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EVALUATION OF A VIROLOGY SPECIMEN TRANSPORT DEVICE WITH SIX VIRUSES USING CLSI STANDARD M40-A

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Abstract

Although PCR is becoming established as the method of choice for the diagnosis of viral infections, to recover live virus for full characterisation and future reference still requires the traditional methods of cell culture. With cell culture facilities being increasingly restricted to a few reference laboratories, it is even more essential that transport devices for viral specimens are reliable for transportation over longer distances.
CLSI's 'Quality Control of Microbiological Transport Systems'; Approved Standard M40-A[1] provides a method for determining whether particular devices are suitable for the transport of viral specimens over extended distances.
In the present study Virocult® swabs (Medical Wire) were evaluated in accordance with M40-A. The panel of viruses was increased to include Adenovirus 1, Influenza A, Parainfluenza 3, Rhinovirus, and Herpes Simplex Types 1 and 2. Survival was studied after holding times of up to 7 days, and at holding temperatures of 4°C (refrigeration) and 22°C (representing ambient temperatures).
Virus strains were all obtained from ATCC,with the exception of influenza A for which a clinical isolate strain was used. Titres were established so that an inoculum close to 5 x 10^4 TCID50 could be used. Appropriate cell lines were used for each virus, for initial growth, and for the detection of live virus following simulated transport. In one case (Adenovirus 1) live virus following transport was detected using fluorescent antigen.
Virocult ® was shown to successfully recover all 6 viruses after holding for up to 7 days, and whether held at 4°C or ambient temperature.

Introduction

The current emergency with a pandemic strain of swine flu[2,3] has highlighted the continuing need for virus culture facilities to be available for the typing, full characterisation and monitoring of previously unknown strains. While PCR and other rapid methods provide convenient and highly effective tools for the diagnosis and monitoring of previously known strains, they are as yet unable to precisely identify new strains which may share those highly conserved target DNA or RNA sequences with their less virulent antecedents, have novel features which allow them to be agressively infectious.

Although molecular techniques such as PCR have become established as the methods of choice for the diagnosis of viral infections, to recover live virus for full characterisation and future reference still requires the traditional methods of cell culture[4]. With cell culture facilities being increasingly restricted to a few reference laboratories, it is even more essential that transport devices for viral specimens are reliable for transportation over longer distances.

CLSI's 'Quality Control of Microbiological Transport Systems'; Approved Standard M40-A provides a method for determining whether particular devices are suitable for the transport of viral specimens over extended distances. In the present study Virocult ® swabs (Medical Wire) were evaluated in accordance with M40-A. The panel of viruses was increased to include Adenovirus 1, Influenza A, Parainfluenza 3, Rhinovirus, and Herpes Simplex Types 1 and 2, to reflect the typical range of organisms expected to be present in submitted specimens. Survival was studied after holding times of up to 7 days, and at holding temperatures of 4°C (refrigeration) and 22°C (representing ambient temperatures).M40-A only requires survival for up to 96 hours, but the test period was extended to reflect situations where specimens might require to be forwarded to a reference facility after initial rapid testing. Virus strains were all obtained from ATCC,with the exception of influenza A for which a clinical isolate strain was used. Titres were established so that an inoculum close to 5 x 10^4 $TCID_{50}$ could be used. Appropriate cell lines were used for each virus, for initial growth, and for the detection of live virus following simulated transport. In one case (Adenovirus 1) live virus following transport was detected using fluorescent antigen.

References
1.NCCLS, 2003, Quality Control of Microbiological Transport Systems: Approved Standard NCCLS Document M40-A
2.WHO - Public Health Emergency of International Concern
Statement by WHO director-general, Dr Margaret Chan [25 Apr 2009] Swine influenza
http://www.who.int/mediacentre/news/statements/2009/h1n1_20090425/en/index.html
3.CDC Health Advisory Distributed via Health Alert Network April 25, 2009, 3:00 EST (03:00 PM EDT) CDCHAN-000281-2009-04-25-ALT-N Investigation and Interim Recommendations: Swine Influenza (H1N1)
4.Rudsdale, A.. & D. Shedden, 20th April, 2009, 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, *POSTER M42 , Clinical Virology Symposium 2009, Daytona Beach*

Virocult swabs, and ATCC virus cultures for this study were provided by Medical Wire

www.mwe.co.uk
www.mwe-usa.com

Methods

Freeze dried virus reconstituted with H_2O

▼

Confirm identity of virus and titrate (see panel below)

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Dilute virus 10^0 – 10^{-6} in Eagles MEM (Biowhittaker)
(0.3ml inoculum +2.7ml Eagles MEM)

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Immerse Virocult swabs in each dilution
(4 swabs per dilution) for 10 seconds

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Insert each swab into its own Virocult tube with medium

▼

Squeeze tubes

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Incubate at 2-8°C or at Room Temperature
for holding period (see table)

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After holding period add 4ml liquid medium (Eagles MEM) to tube, vortex for 10 seconds.

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100µl of medium inoculated onto cell culture (X2)

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Cell culture monitored daily for appearance of CPE

▼

The results shown are for an inoculum of 3 x 10^4 $TCID_{50}$

Identification and Titration of HSV-2

Freeze dried virus reconstituted with H_2O

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Confirmation of identity of stock virus
Reconstituted virus 200µl into 3 tubes of MRC-5 cells 3 days old in EMEM + 1% foetal bovine serum)
Reconstituted virus 100µl into 1 tube of MRC-5 cells 3 days old in EMEM + 1% foetal bovine serum)

▼

Incubated stationary at 37°C for 1 day.
Examined by light microscopy: tttt Cytopathic Effect in all 4 tubes
Stained with Chemicon IF stain HSV 2 or IF stain HSV 1

▼

Examined by IF
HSV-2 tttt
HSV-1 -

▼

Serial dilutions (100µl inoculum to 1ml with diluent)10^0 – 10^{-6} in EMEM (Biowhittaker) + 1% foetal bovine serum (x2)
Incubate stationary at 37C

Observe for CPE

Incubation period	24 hours		48 hours		72 hours	
Dilution	Tube 1	Tube 2	Tube 1	Tube 2	Tube 1	Tube 2
10^0	+++	+++	++++	++++	++++	++++
10-1	+	+	++++	++++	++++	++++
10-2	-	+	+++	+++	++++	++++
10-3	-	-	++	++	++++	++++
10-4	-	-	+	+	+++	+++
10-5	-	-	+	-	++	++
10-6	-	-	-	-	-	+
Cell Control	-	-	-	-	-	-

$TCID_{50}$ = 10^5/100µl @ 48hours

These procedures were carried out for each virus
using appropriate cell lines, IF stains, etc

Results

VIRUS	Holding Time (days)	Holding Temp	Inoculum ($TCID_{50}$)	Cell line	CPE detected or virus detected by FA	Days to CPE
Adenovirus 1	4	4°C	3 x 10^4	Hep-2	YES	n/a
ATCC VR-1	4	RT		Hep-2	YES	n/a
	7	4°C		Hep-2	YES	2
	7	RT		Hep-2	YES	2
HSV Type 2	3	4°C	3 x 10^4	MRC-5	YES	1
ATCC VR-734	3	RT		MRC-5	YES	1
	7	4°C		MRC-5	YES	2
	7	RT		MRC-5	YES	3
HSV Type 1	3	4°C	3 x 10^4	MRC-5	YES	2
ATCC VR-539	3	RT		MRC-5	YES	2
	7	4°C		MRC-5	YES	1
	7	RT		MRC-5	YES	2
Parainfluenza 3	3	4°C	3 x 10^4	PLC	YES	4
ATCC VR-93	3	RT		PLC	YES	7
	7	4°C		PLC	YES	6
	7	RT		PLC	YES	15
Rhinovirus	3	4°C	3 x 10^4	MRC-5	YES	2
ATCC VR-1118	3	RT		MRC-5	YES	3
	7	4°C		MRC-5	YES	3
	7	RT		MRC-5	YES	5
Influenza A	3	4°C	3 x 10^4	PLC	YES	4
Clinical isolate	3	RT		PLC	YES	5
	8	4°C		PLC	YES	5
	8	RT		PLC	YES	8

Virocult® was shown to successfully recover all 6 viruses after holding for up to 7 days, and whether held at 4°C or ambient temperature.

CLSI's document M40-A provides a useful gauge for the assessment of transport devices. For bacteriology where there is a quick turn round of cultures, it is a practical method of judging the suitability of a device for a particular application. However, for virology this is not the case, and the work is extremely time consuming, and expensive in materials, and not practical for a routine laboratory. This may explain why there have been so few reported virology based M40 studies, while there is a plethora of bacteriological studies.

The Virocult® swab has been around for many years, and there have been many studies in the past showing it to be effective for most of the common respiratory and skin associated viruses. The present study has provided the opportunity to assess the Virocult swab in a new era, using current materials and cell lines.

M40-A requires the transport device to maintain live virus at detectable levels starting with a titre of 5 x 10^4 for up to 96 hours. The results shown here are for an inoculum of 3 x 10^4, as being closest to that standard. Other dilutions were also used, and recovery demonstrated, but these are not shown here. In this study the test period was extended to 7 days to reflect situations where specimens might require to be forwarded to a reference facility after initial rapid testing. In the case of influenza Type A, the reading had to be taken at 8 days because of a holiday situation, but survival was still demonstrated.

In the study Virocult® was shown to meet and exceed the requirements of M40-A, by maintaining a selection of viruses in a viable condition when held at 4°C, or at ambient/room temperatures for 96 hours, and beyond until at least 7 or 8 days. The device should be suitable for respiratory or skin lesion specimens that require to be transported from remote sites, even if they cannot be frozen or transported with dry ice.