Laboratory Evaluation of Σ-Virocult® Transport Swabs

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ABSTRACT

Background: Collection and storage of viral specimen is the most important factor in obtaining a reliable and accurate diagnostic result. Swabs are often used to collect specimens for detecting and identifying viral infections
Scope of study: In this study Σ-Virocult® transport swabs (Medical Wire and Equipment) were evaluated using the CLSI Quality Control of Microbiological Transport Systems Approved Standard M40-A. The standard presents a specified culture protocol to assess a viral culture transport device manufactured to facilitate virus preservation. The study was carried out using two different temperatures.
Method: Herpes simplex virus type 2 and Adenovirus type 3 were inoculated into Σ-Virocult® swabs at a concentration of 5×10^4 $TCID_{50}$ and held at 22ºC and 4ºC. The swabs were sampled every day for four days, then at day seven.
Results: The results showed that the swabs maintain the viability of virus as set out in the standard and that survival is better at 4ºC.
Conclusion: The Σ-Virocult® transport swabs may be recommended as a reliable virus transport device.

INTRODUCTION

Viral particles vary widely in composition, structure, morphology size and stability (2). Viral isolation has been widely accepted as the gold standard for laboratory confirmation of viral infection; however, it requires specimen storage and transport in a viral medium maintained at low temperatures to optimally preserve infectious viral particles (1). Clinical specimens for virus isolation should ideally be inoculated into cell culture with as little delay as possible (2). However, many common viruses are able to withstand both storage at room temperature and transport for extended periods of time when placed in a suitable transport medium (2). A suitable transport medium for swabs must maintain viability of viruses, prevent overgrowth of bacteria and fungi, and prevent drying of the specimen (6). Swabs are generally used to collect cells and fluid from nasal passages, the throat, the rectum, vesicles, eyes, and cervical, genital and skin lesions. The Σ-Virocult® is designed for specimen collection, transport and maintenance of virus viability. The device consists of soft polyurethane foam budded swab and a transport tube of liquid virus transport medium which contains antimicrobials to inhibit growth of any bacteria or fungi present in the specimen (5,10). This study evaluates the Σ-Virocult® transport swab to determine whether it is able to maintain the viability of viruses at different temperatures over a certain period of time.

MATERIALS AND METHODS

Materials

Viruses: Stock concentrations of wild-type (clinical isolates) Adenovirus type 3 and Herpes simplex virus type 2 were used in the swab evaluation study.
Cell lines: HEP2 (human larynx carcinoma) and A549 (human lung carcinoma) cell lines routinely utilised in the laboratory were utilised as the cell lines to support the growth of Adenovirus (HEP2) and Herpes simplex type 2 (A549). Fresh working lots of low passage number (<15) were split into tube cell cultures to have a confluent monolayer after 24 hours.
Swabs: Σ-Virocult®, Lot No. 09J30, expiry date Sep 2010 were used in the study.

Methods

The wild-type viruses were inoculated into a flask containing a confluent monolayer of HEP2 for Adenovirus type 3 and A549 for Herpes simplex type 2. Once most of the monolayer showed typical cytopathic effect (CPE), the virus was harvested and stored for use as the stock suspension of the virus. Stock suspensions of Adenovirus type 3 and Herpes simplex

type 2 were serially diluted in cell culture maintenance medium using 10-fold dilutions from 10^{-1} to 10^{-7}. For each dilution, 5 tubes of fresh HEP2 and A549 cell cultures were inoculated with 100µl of diluted viral suspension. The cultures were incubated at 37ºC and examined daily for CPE over a period of 10 days. $TCID_{50}$ (tissue culture infective dose which is the amount of virus which gives a CPE in 50% of inoculated cultures) was calculated as described by Reed and Muench (7). The $TCID_{50}$ for the Adenovirus type 3 was calculated to be 4.22 x 10^5 /0.1ml. And the $TCID_{50}$ for the HSV type 2 was calculated to be 3.16 x 10^5/0.1ml. The Σ-Virocult® swabs contain 2mls of liquid transport medium and the inoculation to achieve 5 x 10^4 $TCID_{50}$ as per the Approved Standard M40-A was calculated to be 230µl of the Adenovirus stock suspension and 300µl for the HSV stock suspension. 40 swabs were inoculated with 230µl of the Adenovirus stock suspension and 40 swabs were inoculated with 300µl of the HSV stock suspension. For each virus, 20 swabs were stored in the fridge at 4ºC and the other 20 were stored at room temperature, 22ºC. The swabs were sampled every day for four days, then at day 7. On each appropriate day, 200µl of the liquid medium from the swabs was inoculated into the appropriate cell culture and incubated at 37ºC. Tubes were removed once CPE was observed.

RESULTS

Adenovirus CPE after being inoculated in swabs

TEMP	4ºC				22 ºC			
Day	**Tube 1**	**Tube 2**	**Tube 3**	**Tube 4**	**Tube 1**	**Tube 2**	**Tube 3**	**Tube 4**
1	4+	3+	4+	4+	3+	4+	4+	3+
2	3+	4+	4+	4+	3+	3+	3+	3+
3	4+	4+	4+	4+	3+	2+	2+	2+
4	4+	4+	4+	4+	2+	3+	2+	2+
7	4+	4+	4+	4+	2+	2+	3+	2+

Herpes simplex virus CPE after being inoculated into swabs

TEMP	4ºC				22 ºC			
Day	**Tube 1**	**Tube 2**	**Tube 3**	**Tube 4**	**Tube 1**	**Tube 2**	**Tube 3**	**Tube 4**
1	3+	2+	2+	3+	2+	2+	2+	2+
2	2+	3+	3+	3+	2+	2+	3+	2+
3	3+	4+	3+	3+	3+	2+	2+	2+
4	4+	4+	4+	4+	3+	3+	3+	2+
7	4+	4+	4+	4+	2+	2+	2+	2+

1+: 25% of cell monolayer showed CPE
2+: 50% of cell monolayer showed CPE
3+: 75% of cell monolayer showed CPE
4+: 100% of cell monolayer showed CPE

DISCUSSION

The Σ-Virocult® swab was able to maintain the viability of the viruses for up to 7 days at two different temperatures. An optimal viral transport system can be defined as that system which preserves the virus in the system, prevents loss of the specimen or test due to microbial contamination, has a long shelf life, is readily available and is inexpensive (4). The results demonstrated that the swabs held at 4ºC provide better viability than those held at room temperature and therefore users should be encouraged to place swabs in the refrigerator if delay in sending the swabs to the laboratory is likely to occur. Typical cytopathic effect was seen in all inoculated tubes which demonstrated the tubes do not inhibit virus viability and that the swab is non-toxic to virus in the specimen.
The Σ-Virocult® transport swab is compact, enclosed and resistant to breakage or damage during shipping therefore viral specimens can be reliably transported to the laboratory at ambient temperatures and viral infections can be routinely diagnosed by culture (9). In the laboratory, the swab containers fit easily into racks, stand alone and may be centrifuged. These features are very useful when processing swab specimens.
In summary, the Σ-Virocult® transport swab complied with the Approved Standard M40-A and may be recommended as a reliable virus transport device.

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