

Comparison of Madin-Darby Canine Kidney Cells (MDCK) with a Green Monkey Continuous Cell Line (Vero) and Human Lung Embryonated Cells (MRC-5) in the Isolation of Influenza A Virus from Nasopharyngeal Aspirates by Shell Vial Culture

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We report a comparative study of the MDCK, Vero, and MRC-5 cell lines in the isolation of the influenza A (IA) virus. We studied 746 samples in which 63 IA viruses were isolated. The MDCK line displayed 100% sensitivity, the Vero line displayed 71.4%, and the MRC-5 displayed 57.1%. The MDCK line showed a statistically significant difference with respect to the Vero line ($P = 0.001$) and the MRC-5 line ($P = 0.001$). The quantitative sensitivity analysis showed the MDCK line to be superior to the other lines. It seems that the MDCK line is still one of the most recommendable for the isolation of the IA virus from respiratory samples.

The influenza A (IA) virus is the principal cause of the outbreaks of flu. Usually, this infection may be considered as self-limiting in the healthy population. Nevertheless, in very young and in immunodepressed patients it may lead to an increase in morbidity and mortality (5, 11).

The diagnosis of IA is largely clinical. Nevertheless, it is necessary to carry out some form of rapid antigenic diagnosis (2, 7) and the culture of respiratory samples to confirm the etiology of the respiratory disease and to determine the antigenic characteristics of the epidemic strains (2, 7, 12). Although the "gold standard" isolation technique is inoculation in embryonated hens' eggs, the technical difficulties involved and the delay in obtaining results mean that this technique is performed only in reference centers (8, 11).

The observation that the IA virus is able to grow in various cell lines, primary and continuous (4), has simplified its isolation. In addition, the report of Bartholoma and Forbes (1) concerning the efficacy of the shell vial assay in the isolation of the IA virus in clinical samples has led to the routine use of this technique, with results very similar to those obtained with the gold standard technique (10, 13).

The Madin-Darby canine kidney (MDCK) continuous cell line seems to be one of the most useful for the isolation of the IA virus in respiratory samples (9, 11). Two recent studies have reported that other cell lines may be of certain diagnostic utility (3, 6). As a result, we have performed a prospective study of the efficacy of three commercial cell lines in the isolation of the IA virus from nasopharyngeal aspirates of patients with lower respiratory tract infections.

Over a 16-month period (January 1995 to April 1996), we studied all the nasopharyngeal aspirates sent to the virology laboratory for the diagnosis of viral respiratory infections.

Each sample was homogenized with 3 ml of phosphate-buffered saline (pH 7.4). For the shell vial cultures, 200 μ l of the sample was inoculated into MDCK, Vero, and MRC-5 vials (Viracell; Ingelheim Diagnostica, Barcelona, Spain). The

vials were then centrifuged at 700 \times g for 45 min. They were allowed to rest at 36°C for 60 min, and the supernatant was discarded. To each sample, 1 ml of maintenance medium (minimal essential medium with 1% fetal bovine serum with 2 μ g of trypsin per ml) was added. The vials were incubated for 3 days at 36°C and subsequently stained for an indirect immunofluorescence assay with a monoclonal antibody against the IA virus (clone IA-52) (Monofluokit Influenza; Diagnostic Pasteur, Marnes-la-Coquette, France). Two types of positivity were considered: qualitative (presence of cells with specific fluorescence) and quantitative (number of infectious foci [IFs] present in each shell vial).

Statistical analysis was carried out on results of different comparison by performing the Student *t* test on paired data. All *P* values are two tailed and considered significant if less than 0.05.

Over the study period, 746 nasopharyngeal aspirate samples were analyzed, of which 359 (48.2%) were considered positive (isolation of a respiratory virus). In 63 cases, the IA virus was isolated. Only one sample per patient was evaluated.

In 57.1% of the samples, the IA virus was isolated simultaneously in the three cell lines (Table 1). Only the MDCK cell line enabled us to isolate all the IA virus. For this reason, it was used as a reference cell line to which the other cell lines were compared. Statistically significant differences were observed between the MDCK and the Vero lines ($P = 0.001$) and the MDCK and MRC-5 lines ($P = 0.001$), as well as between the Vero and the MRC-5 lines ($P = 0.04$).

With reference to quantitative sensitivity (Table 2), the MDCK line was found to be superior to the other two, based on the number of IFs present in the monolayers. The MDCK line's greatest yield was obtained in the moderate (11 to 25 IFs) and low (1 to 10 IFs) quantification values. In samples with a high viral load (>26 IFs), the three cell lines displayed the same diagnostic yield.

In the comparison carried out by De Oña et al. (3) of the MDCK cell line with the MRC-5 cell line, these authors report that the former permits the isolation of only 63.6% of IA virus, against the 77.3% for the MRC-5 cell line. Nevertheless, these authors used the MDCK line in the form of tube or conventional culture. We demonstrated that centrifugation of the

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TABLE 1. Qualitative results obtained in a comparative study of three commercial cell lines for the isolation of IA virus^a

MDCK	Result for cell line:			No. (%)
	Vero	MRC-5		
+	+	+		36 (57.1)
+	+	-		9 (14.3)
+	-	-		18 (28.6)
63 (100)	45 (71.4)	36 (57.1)		63 (100)

^a The last column shows the number of samples (percent of total) positive for IA virus by all cell lines marked as positive. The bottom row shows the total number of samples positive for IA virus in the respective cell line (sensitivity [percent]).

sample onto an MDCK monolayer increases the sensitivity in the isolation of the IA virus (10, 13). In our study, we preferred to compare the three cell lines by the shell vial technique, which could explain why our data did not coincide with those of these authors.

By shell vial assay, we found the MDCK line to show the highest sensitivity (100%), for the isolation of the IA virus. In no case did we isolate the IA virus only in the MRC-5 line, a phenomenon observed by De Oña et al. (3) in eight cases (36.3%). In our study, whenever the isolate grew in the MRC-5 line, it did so simultaneously in the reference line (MDCK).

It is more difficult to compare our results with those reported by Govorkova et al. (6), as these authors studied only the growth capacity of a determined number of IA viruses in the continuous Vero line in an attempt to use this cell line for the production of vaccines. However, of the nine clinical samples analyzed, they report that seven (77.7%) grew in the MDCK line and six (66.6%) grew in the Vero line. In our study, the Vero line permitted the isolation of 45 IA viruses (71.4%), a percentage not very different from that obtained by these authors.

In analyzing the quantitative sensitivity (Table 2) in each of these cell lines, the differences observed are similar. The MDCK line displays the greatest sensitivity, whatever the viral load, while the Vero and the MRC-5 lines performed similarly

TABLE 2. Quantitative results obtained in a comparative study of three commercial cell lines for the isolation of IA virus

No. of IFs	No. (%) of samples in cell line:		
	MDCK	Vero	MRC-5
1-10	30 (100)	18 (60)	12 (40)
11-25	6 (100)	0	0
>26	27 (100)	27 (100)	24 (89)
Total	63 (100)	45 (71.4)	36 (57.1)

to the MDCK line in samples with a high viral load. In samples with a low viral load, the MDCK line was significantly superior to the other cell lines.

In view of these results, the MDCK cell line has a greater capacity to yield IA virus from nasopharyngeal aspirates. This cell line has been shown to be the most sensitive, from both the qualitative and the quantitative points of view. Its routine use, therefore, seems necessary in order to obtain the maximum diagnostic yield. The other two lines studied may be used to complement the MDCK line, especially the Vero line. However, according to the results obtained, the Vero cell line should not be used as a substitute. Furthermore, we believe that the routine use of the MRC-5 line is not advisable in the isolation of the IA virus, because of the low percentage of isolations from this cell line.

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