Evaluation of different continuous cell lines in the isolation of mumps virus by the shell vial method from clinical samples

J Reina, F Ballesteros, M Mari, M Munar

Abstract

***Aims*—To compare prospectively the efficacy of the Vero, LLC-MK2, MDCK, Hep-2, and MRC-5 cell lines in the isolation of the mumps virus from clinical samples by means of the shell vial method.**

***Methods*—During an epidemic outbreak of parotiditis 48 clinical samples (saliva swabs and CSF) were studied. Two vials of the Vero, LLC-MK2, MDCK, MRC-5, and Hep-2 cell lines were inoculated with 0.2 ml of the samples by the shell vial assay. The vials were incubated at 36°C for two and five days. The vials were then fixed with acetone at −20°C for 10 minutes and stained by a monoclonal antibody against mumps virus by means of an indirect immunofluorescence assay.**

***Results*—The mumps virus was isolated from 36 samples. The Vero and LLC-MK2 cell lines showed a 100% isolation capacity, MDCK showed 77.7%, MRC-5 showed 44.4%, and Hep-2 showed 22.2%. The Vero and LLC-MK2 lines were significantly different to the other cell lines ($p < 0.001$). The sensitivity for the Vero and LLC-MK2 lines at two and five days of incubation was identical (100%). The values obtained in the study of the quantitative isolation capacity (positive isolation with > 5 infectious foci) were 94.4% for Vero, 97.2% for LLC-MK2, 5.5% for MDCK, 5.5% for Hep-2, and 0% for MRC-5.**

***Conclusions*—The Vero and LLC-MK2 cell lines are equally efficient at two and five days incubation for the isolation of the mumps virus from clinical samples, and the use of the shell vial method considerably shortens the time of aetiological diagnosis with higher specificity.**

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Keywords: mumps virus; Vero cell line; LLC-MK2 cell line; MDCK cell line; Hep-2 cell line; MRC-5 cell line; isolation; shell vial

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Parotiditis (mumps or infectious parotitis) is an usually benign viral disease characterised by parotid swelling and pain. It is caused by the mumps virus, which belongs to the paramyxovirus genus of the paramyxoviridae family.[1] The clinical manifestations of parotitis are so typical that a laboratory diagnosis is seldom necessary, especially when it presents in the form of an epidemic outbreak. However, sporadic cases, above all when occurring in a population with a high vaccination rate, are more difficult to diagnose and require specific techniques.[1 2] In general, the diagnosis of infection by the mumps virus is carried out by means of the detection of a specific IgM. However, on many occasions, this immunoglobulin cannot be detected, particularly in previously vaccinated individuals.[2–4]

As a result, isolation in cell culture is recommended as the gold standard for a definitive aetiological diagnosis.[5–9] For a long time, conventional or tube cell cultures have been used for the isolation of the mumps virus. However, this method is slow and labourious and requires a prolonged incubation period. Therefore, recently, the shell vial culture method has been recommended to obtain rapid isolation and a specific diagnosis.[10] Most authors recommend the use of different cell lines for the isolation of the mumps virus. Primary rhesus monkey kidney and human embryonic kidney cells have proved to be those that provide the highest yield.[7 9–11] However, certain continuous human and animal cell lines, such as HeLa or Vero, provide very similar detection percentages.[7 8 10]

Taking advantage of an epidemic outbreak of mumps in our community, we carried out a prospective comparative study of the efficacy of different cell lines by the shell vial method in the isolation of mumps virus from clinical samples.

Material and methods

During an epidemic outbreak we studied 48 patients with clinical parotitis (salivary gland inflammation, fever, and/or meningeal symptoms). In all cases, a sample of saliva or swab was taken from Stensen's duct area of the most affected gland within the first 48 hours of illness. All samples were placed in virus transport medium and sent to the laboratory immediately. A lumbar puncture was performed in patients with suspected meningitis.

Two vials of Vero (green monkey continuous), LLC-MK2 (continuous monkey kidney cell), MDCK (Madin-Darby canine kidney), MRC-5 (human lung embryonated), and Hep-2 cell lines (Vircell, Granada, Spain) were inoculated with 0.2 ml of the samples, by the shell vial assay. The vials were centrifuged at 700 ×*g* for 45 minutes and, after the addition of maintenance medium, were incubated at 36°C for two and five days. The vials were then fixed with acetone at −20°C for 10 minutes and stained by a monoclonal antibody against mumps virus (clone 75; Argene-Biosoft, Varilhes, France) by means of an indirect immunofluorescence assay. The presence of a cytoplasmic, type specific immunofluorescence (clod or

Table 1 The isolation of mumps virus in the different cell lines studied after five days of incubation

Vero	*LLC-MK2*	*MDCK*	*MRC-5*	*Hep-2*	*No. (%)*
+	+	+	+	+	6 (16.6)
+	+	+	−	−	16 (44.4)
+	+	+	+	−	6 (16.6)
+	+	−	+	+	2 (5.5)
+	+	−	+	−	2 (5.5)
+	+	−	−	−	4 (11.1)
+	+	+	−	+	0
+	+	−	−	+	0
36	36	28	16	8	36
(100)*	(100)	(77.7)	(44.4)	(22.2)	(100)

*Percentage of isolation.

patch fluorescence) was considered to be a positive reaction. The mumps Rubini strain from the attenuated vaccine used in our community for routine vaccination was used as a positive control.

First, we studied the qualitative isolation capacity of the cell lines in their efficacy to isolate the mumps virus. Second, we analysed the quantitative isolation capacity of the cell lines with positive viral isolation, establishing the isolation efficacy in the detection of > 5 infectious foci in the monolayer.

Statistical analysis was carried out using the Student's *t* test on paired data. All p values are two tailed and considered significant if < 0.05.

Results

During the study period, 48 samples were studied. In 36 it was possible to isolate the mumps virus. The remaining 12 were considered to be negative. Of the 48 samples, 41 were saliva swab samples, with 35 being positive, and seven were cerebrospinal fluid, with one being positive.

The Vero and LLC-MK2 cell lines were positive in all 36 samples (isolation capacity, 100%), whereas MDCK had a sensitivity of 77.7%, MRC-5 of 44.4%, and Hep-2 of 22.2% ($p < 0.001$). Table 1 shows the combined positivity of the different cell lines studied. A comparison of the different cell lines with the Vero or LLC-MK2 lines shows a negative predictive value of 60% for the MDCK cell line, 37.5% for MRC-5, and 30% for Hep-2, with a specificity and positive predictive value of 100%. The isolation capacity for the Vero and LLC-MK2 cell lines at two and five days of incubation was identical because both lines detected all of the positive samples.

The quantitative isolation capacity was studied for all the cell lines only at five days of incubation. The values were 94.4% for Vero, 97.2% for LLC-MK2, 5.5% for MDCK, 5.5% for Hep-2, and 0% for MRC-5. The Vero and LLC-MK2 cell lines were significantly different to the other cell lines but there was no significant difference between these two cell lines, either at two days of incubation (72.2% for both lines) or at five days ($p > 0.05$).

Discussion

Of the various cell lines studied, Vero and LLC-MK2 displayed the best isolation capacity (qualitative sensitivity). The high efficacy of the Vero cell line has been reported previously and this cell line was used by Germann *et al* in their comparative study of isolation methods.[10] We found no reports concerning the efficacy of the LLC-MK2 cell line in the isolation of the mumps virus, so that we considered this observation to be of interest. LLC-MK2 displayed the same efficacy as the Vero cell line, with both cell lines being significantly higher than the other cell lines studied, including HeLa-Hep-2, which is recommended by some authors.[7–11]

The two cell lines (Vero and LLC-MK2) displayed identical efficacy in the early detection of the mumps virus, with 100% of the samples showing positive in both cell lines at 48 hours of incubation. Thus, the shell vial method provides a notable reduction in the time needed for the isolation of the virus compared with that required by the conventional tube culture method. Germann *et al* showed that the shell vial method (Vero line) detects 66% of the viruses at 48 hours of incubation and 95.8% at five days, compared with only 21.4% at five days with the conventional tube culture method.[10] In our study, an incubation period of 48 hours was sufficient. We believe that this was because most of our patients were in the acute phase of the disease and presented with a high viral load in the clinical specimen processed (saliva). It is probable that a five day incubation period would be more efficacious in samples taken in the late phase of the illness.[7 10]

None of the other cell lines was able to isolate the mumps virus at two days of incubation, for which reason their final evaluation could only be carried out at five days. At five days, the MDCK cell line had the isolation efficacy value (77.7%) that was closest to that of the Vero and LLC-MK2 cell lines, although its sensitivity was insufficient to recommend it as an alternative to the Vero cell line.

An analysis of the quantitative isolation capacity shows that at two days of incubation there was no difference between the Vero and LLC-MK2 cell lines. However, LLC-MK2 was slightly superior (97.2% *v* 94.4%; $p > 0.05$) to Vero at five days. In view of the low number of samples studied, it is not possible to establish definitive conclusions regarding the greater growth capacity of the mumps virus in the LLC-MK2 cell line. This possibility should be investigated in larger studies.

In summary, the comparison between the different cell lines in the isolation of the mumps virus from clinical samples has shown that the Vero and LLC-MK2 cell lines are equally efficient, both in sensitivity at two and five days of incubation and in growth capacity for this virus. The use of the shell vial method considerably shortens the time required for the aetiological confirmation of cases of infectious viral parotitis.

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