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Using A Swab Based System (Sigma Virocult®) As An Alternative To Aspirates For The Recovery Of Human Respiratory Syncytial Virus

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Abstract

In this study the capability of a new swab based specimen transport system for the recovery of Human Respiratory Syncytial Virus (hRSV) was assessed.

Sigma Virocult® from MWE is a novel swab device in which wound linear fibre is replaced by a polyurethane cellular foam bud. The transport medium is in a purely liquid form, and the format may be more appropriate for small virus particles such as hRSV. Claims for particular devices can now be assessed using Clinical Laboratory Standards Institute's Standard M40-A (1). For acceptable performance with hRSV, recovery of live virus has to be demonstrated for up to 96 hours for specimens held at either ambient or refrigerator temperatures.

Virus stock was hRSV ATCC VR-1540 grown on MRC-5 cells For each swab to be tested 88 µl ($10^{4.7}TCID_{50}$) was absorbed using the swab supplied with the Sigma Virocult. The swab was placed in the tube of liquid virus transport medium. The tube was held at 4°C or at room temperature (20°C to 25°C) for 0 hours or 96 hours. After the holding period each tube was titrated using MRC-5 cells. Cells were examined daily for the presence of cytopathic effect (CPE).
All Time zero swabs showed over 75% CPE by 6 days, while the 96 hours swabs all showed over 75% CPE after 10 days, for both temperatures.
The study has shown that Sigma Virocult transport swabs will recover hRSV at clinically significant concentrations.

1. CLSI: Quality Control of Microbiological Transport Systems; Approved Standard 2003

Topics: 1. Respiratory virus infections, 2. Viral diagnosis (innovative methods, quality control)

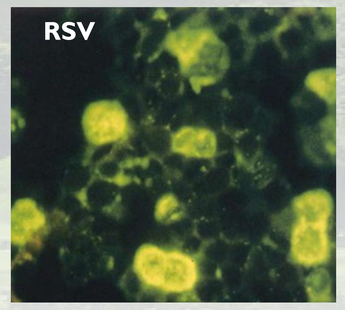
Introduction

Human Respiratory syncytial virus (hRSV) is a leading cause of viral lower respiratory tract illness in infants and children, as well as the elderly. Global hRSV disease is estimated at 64 million cases annually, with 160,000 deaths (WHO, Acute Respiratory Infections (Update September 2009)).

hRSV is a negative sense RNA virus, and belongs to the genus *Pneumovirus*. Although diagnosis is generally based on symptoms, it is important to collect some specimens for confirmation and epidemiology. Because of the small size of the virus particle, swabs have not been regarded as a suitable collection device, with more reliance placed on nasal aspirates. However most of the patients are infants or small children, and the process of collecting aspirates is unpleasant and distressing.

Recently some novel swab devices have become available, with buds made from cellular foam, or flocked polyester fibres, rather than the previously conventional spun fibre such as rayon or cotton. An example is Sigma Virocult® from MWE. This uses a cellular polyurethane foam bud, in combination with Virocult® medium which had previously been shown to be suitable for the recovery of viable hRSV.

The present study was designed to demonstrate whether or not Sigma Virocult® could reliably recover and maintain hRSV for at least 96 hours (as required by CLSI's standard M40-A), and allow them to be released and assayed in cell culture.



Methods

The basis of the study was M40-A, CLSI's standard for microbiology transport devices, which requires virus transport devices for hRSV to be able to recover and maintain a defined titre of infective virus for 96 hours at either room temperature or refrigerated temperatures.

1. A stock was prepared of HRSV (ATCC VR-1540) in MRC-5 cells, with a titre of $10^{4.5}TCID_{50}$ per 50µl. The required titre for inoculation is $10^{4.7}TCID_{50}$, so an inoculum of 88µl was used.
2. 88µl of virus suspension was added to the required number of wells in a sterile tissue culture plate. 3 swabs were used for each incubation condition.
3. Swabs were inoculated by inserting the tip into the well and moving around until all of the virus suspension had been absorbed. The swab was then placed in its tube of transport medium, the shaft snapped at its breakpoint, and the cap screwed onto the tube.
4. Each tube was gently shaken, then incubated at either 4°C, or room temperature (20-25°C) for 96 hours.
5. A reference Time Zero swab was also inoculated in the same way, but 250 µl was removed from the tube for titration.
6. After 96 hours the transport medium from each tube was titrated in cultures of MRC-5 cells. Control virus and Time zero samples were also titrated in this way. 4 replicates from each swab were examined.
7. Cells were examined for the presence of cytopathic effect (CPE) on each day following inoculation for a maximum of 2 weeks.
8. The degree of CPE was recorded as follows

No CPE	-
<25% CPE	+
26-50% CPE	++
51-75% CPE	+++
>75% CPE	++++

Results

Holding Time	Holding Temperature	Swab	CPE			
			Replicate			
			1	2	3	4
0 hrs		1	++++	++++	++++	++++
96 hrs	Room Temperature	1	++++	++++	++++	++++
96 hrs	Room Temperature	2	++++	++++	++++	++++
96 hrs	Room Temperature	3	++++	++++	++++	++++
96 hrs	4°C	1	++++	++++	++++	++++
96 hrs	4°C	2	++++	++++	++++	++++
96 hrs	4°C	3	++++	++++	++++	++++

Conclusion

Sigma Virocult® showed acceptable recovery of hRSV at both holding temperatures.

Discussion

Although nasopharyngeal aspirates (NPA) are considered the best type of specimen for the detection of Human Respiratory Syncytial Virus (hRSV) it is often impractical. Specimens may have to be collected in the community without access to vacuum required for NPA. The procedure for collecting NPA can be painful and distressing for the patient, often an infant. A nasal swab is more acceptable to the patient, but have often been considered unsatisfactory because of lower yields of detectable virus. It is thought that the very small virus particles are more likely to be entrapped in the fibre matrix of the swab bud.

In recent years new materials have been used in the manufacture of swab buds. These include flocked polyester fibres such as the multifilament polyester fibres in PurFlock® and HydraFlock® swabs from MWE, and the open cell polyurethane foam used with MWE Sigma Swabs®.
In the present laboratory study, the ability of Sigma Virocult® to recover hRSV was investigated. Sigma Virocult® combines a novel swab (Sigma Swab® with plastic shaft and soft polyurethane foam bud) with Virocult®, a long established virus transport medium from MWE. The soft foam bud is particularly suited for infant patients, and the cellular structure allows free movement and optimum release of the small hRSV virus particles.
In the study Sigma Virocult® was shown to give excellent recovery of hRSV in swabs held for up to 4 days at either room temperature, or refrigerated temperature, and so would be capable of maintaining the viability and infectivity of hRSV at the limits of the feasible transport periods.
The study was performed in accordance with CLSI M40-A[4].

References

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4. CLSI M40-A: Quality Control of Microbiological Transport Systems; Approved Standard 2003