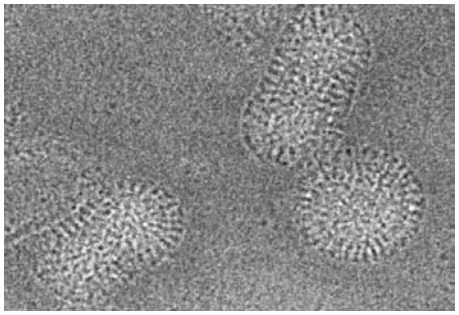

Influenza A Virus

COMPARISON OF VIROCULT® SWAB, Σ-SWAB® AND Σ-VIROCULT® FOR INFLUENZA A VIABILITY FOR CELL CULTURE AND MOLECULAR DETECTION

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ABSTRACT

Influenza surveillance in the general population relies on clinical samples performed by general practitioners or paediatricians from the GROG network using Virocult® swab. These clinical samples are simply sent to the laboratory by post. This shipment may last for up to 4 days. Even if the virological diagnosis is based on RT-PCR, cell culture remains essential for virus isolation and complete characterization. Moreover, the surveillance and cell culture were of particular importance in the early stages of the recent novel A H1N1 pandemic. This isolation may be impaired when the delay between collection and culture is too long.

In the present study, we compared the respective performance of Virocult® swab, the Σ-swab™ and the Σ-Virocult®. The A/Uruguay/716/2007 (H3N2) prototype strain was selected for this study. The virus was grown on MDCK cells to prepare a fresh viral stock. The virus was seeded at different virus concentrations on the 3 systems. The spiked transport devices were subsequently stored for 1 to 4 days at ambient temperature and at +4°C before being inoculated onto MDCK cells. Each sample was then processed as a GROG specimen either by RT-PCR, antigen detection using an ELISA technique and virus isolation.

The results showed that viral detection and virus growth were possible with the three transport devices for up to 4 days. However, the Virocult® swab and Σ-Virocult® devices spiked with low levels of virus appeared to be better to preserve infectivity of the influenza A virus.

INTRODUCTION

Influenza epidemics occur almost every winter in temperate countries. The epidemic intensity varies widely according to the virus but is associated with excess morbidity and mortality. Laboratory diagnosis is an important tool for the control of the outbreak and the characterization of the circulating viruses. In France, the influenza surveillance in the general population relies on the GROG network. The general practitioners or paediatricians perform clinical specimens on patients presenting with acute respiratory infection. The sampling consists of nasal or nasopharyngeal swab collected within 36 hours of the onset of the disease. Clinical samples collected during outpatient consultation are sent by post. To optimize the recovery of the specimen, doctors used Virocult® transport medium.

The objectives of virological diagnosis are the early identification of the circulating viruses, the detection of new variants or emerging viruses and assessment of the epidemic target population, and intensity.

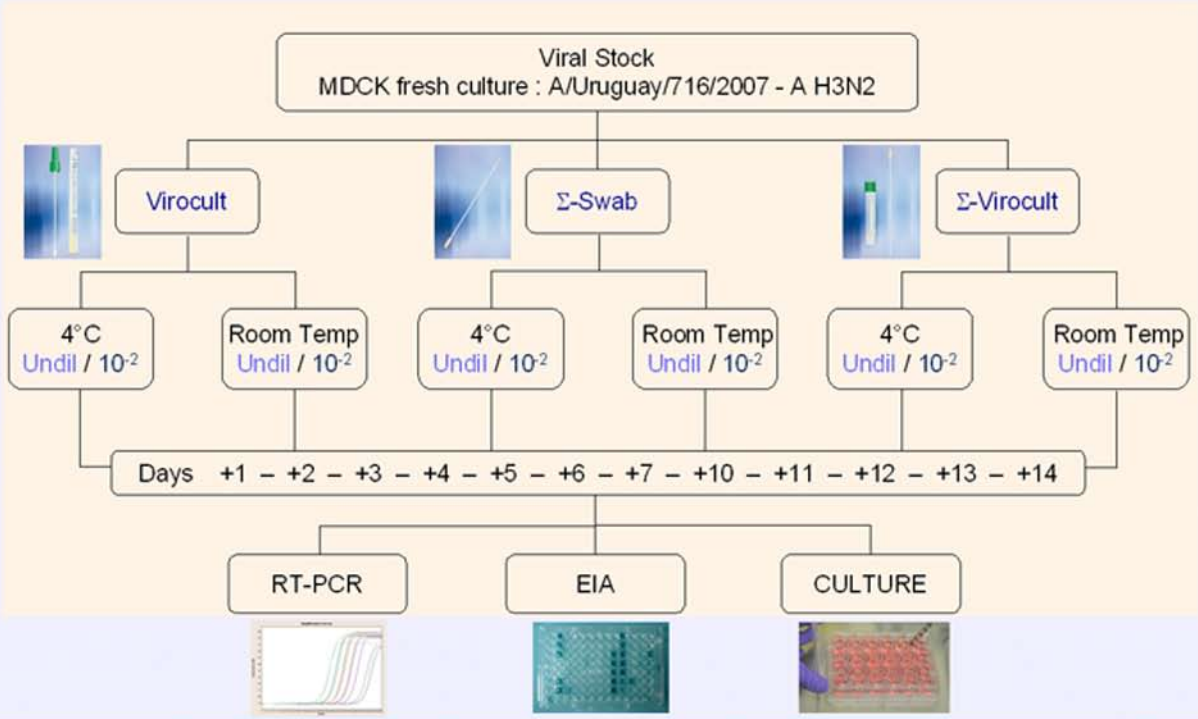
In practice, each clinical sample is tested for the detection of influenza virus antigens either directly in clinical specimens by ELISA (EIA) and for the detection of viral RNA by real time RT-PCR. The National Influenza Centre performs cell culture on MDCK of all clinical specimens for the isolation and complete characterization of the virus. The molecular technique is rapid, specific and sensitive but the cell culture isolation of live virus for full identification of the virus remains the reference technique.

The present study was designed to compare the survival of influenza A/Uruguay/716/2007 on 3 transport devices according to the delay and temperature storage.

The selected influenza strain was an A H3N2 prototype obtained from Dr A. Hay, World Influenza Centre, London. The virus was cultivated on MDCK cells and seeded on the swabs. The experiment mimiced the clinical specimen for viral concentration and storage temperature during transport to the laboratory.

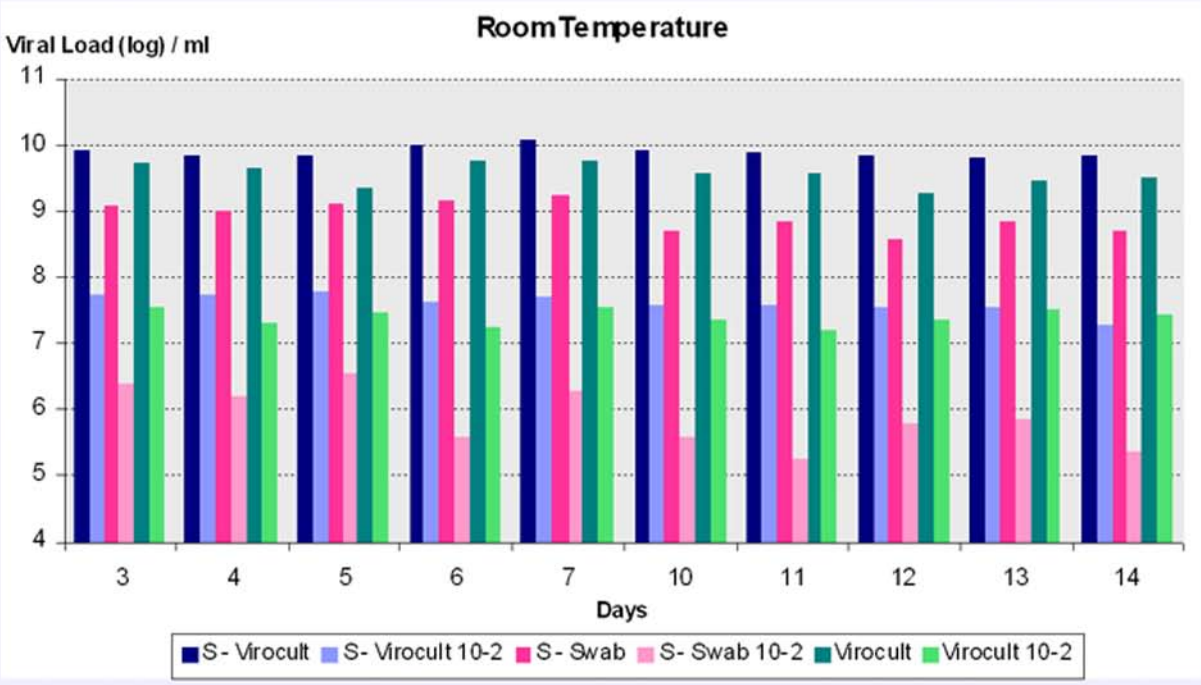
The design protocol is described below.

METHOD



RESULTS

Viral RNA Detection by RT-PCR



The A H3N2 A/Uruguay/716/2007 MDCK cell culture harvested after 3 days culture constituted the seed stock. According to the protocol, all the transport devices were seeded with 100µl of the viral culture either undiluted or 10^{-2} dilution and held at ambient temperature or at 4°C. After the dedicated holding period the experimental sample was tested as for clinical samples from GROG surveillance by adding 2 ml Eagle MEM for Virocult® and Σ-Swab™ but 1 ml for Σ-Virocult®. The samples were tested for viral load by RT-PCR and by EIA for rapid antigen detection, then inoculated onto MDCK cell. Viral growth was monitored after 3-5 days incubation at 33°C for CPE and confirmed by EIA on the supernatant.

Antigen Detection by EIA directly in Samples & after Propagation in Cell Culture for swabs held at Room Temperature

	Σ - Virocult				Σ - Swab				Virocult			
Holding period	Undiluted		10^{-2}		Undiluted		10^{-2}		Undiluted		10^{-2}	
(days)	P0	P1	P0	P1	P0	P1	P0	P1	P0	P1	P0	P1
3	+	+	-	+	+/-	+	-	+/-	+	+	-	+/-
4	+	+	-	+	+/-	+	-	-	+	+	-	-
5	+	+	-	+	+/-	+	-	-	+	+	-	-
6	+	+	-	+	+/-	+	-	-	+	+/-	-	-
7	+	+	-	+	+/-	+	-	-	+	+/-	-	-
10	+	+	-	-	-	+	-	-	+	+	-	-
11	+	+	-	-	-	-	-	-	+	+/-	-	-
12	+	+	-	-	-	-	-	-	+	+/-	-	-
13	+	+	-	-	-	-	-	-	+	+/-	-	-
14	+	+	-	-	-	-	-	-	+	+/-	-	-

P0 - Samples tested immediately after holding period by EIA

P1 - Detection of CPE (confirmed by EIA of supernatant) after 3-5 days culture on MDCK cells

CONCLUSIONS

The Virocult® swab has been used for many years in France for the sampling of influenza surveillance in the general population. The transport of the clinical specimens at ambient temperature usually last for 4 days but eventually longer. The device was effective for the viral diagnosis either for molecular technique, direct antigen detection by EIA and cell culture.

The comparison of 2 devices versus Virocult® showed the same performance at 4°C storage with a high viral load. The device systems' efficiencies differ for virus storage at ambient temperature. Utilizing RT-PCR we found similar results for Virocult® and Σ-Virocult® but the viability of the virus on cell culture was maintained longer with Σ-Virocult®. The shorter survival and detectability with the dry polyurethane foam swab could be associated with the absence of transport medium. These results prove that Σ-Virocult® is reliable and improves the efficiency of influenza virus A H3N2 viability when held at ambient temperature compared to Virocult®. The dry Σ-Swab™ device can also be useful as the others if it can be processed within 2 days (for example for specimens taken in clinics within the same town as the laboratory). Σ-Virocult® should be suitable for the diagnosis of new Influenza viruses and other respiratory viruses for differential diagnosis of acute respiratory infections.

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