

INVESTIGATION OF THE SUITABILITY OF THE VIROCULT® SWAB TRANSPORT DEVICE FOR INFLUENZA A SPECIMENS WHICH ARE TO BE ANALYZED BY CELL CULTURE OR MOLECULAR TECHNIQUES



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ABSTRACT

Introduction: In recent years many clinical microbiology laboratories have discontinued the use of cell culture for the identification of viruses, relying instead on the more rapid molecular techniques now widely available. Nevertheless it is often necessary to submit specimens to reference laboratories for further identification or confirmation using cell culture. The present study is intended to investigate whether Virocult® swabs could be used as a single source for both types of testing, thus avoiding the need for multiple specimens and collection devices.

Methods: A new cell culture based study was performed for Influenza A virus, using the methods described in CLSI Quality Control of Microbiological Transport Devices Approved Standard M40-A, measuring recovery for up to 8 days holding time on Virocult® swabs, both at ambient and refrigeration temperatures. The results were compared with clinical studies using molecular techniques for the identification of Influenza A on Virocult®-collected specimens.

Results: It was shown that Virocult®-collected specimens recovered Influenza A virus for at least 8 days, both at ambient and refrigeration temperatures, and in addition worked acceptably with each of the molecular techniques assessed. From this study, it is shown that for Influenza A, an important respiratory pathogen, the Virocult®-collected specimens could be used for both cell culture and molecular testing.

INTRODUCTION

Influenza is an acute upper respiratory tract infection, normally associated with the winter months in temperate climates. Although normally self-limiting in otherwise healthy adults, there can be a high mortality and morbidity for vulnerable groups including the elderly and the very young. There is also a considerable economic burden in terms of loss of productivity due to absence in business, and the considerable cost of hospitalisation.

Traditional methods for detecting influenza virus include cell culture, complement fixation, and haemagglutinin inhibition. Such methods, however, are slow and often of little value in determining treatment for the patient. They are of more relevance in providing epidemiological data for monitoring the spread of particular strains. The more recent development of rapid methods of influenza detection such as Reverse Transcriptase PCR (RT-PCR), and Direct Antigen Immunofluorescence allows more rapid detection and identification of the infecting virus, providing the strain is already known, and its characteristics are already stored within the test's database.

Although cell culture is being or has been phased out in many laboratories, there is still a need in reference laboratories for culture as the gold standard final identification and confirmation step, and for the isolation and characterisation of new strains. This is particularly important for influenza virus which is inherently variable due to antigenic drift, the result of the high frequency of point mutations within certain

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genes, and the more drastic antigenic shift when genetic re-assortment occurs between different co-infecting subtypes.

For many years Virocult® swabs have been used for the collection of respiratory specimens for investigation by cell culture. The device include a transport medium which will keep many species of virus viable for many days. One of the advantages of the Virocult® device is that it can be transported at ambient temperatures. In a recent survey of methods in 20 European countries, it was reported that in 16 countries viral specimens were submitted under ambient conditions, including 13 where submission was by post. Specimens were taking between 24 and 48 hours in transit.

For many of the new molecular techniques, it is not required to keep the viruses alive, but the tests involve antibodies or enzymes which could be sensitive to interference from components of a transport medium. In most situations it would be preferable to have a transport device that would be compatible with both methods, allowing the specimen to be initially tested by a rapid method, with the result providing the basis for any recommended treatment of the patient. Subsequently the device could be forwarded to a reference laboratory for isolation of virus by culture and further characterisation.

The present study was designed to assess the suitability of Virocult® for the transport of Influenza Type A specimens for culture, using the CLSI M40-A standard for transport devices. In addition, a survey was made of literature references over the previous 15 years comparing overall identification rates for Influenza Type A, and Influenza Types A and B combined, for all methods, with those obtained when Virocult® was used as the main collection device.

METHODS - Cell Culture

Influenza A Strain 3524/08 (Clinical Isolate) Strain 3524/08 (H1N1) 250µl into 4 tubes PLC cells

Incubate @32-34°C

Observe daily until Cytopathic effect observed (5 days)

Virus identity confirmed as Influenza A (Light Diagnostics Influenza Reagent 5017 Simofluor Reagent 5296)



Virus diluted $10^0 - 10^6$

(0.3ml Virus suspension + 2.7ml EMEM (Biowhittaker BE12-136F))



4 Virocult swabs immersed in each dilution for 10 seconds, then immediately placed in Virocult medium



Then held as follows:

A 3 days @ 2-8°C

B 8 days @ 2-8°C

C 3 days @ RT (19-21°C)

D 8 days @ RT (19-21°C)



After holding time

Add 4ml EMEM to Virocult tube.

Vortex 10 seconds



100µl from tube into cell culture (PLC cells maintained in 1ml EMEM + 1% Foetal Calf Serum) (x2)



Incubate @ 32-34°C

Observe daily for appearance of CPE

RESULTS

Limit of detection (lowest starting concentration / earliest full CPE)

A 3 days @ 2-8°C	10^{-3} @ 7 days confirmed by immunofluorescence
B 8 days @ 2-8°C	10^{-2} @ 7 days confirmed by immunofluorescence
C 3 days @ RT(19-21°C)	10^{-3} @ 11 day confirmed by immunofluorescence
D 8 days @ RT(19-21°C)	10^0 @ 8 days confirmed by immunofluorescence

METHODS- Molecular

An analysis was made of reports published since 1995 where non-culture methods were used to detect influenza virus in specimens from patients presenting with respiratory symptoms. Some of the studies were epidemiological, with large numbers of patients, with the objective of monitoring the spread of influenza, including particular serotypes. Others were studies devised to assess the performance of various tests. In some of the studies Virocult[®] swabs were used as the collection device, while in the others the devices were throat swabs, nasopharyngeal swabs or nasopharyngeal aspirates.

The intention of this study is to demonstrate whether the overall proportion of positive specimens of influenza type A, or influenza types A & B, observed when using molecular techniques was similar or significantly different between specimens collected using Virocult[®], specimens collected using other devices, and for all specimens.

The results of all the studies were collated, adding together the total numbers of valid specimens, and the total number of specimens shown to be positive for influenza virus by the methods being used or assessed. Overall 10812 specimens were examined, including 4310 specimens collected and transported using the Virocult[®] device, Virocult[®] transported specimens, and 6647 specimens where other devices were used for collection.

RESULTS

Table 1 Influenza Types A & B

Method of collection	Number of specimens	Number of specimens positive for Influenza virus	Detection rate for influenza
All methods	10904	2935	26.9%
All methods excluding Virocult	6647	1822	27.4%
All methods using Virocult [®] as collection device	4310	1146	26.6%

Table 2 Influenza Type A

Method of collection	Number of specimens	Number of specimens positive for Influenza virus	Detection rate for influenza
All methods	10029	2163	21.6%

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All methods excluding Virocult	5852	1256	21.5%
All methods using Virocult® as collection device	4177	907	21.7%

CONCLUSIONS

Cell culture technique

Live virus was detected by the appearance of cytopathic effect, confirmed by immunofluorescence, in the cell layer inoculated from Virocult® swabs after holding periods of 3 days and 8 days at ambient temperatures or refrigeration temperatures. This exceeded the requirements of Standard M40-A.

Molecular techniques

There was a remarkable convergence of the overall detection rates for influenza Type A, and for Types A & B, from diverse populations of respiratory patients, with almost identical rates being demonstrated whether samples were obtained by Virocult® swabs, or by other methods, or by all methods.

While further statistical analysis may be required to assess the true significance of the convergence of the molecular results, it does seem evident that the Virocult® swab is a reliable specimen collection device for influenza Type A virus, whether investigation is by traditional culture methods, or by the newer rapid molecular techniques.

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