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Detection of viral DNA when comparing two bacterial transport systems

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mwe medical wire

ABSTRACT

Assessing the ability of two commercially available bacterial storage and transport systems to be used in the detection of viral DNA. Storage conditions and the presence of contaminating bacteria were analysed in this project. Viral DNA could be detected following storage of up to 144 hours at 4ºC and room temperature for both systems. However, the presence of bacteria had a significant effect on one of the transport systems, reducing virus recovery rates.

INTRODUCTION

Many commercial swab transport systems have been developed to preserve and stabilise clinical material prior to downstream microbiological investigation. Some have been designed to enhance viral or bacterial stability using growth supplements or suppressants, some of which may have an impact or limit their potential use. Traditionally, swabs taken for viral investigations were required to be placed in a specialised viral transport medium containing antibiotics to prevent or reduce bacterial contamination which may have a deleterious effect on downstream detection methods.

Two such microbiological swab transport systems are the COPAN ESWAB® with a nylon flocked swab and the MWE ∑-Transwab® incorporating a foam-tipped swab. Both are liquid culture systems based on modified liquid Amies transport medium with proven applications in bacteriology.

This investigation looks at the potential use of these bacterial transport systems for the detection of viral pathogens in clinical samples and the effect that storage conditions and the presence of bacterial contamination may have on the detection of viral DNA.

METHODS

100µl Herpes Simplex Virus 1 (HSV 1) positive control material was added to four tubes of each respective transport medium. 100µl of *E. coli* suspension was added to two of each of these HSV 1 inoculated tubes. Two of each (two inoculated with HSV 1, and two inoculated with HSV 1 and *E. coli*) were incubated at room temperature and at 4ºC and then examined for the presence of HSV 1 DNA at 24hr intervals from 0hrs to 144hrs. To do this, a 200µl sample of each transport medium was extracted using the BioMerieaux easyMAG automated extraction platform and examined for the presence of HSV 1 DNA using an in-house HSV1/HSV2 duplex real-time PCR performed on the ABI 7500 TaqMan platform. Using this assay, the HSV 1 control material is known to give a crossing threshold (Ct) of approximately 32-34 cycles. A 10µl sample of each transport media inoculated with *E. coli* was taken after 72 and 96 hours incubation at both room temperature and 4ºC, and then plated onto Blood Agar and incubated overnight at 37ºC.

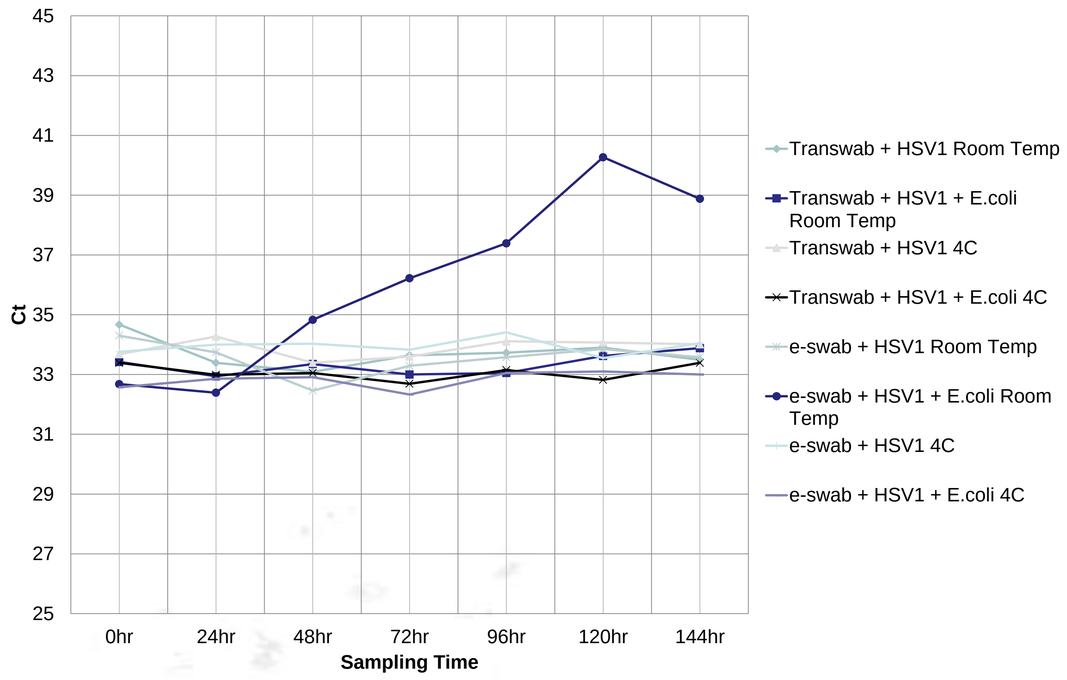
RESULTS

HSV 1 DNA could be detected following storage for up to 144 hours at 4ºC and at room temperature from both transport systems with Ct values of between 32 – 35 cycles . Inclusion of *E. coli* resulted in increased Ct values for the Copan ESWAB® system, up to 40.27 cycles, when stored at room temperature for longer than 72 hours. Viable *E. coli* was recovered following sampling and culture at 72 and 96 hours when plated onto blood agar. Results are presented in the following table and graph.

Table 1 and Graph 1. Ct values of HSV 1 from each transport system when stored at either room temperature or 4ºC sampled at 24 hour intervals, with and without *E. coli.*

	0hrs	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
A HSV RT	34.67	33.39	33.09	33.64	33.73	33.9	33.49
A HSV/EC RT	33.42	32.94	33.35	33.00	33.05	33.62	33.88
A HSV 4C	33.68	34.27	33.39	33.60	34.11	34.07	34.01
A HSV/EC 4C	33.39	32.99	33.05	32.69	33.15	32.82	33.39
B HSV RT	34.30	33.74	32.46	33.29	33.58	33.85	33.58
B HSV/EC RT	32.68	32.39	34.83	**36.22**	**37.39**	**40.27**	**38.88**
B HSV 4C	33.76	34.00	34.03	33.83	34.41	33.55	34.05
B HSV/EC 4C	32.57	32.86	32.91	32.33	33.05	33.10	33.00

A = MWE ∑-transwab
B = COPAN ESWAB
HSV = HSV 1
EC = *E. coli*
RT = Room Temperature
4C = 4ºC



CONCLUSIONS

On examination of the results, recovery of HSV 1 DNA at a constant level (CT 32-35) following storage of up to 144 hours at both room temperature and 4ºC, in the presence or absence of bacterial contamination was achieved using the MWE ∑-transwab system only. Recovery of HSV 1 DNA from COPAN ESWAB system could be achieved for up to 144 hours when stored at 4ºC with or without bacterial contamination, or at room temperature without the addition of *E. coli.* At room temperature the inclusion of *E. coli* began to reduce the ability to detect the HSV 1 DNA after 24 hours and was significantly reduced by 120 hours, although it was still detectable.

It can be concluded that, although specialised viral transport are recommended for the storage and enhanced recovery of viral DNA, systems designed for other microbiological investigations may be of use. However, prolonged storage at non-refrigerated temperatures and the potential for bacterial contamination may lead to poor or reduced recovery and possible misdiagnosis.

Disclaimer: Use of trade names is for identification only and does not imply endorsement by Public Health England