Bulletin of the World Health Organization, 56 (6): 991-993 (1978)

The use of a continuous cell line for the isolation of influenza viruses*

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Abstract

Cultures of MDCK cells exposed to trypsin were as efficient as cultures of rhesus monkey kidney cells for detecting influenza virus, both in dilutions of infected allantoic fluids and in nose and throat swabs. We suggest that the MDCK cell/trypsin system provides a satisfactory alternative to monkey kidney cultures for the isolation of influenza viruses from clinical specimens.

The isolation of virus from cases of influenza plays an essential part in the worldwide surveillance of this disease, allowing the detection of antigenic changes and the development of appropriate vaccines at the earliest possible time. Influenza viruses are usually grown from clinical specimens in fertile hens' eggs or in cultures of rhesus monkey kidney cells. Both methods are well-tried and effective, but their expense puts them beyond the resources of many laboratories. Moreover, there is increasing resistance to the use of primate cultures for this purpose because of progressive depletion of the species. An alternative system for the primary isolation of influenza viruses is urgently needed.

The ability of influenza viruses to grow and form plaques in cultures of chick embryo cells can be greatly enhanced by including trypsin in the overlay medium (*1*, *7*). Trypsin acts by cleaving the haemagglutinin polypeptide of progeny virus, converting the predominantly noninfective particles to an infective form and thus allowing the more rapid spread of infection (*3*, *4*). Tobita et al. (*8*) demonstrated a similar effect of trypsin on the growth of influenza A viruses in the continuous canine kidney cell line, MDCK, which Gaush & Smith (*2*) had earlier found to be susceptible to certain strains of influenza virus. They showed that MDCK cells with trypsin could be used for the primary isolation of virus from clinical cases of influenza and that the method was as sensitive as amniotic inoculation of eggs. This system seemed to hold such promise that it is surprising that it has not been more widely exploited. We have, therefore, compared the efficiency of MDCK cultures plus trypsin with that of conventional rhesus monkey kidney cultures for detection of small amounts of influenza virus. Preliminary tests were performed with dilutions of infected allantoic fluids, and later ones with throat and nose swabs taken during the winter of 1976–77 from patients with suspected influenza.

Materials and methods

Viruses. Influenza strains A/England/6/74, A/England/42/72 and A/England/954/73 were used as infected allantoic fluids. The viruses had been passed once or twice in rhesus monkey kidney cultures and up to six times in eggs.

Nose and throat swabs from cases of suspected influenza were kindly supplied by Dr H. Mair at the Public Health Laboratory, Leicester, Dr J. Smith, Public Health Laboratory, Guildford, and Dr D. McSwiggan, Public Health Laboratory, Central Middlesex Hospital. The swabs had been stored either in liquid nitrogen or at −20°C since their original examination several months previously.

Cell cultures. MDCK cells were grown in tubes in medium 199 plus 5% or 10% fetal calf serum. Secondary rhesus monkey kidney (RMK) cultures were grown similarly.

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Virus detection. For tests on infected allantoic fluids, cultures of MDCK cells were washed once with medium 199, the original medium and the washing fluid being removed as efficiently as possible by means of a Pasteur pipette attached to a vacuum line. The cultures were then inoculated in triplicate with 1 ml of serial ten-fold dilutions of virus in medium 199 containing 5 μg/ml of crystalline trypsin (Armour). After incubation for four days at 33–35°C, infected cultures showed partial or complete disintegration of the cell sheet, whereas control cultures showed only some distortion due to the trypsin. The presence of influenza virus was detected most conveniently and sensitively by the addition of 0.25 ml of 0.5% fowl red cells direct to the culture tubes; after standing vertically for about 2 h, infected cultures showed the characteristic pattern of haemagglutination in contrast to the 'button' of red cells seen in uninfected cultures.

For tests on clinical specimens, the method was modified slightly. MDCK cell cultures were washed twice before infection; the medium contained only 1.25 μg/ml of crystalline trypsin (BDH); and 0.1-ml volumes of undiluted throat and nose swab materials were added to medium already in the culture tubes.

In both types of test, RMK cultures were treated in the same way as MDCK cultures, except that trypsin was not included in the medium.

Results

The infectivities of three strains of influenza virus were titrated in RMK cultures and in MDCK cultures both with and without trypsin in the medium (Table 1). In the absence of trypsin, MDCK cultures were relatively resistant to infection. However, when trypsin was included in the medium, the sensitivity of MDCK cultures became equivalent to that of RMK cultures. Numerous tests gave similar results. It was also found that trypsin did not further increase the growth of influenza viruses in RMK cultures.

Table 1. Comparison of influenza virus infectivities in cultures of MDCK cells and rhesus monkey kidney cells

Virus strain	Infectivity end points in:		
	RMK cells	MDCK cells	MDCK cells + trypsin
A/England/6/74	10^{-7}	10^{-3}	10^{-7}
A/England/42/72	10^{-7}	$10^{-3.5}$	10^{-7}
A/England/954/73	$10^{-7.5}$	$10^{-4.5}$	$10^{-7.5}$

Specimens from 118 patients were examined in parallel in MDCK cultures plus trypsin and in RMK cultures (Table 2). The two systems were approximately equal in sensitivity. Most isolates were detected in both types of culture, although virus from some specimens grew preferentially in one or the other.

Table 2. Comparison of MDCK cells with trypsin and rhesus monkey kidney cells for the isolation of influenza virus from clinical specimens

Number of swabs tested	Number of swabs positive in both	Number of swabs negative in both	Number of swabs positive in MDCK only	Number of swabs positive in RMK only
118	35	50	14	19

Discussion

A cheap and easily maintained culture system for isolating influenza viruses from clinical material has long been sought, and many types of primary and continuous cell cultures have been tested for suitability. However, none has reached a sensitivity comparable with that of fertile hen's eggs or rhesus monkey kidney cultures. The use of MDCK cells with the addition of trypsin now seems to offer a satisfactory alternative. Tobita et al. (*8*) found this system to be as effective as eggs and we have found it to be as effective as rhesus monkey kidney cultures for the isolation of H3N2 strains. The use of MDCK cells for isolation of the current H1N1 strains is now under investigation.

Some simple precautions must be observed when carrying out the test. Cultures should be well washed before the addition of maintenance medium containing trypsin; we have found that the presence of as little as 0.1% of fetal calf serum can nullify the effect of 5 μg/ml of trypsin. The optimum concentration of trypsin may need to be determined; it is the maximum that can be tolerated without causing detachment of the monolayer, MDCK cells having the advantage over most cell types of being fairly resistant to this action of trypsin.

Preliminary tests suggest that the MDCK cell system may not be entirely satisfactory for the isola-

tion of other viruses infecting the respiratory tract although several prototype strains and recent isolates of influenza B virus grew well in MDCK cultures even without trypsin, confirming Tobita's earlier results (*6*). The C/Paris/1/67 strain of influenza C virus was also found to grow in MDCK, confirming the results of Nerome & Ishida (*5*). However, the prototype strains of parainfluenza viruses grew to much lower titres than in rhesus monkey kidney cultures and it is doubtful if this system will prove useful for isolating this group of viruses.

It is not recommended that the MDCK system should totally replace embryonated eggs for isolation of influenza viruses since only egg isolates are acceptable as candidate vaccine strains in many countries. However, the use of MDCK cells will enable many laboratories with limited resources to expand their influenza virus isolation capabilities and, thus, to play a greater and more effective part in influenza surveillance.

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