normally detected a few days after disease onset, reach a peak at seven days and persist in blood for some months (Fig. **1**). In some cases, IgM antibodies are detected from the first day, but testing of samples obtained during the six first days of the disease may give rise to false negatives [2, 3]. A recent study [3] found no mumps-specific IgM antibodies in five out of nine samples of infected patients obtained during the first six days of the disease, although IgM antibodies were detected in later samples in all five cases. Recent studies have shown that mumps virus infection may also be reliably identified by the detection of mumps-specific IgM antibodies in saliva samples, with a specificity of 97% and a sensitivity of 90% [2].

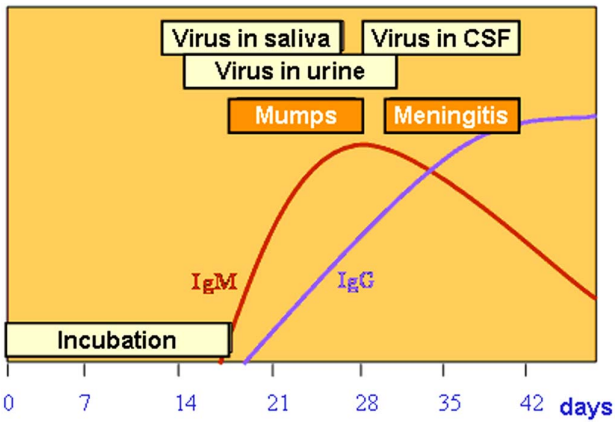

Fig. (1). Chronology of mumps virus infection.

Alternatively, mumps can be diagnosed by demonstrating seroconversion or a significant increase (at least four-fold) in IgG antibody titres between acute and convalescent phase serum samples. IgG antibodies are normally detected one week after symptom onset, with a rapid increase in blood levels. To compare antibody levels in the acute and convalescence phases, samples obtained a few days after symptom onset and at two weeks should be analysed.

EIA is the most-commonly used serological test for the diagnosis of mumps due to its high sensitivity and specificity and its simplicity. Other serologic tests, with an equal sensitivity and specificity, such as the neutralization test or indirect immunofluorescence (IFI) are used less frequently as they are more time-consuming. In addition, the neutralization test does not distinguish between IgG and IgM antibod-

*Address correspondence to this author at the Microbiology Service, Hospital Clínic, University of Barcelona, Ciberehd Spain; Tel: 34 932275522; Fax : 34 93 2279372; E-mail: jcostac@clinic.ub.edu

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ies. Methods often used in the past, such as complement fixation or the hemagglutination test, are now practically obsolete.

In spite of their high diagnostic yield, serologic tests have important limitations. The detection of mumps-specific IgM antibodies is very sensitive in diagnosing primary infection, but when the infection affects already-vaccinated people, as frequently occurs, the sensitivity decreases precipitously, since the antibody response is irregular and tests often provide indeterminate or negative results.

A Spanish study in 2003 [4] found that the sensitivity of IgM in unvaccinated patients infected for the first time was 100%. However the sensitivity fell to < 30% in vaccinated patients and 15% in those who had received two doses of vaccine. Analysis of the increase in antibody titres between samples from the acute and convalescent phases may be very useful in diagnosing mumps in vaccinated people. However, as mumps is a self-limiting disease that normally does not present complications, obtaining a second specimen in the convalescent stage is generally difficult.

It has also been suggested that the detection of high IgG titres in a single sample may be indicative of infection [5]. However more exhaustive studies to accurately define the titres associated with infection with sufficient specificity are required.

When mumps virus infection cannot be confirmed by serologic tests, the diagnosis should be made by direct methods such as isolation of the virus in cell cultures or detection of the viral genome by molecular tests.

Cell Culture

The mumps virus can be isolated in oral mucosa from seven days before to nine days after the appearance of the symptoms and in urine from six days before to fifteen days after symptom onset [6, 7]. In cases of aseptic meningitis and encephalitis, the mumps virus can be isolated in cerebrospinal fluid (CSF) up to five days after the appearance of signs of infection of the central nervous system.

Isolation of the virus provides a greater diagnostic yield if the samples are collected very carefully at the onset of disease and processed rapidly. Ideally, samples should be inoculated in culture shortly after their obtention. As the mumps virus is heat-labile, samples should be maintained at 4ºC until inoculation. If the samples cannot be processed in a few hours, they should be diluted in a salt solution (Hank' s BSS) supplemented with inactivated fetal serum 1-2% and frozen at -70 ºC. When isolating virus from non-sterile samples such as saliva and urine, antibiotics should be added to avoid bacterial growth.

The virus can normally be isolated in saliva 48-72 hours after symptom onset. Culture samples should be obtained by vigorous application of a cotton swab to the oropharynx and salivary glands and around the openings of the parotid ducts, located anterior to the second upper molars, where the secretions of the parotid glands are released. The swabs should be eluted in 2-3 ml of culture medium before inoculation.

Urine samples should be preserved immediately in ice and complemented with antibiotics (500 U/mL of penicillin, 200 U/mL of nystatin and 200 U/mL of streptomycin) to avoid bacterial growth. Culture yields improve noticeably if the specimen is concentrated by ultracentrifugation before inoculation.

CSF may be inoculated directly in the cell culture with no previous treatment.

Good results are generally obtained with primary lines of *Macaca mulatta* kidney cells, which may be acquired commercially and are often used in clinical virology laboratories. Primary lines of human or *Macacus cynomolgus* embryonic kidney cells are a good alternative. All isolates must be confirmed by immunofluorescence with mumps-specific monoclonal antibodies.

The diagnostic efficiency of cell cultures is generally low, with a sensitivity of <50%. Various factors affect the sensitivity of this method: the concentration of infectious viral particles in the original sample, the presence of mumps antibodies and the loss of viability of the virus during sample handling.

Gene Amplification

A good alternative to isolation by culture is detection of the viral genome in saliva and CSF samples in cases of meningitis by genetic amplification techniques such as reverse transcriptase polymerase chain reaction (PCR) [8,9]. These methods have a very high specificity and a sensitivity greater than cell cultures in urine, saliva and, especially, CSF samples (Table **1**) [10-12]. The greater sensitivity is because the above-mentioned factors that affect cell cultures do not influence reverse transcriptase PCR results. Even so, samples should be collected as rapidly as possible after the onset of symptoms, preferably during the three first days. The sensitivity of reverse transcriptase PCR decreases rapidly from seven days after symptom onset. A recent study suggests that levels of the virus in saliva decrease rapidly after the third day in vaccinated people.

Recently, methods for detection of mumps virus RNA by Real Time PCR have been reported [10, 12]. The advantages of this procedure are rapidity, high sensitivity, the smaller risk of cross-contamination and the possibility of quantifying viral levels in the sample. PCR also permits sequencing of the viral genome for genotyping and can distinguish between the wild and vaccine-induced virus.

Since isolation by cell culture is not very sensitive, is slow and laborious and requires significant infrastructure that is available to few clinical microbiology laboratories, Real Time reverse transcriptase PCR is an excellent alternative method for the diagnosis of mumps.

SUMMARY

The identification of mumps-specific IgM antibodies by serologic testing in a single sample obtained seven days after the onset of symptoms is the method of choice for primary infections due to its high sensitivity and specificity.

Table 1. Sensitivity of Culture Versus PCR Methods

	Oral Fluid			CSF
	n=46 (1)	n=36 (2)	n=180 (3)	n=27 (1)
Culture	24 (52.2%)	7 (20%)	90 (50.0%)	6 (22.0%)
RT-nPCR	33 (71.7%)	18 (51.4%)	92 (51.0%)	19 (70.4%)
RealTime	33 (71.7%)	27 (77.1%)	119 (66.0%)	20 (74.1%)

(1) Uchida *et al*. (2005).
(2) Jin *et al*. (2007).
(3) Krause *et al*. (2006).

However, when an earlier diagnosis is required or in the case of vaccinated subjects, identification of the viral genome by Real Time PCR in samples taken during the first days of infection is an excellent, simpler diagnostic method, with a greater sensitivity than isolation in cell culture.

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