

Comparison between Indirect Immunofluorescence Assay and Shell Vial Culture for Detection of Mumps Virus from Clinical Samples

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We report a prospective comparison of the efficacies of an indirect immunofluorescence assay (IFA) and shell vial culture (SVC) of throat swab and urine samples from patients with mumps. Throat swab samples were used for the IFA; the urine samples and throat swabs were inoculated into vials of Vero cells. We studied 62 patients by using 62 throat swabs and 50 urine samples (50 patients with both samples). Sixty (96.7%) throat samples were positive in the SVC, and 61 (98.3%) were positive in the IFA. For the 50 patients from whom both samples were available, the IFA was positive in 50 (100%) cases, the urine sample was positive in 49 (98%) cases, and the throat swab was positive in 48 (96%) cases ($P > 0.05$). This comparison of throat swabs and urine samples has shown that the two clinical samples are similar in efficacy.

Parotiditis (mumps or infectious parotitis) is a disease, caused by the mumps virus, that affects especially children less than 15 months old who have not been vaccinated or children or young adults living in geographical areas with low vaccination rates (1, 3, 10, 14). Nevertheless, there have been reports of mumps outbreaks in vaccinated populations although it was later found that, in the majority of the cases, the vaccine used was the Rubini strain, which offers slight protection against this disease (7, 13).

The diagnosis of mumps is generally clinical (parotid gland inflammation, swelling, and pain) when associated with a community epidemic outbreak. However, it is always advisable to perform a serological study of the patients and to try to isolate the mumps virus in order to establish its antigenic characteristics (2, 10, 14, 15). There is no specific rapid diagnostic technique for the mumps virus. Immunofluorescence assay (IFA) and the reverse transcription-PCR technique appear to be the methods with the greatest clinical efficacy (5, 9–11). Isolation in cell culture, especially by the shell vial method, has been recommended for a rapid and specific diagnosis, but it takes a minimum of 2 to 3 days (6, 12, 16).

Taking advantage of an epidemic outbreak of mumps in our community, we performed a prospective and comparative study of the direct antigen detection technique (IFA) versus shell vial culture from throat swab and urine samples from patients with clinical symptoms of mumps.

All throat swabs were placed in a virus transport medium and sent to the laboratory immediately. Sample volumes of 250 μ l were used for cytospin preparations by centrifugation onto glass slides at 700 rpm for 10 min with a cytocentrifuge (Cytospin 3; Shandon Scientific, Runcorn, England). After being air dried, the slides were fixed with acetone at -20°C for 10 min and then stained with a mouse monoclonal antibody

against mumps virus (clone 75; Argene-Biosoft) by an indirect IFA. The presence of cytoplasmic or membrane type-specific immunofluorescence was considered a positive result.

Urine samples were first centrifuged at $1,000 \times g$ for 15 min to remove cells and debris and then inoculated (250 μ l per sample) into two vials of Vero cells (Vircell, Granada, Spain) for the shell vial assay. Pharyngeal swabs were also inoculated (250 μ l per sample) into two vials of the same cell line. The vials were centrifuged at $700 \times g$ for 45 min and, after the addition of maintenance medium, incubated at 36°C for 2 to 5 days. The vials were then fixed with acetone at -20°C for 10 min and stained with a monoclonal antibody against mumps virus (the same as that used in the above-described assay) for the IFA.

Statistical analysis of the results of different comparisons was carried out by performing a two-tailed Student *t* test with paired data. *P* values were considered significant if they were less than 0.05.

Sixty-two patients with clinical symptoms of mumps virus infection were studied. Sixty-two throat swabs and 50 urine samples were used. Both types of sample were available for only 50 patients.

Of the 62 throat swabs studied, 60 (96.7%) were positive in the shell vial culture (viral isolation) and 61 (98.3%) were positive in the IFA (antigen detection) ($P > 0.05$). Of the 50 patients for whom both types of clinical sample were available, the IFA was positive in 50 (100%) cases, the urine sample was positive in 49 (98%) cases, and the throat swab was positive in 48 (96%) cases ($P > 0.05$) (Table 1).

Although the symptoms are sufficiently clear for the diagnosis of viral parotitis (mumps), it is necessary, at least in the first cases of an epidemic outbreak, to obtain etiological confirmation by means of a specific diagnostic method (immunoglobulin M detection or virus isolation) (1, 3, 10, 14).

The mumps virus is excreted in the oropharynx of infected patients and may be isolated in saliva (or by a throat swab) from 9 days before to 8 days after symptoms appear (8, 15). As a result, respiratory transmission (droplet spread) of the virus

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TABLE 1. Comparison of the IFA and shell vial culture results of 50 patients with clinical parotiditis

IFA result or no. (%) positive	Shell vial culture result or no. (%) positive		No. (%) of patients
	Pharyngeal swab	Urine	
+	+	+	47 (94)
+	-	+	2 (4)
+	+	-	1 (2)
50 (100)	48 (96)	49 (98)	50

may occur during a period of 7 to 10 days, including the 2 or 3 days before symptoms appear (8). Detection and isolation of the mumps virus in the saliva or throat swabs of patients is the best method for etiological confirmation and may be carried out in the first 4 to 5 days after infection (4).

There are few studies concerning the efficacy of rapid antigen detection techniques applied to the mumps virus. In 1975, Lennette et al. (9) studied the efficacy of an IFA using in-house polyclonal antibodies. In that study, 75% of the throat swabs were IFA positive and 100% were culture (not shell vial culture) positive. Our study shows a higher percentage of positivity, with 98.3% of the throat swabs positive in the IFA, probably as a result of the greater sensitivity of the monoclonal antibody used. One of the advantages of the IFA is its diagnostic rapidity, since this technique provides the result in a maximum period of 2 h, against the 2 days required for the shell vial culture method (12).

The mumps virus may be detected in urine for up to 2 weeks after symptoms appear, especially if centrifugation techniques are used (17, 18). This virus, as a result of viremia, reaches various organs and tissues of the human body, and especially the kidneys, where the epithelial cells of the distal tubules, calyces, and ureters appear to be the primary sites of virus replication (19). The presence of mumps virus in urine (viruria) is a manifestation of renal involvement. In general, dissemination and replication of the mumps virus in the parotid gland and the kidneys occur simultaneously. However, replication in kidney tissue is more prolonged and continues after the appearance of a serological response in the patient's serum (17, 18).

In our study, the percentage of mumps virus isolation in urine was similar to that obtained by the throat swab method ($P > 0.05$). In only one patient was the urine sample positive and the throat swab negative, and in this patient, both samples were obtained 12 days after the start of symptoms. This case demonstrates, once more, the prolonged excretion of the mumps virus in urine compared with its lesser presence in the upper respiratory tract (8, 17).

In summary, the IFA used in this study proved to be a sensitive and specific technique for the rapid etiological diagnosis of mumps virus infection. A comparison of throat swabs

and urine samples has shown that the two clinical sample types are similar in efficacy when obtained during the first 7 to 10 days after symptoms appear. For a specific diagnosis by cell culture once this period has passed, urine seems to be the more appropriate sample.

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