

The use of shell vial fluid to facilitate early detection and serotyping of influenza viruses.

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Abstract

The use of Madin-Darby canine kidney cells (MDCK) in a shell vial system for the rapid detection of influenza viruses has been well established^{1,2}. Generally, at the end of the incubation period, the fluid (medium) inside the shell vial tube is discarded and the coverslip is rinsed with PBS and then subjected to an immunofluorescence (IMF) test, using anti influenza virus antibodies. A positive fluorescence test will indicate the presence of an influenza virus in the specimen. This technique, however, does not determine the subtype of the influenza virus. To determine the influenza virus subtype, one still needs the use of embryonated eggs. In this report we describe the successful use of the fluid in the shell vial tube at the end of the incubation period, to detect and subtype influenza viruses. With this approach, there is no need for the use of embryonated eggs and the final result, including the identity of the influenza virus subtype, is obtainable within 48 hours.

Introduction

Influenza is a major cause of morbidity and mortality in the developed world and is a major cause of school and work absenteeism³. Rapid and reliable laboratory diagnosis of the influenza virus is of the utmost importance in patient management and for containment of the disease. Furthermore, rapid subtyping of the prevalent influenza virus strains is of crucial importance for the annual production of the influenza vaccines and for disease prevention.

The use of embryonated eggs in the laboratory diagnosis of influenza is a sensitive method for virus isolation and for many years was the method of choice for the subtyping of influenza viruses⁴. This procedure however, presents some logistical problems due to the cumbersome and time-consuming nature of the method as well as the non-availability of sufficient embryonated eggs at times. The use of MDCK cells in a shell vial set-up for the rapid detection of influenza viruses has proven to be equally sensitive in a majority of cases⁵. The use of embryonated eggs takes 12-18 days to detect and subtype the isolated influenza virus whereas the MDCK shell vials, usually takes 48 hours to detect the virus⁵.

The aim of this study was to investigate the possibility of using the MDCK shell vial system exclusively for both the identification and subtyping of influenza viruses.

Patients and methods

Patients:

A total of 498 respiratory specimens from patients exhibiting symptoms of influenza were tested for influenza viruses by the embryonated-egg method and the MDCK shell vial method over a period of three years.

Methods:

The procedures for both the embryonated eggs and the MDCK shell vial cultures were performed using techniques previously described^{6,7}, with the following modification for the MDCK shell vial procedure. Instead of discarding the fluid inside the shell vial cultures at the end of the incubation period. (Note that the shell vials were incubated for 48-72 hours. On very rare occasions the shell vials were left for 7 days to obtain a higher titre) the fluid was harvested and used in a haemagglutination (HA) assay to detect and subtype the isolated influenza viruses. The haemagglutination test was performed by adding 25µl of MDCK harvest to a v-shaped microtitre plate (Sero-Wel, Bibby Sterilin Ltd) already containing 25µl normal saline. Thereafter, 50µl of a 0.5% dilution of turkey red blood cells were added and the plate contents were mixed by gentle agitation and then left at room temperature for 45 minutes. A fuzzy appearance in any of the wells will indicate a positive HA test, which is indicative of the presence of an influenza virus. The HA-positive shell vial fluids were then titrated in a haemagglutination inhibition test (HAI) which is the procedure usually performed for the subtyping of the influenza viruses⁸. The HAI was performed using the World Health Organization (WHO) influenza reagent kit (Reagents for Influenza virus diagnosis prepared and distributed by WHO Collaborating Center for Research on Influenza, Melbourne Australia). The shell vial coverslip was

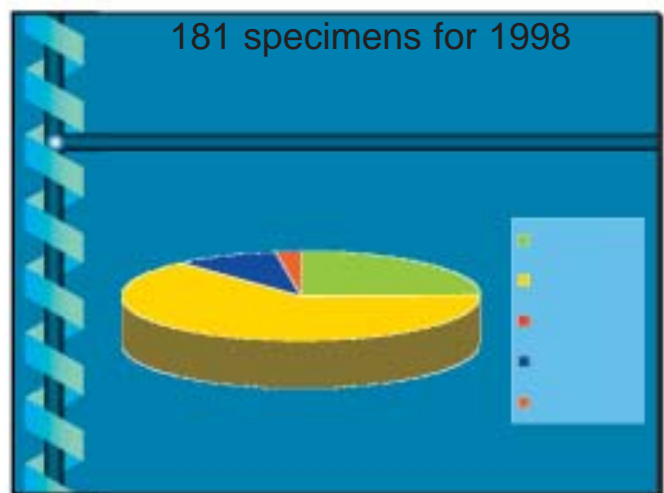
treated according to procedures previously described for the performance of the IMF test⁷ and a direct IMF test was performed on the coverslips for influenzavirus A and B using the SimulFluor direct monoclonal antibody kit (Chemicon). The HA and HAI tests were also performed on the egg harvests at the end of the incubation period for the eggs.

Results

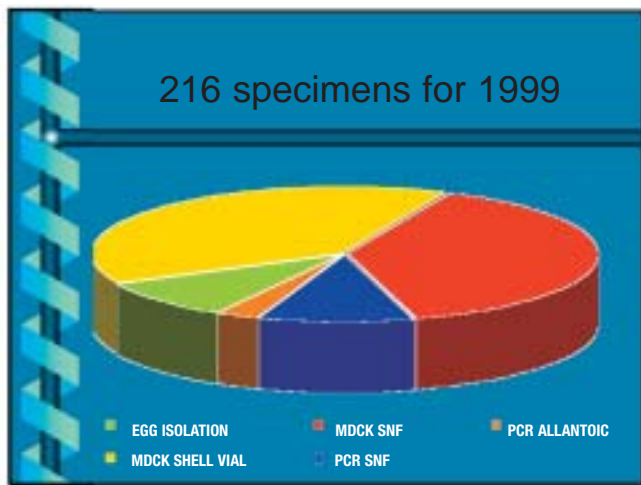
181 specimens were positive for influenza in 1998. From these positives we were able to obtain subtypes from 59 of them by means of the egg isolation method. Even though at this stage we did not know that we could do HAI on the (shell vial fluid) SVF it must be noted that the other 151 positives obtained were typings done by means of the shell vial technique and read by and immunofluorescence microscope. In this year however we sent 28 specimens for molecular studies using PCR. 23 of these samples were SVF and all showed very good bands on the gel. This is what made us consider doing HA on the SVF for the following year. We found that the positive result on the HA compared very well with the IFA result and when doing a titration we were able to obtain titers suitable for HAI. The titers on the initial titration ranged from 1:10 to 1:160. The results obtained on the HAI during subtyping extended as far as ≥ 2560 , concluding thus that the lower initial titer did not interfere with the accuracy of the subtyping at all. Thus in 1999 from the 216 positive specimens obtained we were able to do subtyping on all the positive IFA specimens. We then started sending more SVF for PCR as they were happy with the results they were receiving and were no longer dependant on allantoic positives for their molecular studies. For example in 2000 from the 101 positive specimens obtained 65 SVF were selected for PCR and only 1 allantoic.

All in all 153 randomly selected samples were sent for PCR testing and both the egg grown isolates and the tissue culture derived samples were comparable.

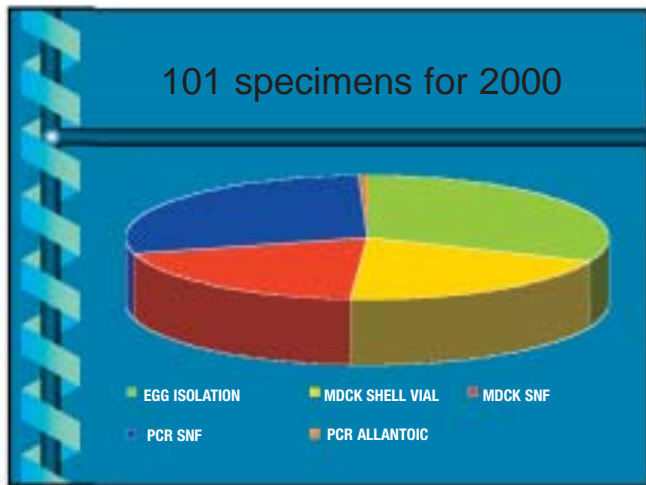
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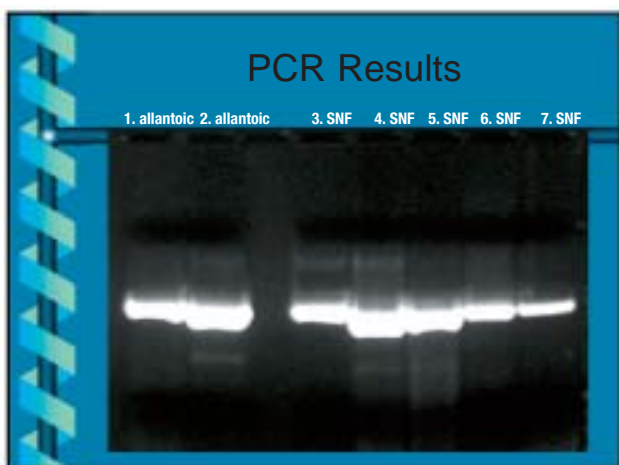
59 egg green, 151 types by shell vial yellow, 0 HAI by SNF of MDCK red, 23 PCR with SNF blue, 5 PCR with allantoic orange



44 subtypes by eggs **green**, 181 types by shell vial **yellow**, 181 subtypes by HAI **red**, 46 PCR of SNF **blue**, 13 PCR from allantoic **orange**



71 subtypes by eggs **green**, 45 types by shell vial **yellow**, 45 subtypes by HAI of SNF **red**, 65 PCR from SNF **blue**, 1 PCR from allantoic **orange**



The bands shown here portray comparable results for both egg grown cultures and tissue culture derived samples.

The subtypes identified in 1998 were :

1. A/Sydney/5/98 H3N2
2. A JHB/82/96 H1N1
3. B/Harbin/7/94 (B/Beijing/184/93-like)

The subtypes identified in 1999 were:

1. A/Sydney/5/97 H3N2
2. B/Harbin/7/94-like

The subtypes identified in 2000 were:

1. A/Sydney/5/97 H3N2
2. A/Beijing/262/95 H1N1
3. A/new Caledonia/20/99 H1N1

No influenza B virus was identified in 2000

Discussion and Conclusions:

The conventional use of the shell vial MDCK culture for influenza virus diagnosis enables laboratories to obtain a result in two days. This result, however, is incomplete because it is only an indication of the presence of an influenza virus in the sample and not an identification of the subtype. For a complete result, including the influenza virus subtype, one had to wait 12-18 days for the egg harvests, or use the more costly polymerase chain reaction (PCR) to identify the subtype⁹. The results of this study clearly indicate that successful subtyping of influenza viruses can be achieved by using the SVF. With this approach, we were able to confirm and subtype all isolated influenza virus in 48 hours as compared to the 12-18 days usually taken by the embryonated egg culture method. The IMF test on the shell vial coverslips can then be performed as an additional confirmation of the HA test obtained on the SVF.

This study has highlighted the fact that the SVF can be used successfully for both the identification and subtyping of influenza viruses in just 48 hours, which will be beneficial to both the laboratory and the patient. This approach also reduces the need for the routine use of embryonated eggs for diagnostic purposes to an absolute minimum.

Acknowledgements

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References:

1. Reina J, Fernandez-Baca V, Blanco I, Munar M. 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. *J Clin Microbiol* 1997; **35**: 1900-1901.
2. Shih SR, Tsao KC, Ning HC, Huang YC, Lin TY. Diagnosis of respiratory tract viruses in 24h by immunofluorescence staining of shell vial cultures containing Madin-Darby canine kidney (MDCK) cells. *J Virol Methods* 1999; **81** (1-2):77-81.
3. Besselaar T, Schoub B. *Influenza Science in Africa*. Online magazine, June 2002.
4. FJ Fenner, DO White, Cultivation and Assay In: Davis BD, Dulbecco R, Eisen HN, Ginsberg HS, and Wood WB, Microbiology. Harper, New York, *Med Virol* 1976, **2**: 35-36.
5. Klenk HD, Rott T, Orlinich M, Blordom J. Activation of influenza A virus by trypsin treatment. *Virology* 1975; **68**: 426-439.
6. J Versteeg, Chick Embryo Techniques, *A colour atlas of Virology*, 1985; 38-53.
7. Ray CG, Minnich LL. Efficiency of immunofluorescence for rapid detection of Common respiratory viruses. *J Clinical Microbiol.* 1987; **27**: 355-357.
8. WHO collaboration center for the surveillance, epidemiology and control of influenza 2001-2002. *WHO Influenza Reagent Kit insert*.
9. Wright, K.E., Wilson, G.A.R., Novosad, D., Dimock, C., Tan, D., Weber, J.m., 1995. Typing and subtyping of influenza viruses in clinical samples by PCR. *J. Clin. Microbiol.* **33**, 1180-1184