A study of various commercially available transport swabs for the recovery of fastidious organisms

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Abstract

Abstract Content: Key words: Sigma Transwab®, viability, fastidious.

For accurate laboratory diagnosis of infections, it is important to use the appropriate specimen and to transport it to the laboratory using a transport medium which will maintain the viability of the microorganisms.

Objectives:
The Sigma Transwab ® is a liquid medium transport swab and uses 1ml of Liquid Amies Transport medium, which is based on the original formulation of Amies, but without the charcoal and is intended for processing by some of the new automated plating systems. The swab is foam tipped which allows the flow through of the liquid medium, reagents and microorganisms, thus increasing the sensitivity and recovery of organisms.
The preliminary study used the principles of CLSI's M40-A standard, inoculating the swabs with specified dilutions of target organisms, and holding at either room temperature or 4^{O}C.
For the preliminary study the organisms used were:
Staphylococcus aureus
Haemophilus influenzae
Beta Haemolytic Streptococcus.
Fresh overnight cultures were used in each case. The organisms were from Clinical specimens not NCTC or ATCC organisms.

Method:
A McFarland standard 0.5 was made in sterile 0.85% saline from a fresh overnight culture of each of the organisms used.
(0.5 McFarland's standard = 1.5 x 10^8 CFU)
Tenfold dilutions were made for the viability studies from the 0.5 McFarland's standard. 100µl of these dilutions were lawned on to the appropriate agar plate to ensure the organism was viable from the saline dilution and used as the Growth Control.
The 10^{-5}, 10^{-6} and 10^{-7} cfu/ml dilutions were used to inoculate the swabs with 100ul
(0.1ml) using 3x 1/2 tubes.
The swab was placed in the 100µl inoculum for 10 seconds and then returned to the transport media.
Swabs were kept at room temperature and at 4^{O}C for the following holding times.
5-15 minutes (Zero Time), 4 hours, 24 hours, 48 hours, and 72 hours.
Each set of dilutions were performed in duplicate.
On completion of the holding time, the swab inoculated on to

Organism	Inoc-ulum	Con-trol Plate	Room Temperature		4^{O}C	
			24hr	48hr	24hr	48hr
H. influenzae	1.5 x 10^6	>1000	80	0	>100	100
	1.5 x 10^7	>1000	>100	>100	>500	>200
S. aureus	1.5 x 10^6	>500	>500	>500	>500	>500
	1.5 x 10^7	>1000	>1000	>1000	>1000	>1000
Beta-haemolytic Strep	1.5 x 10^5	>200	44	40	50	16
	1.5 x 10^6	>500	>200	>200	>200	>100
	1.5 x 10^7	>1000	>500	>500	>500	>200

a plate appropriate to the organism (Blood agar, Chocolate blood agar) in a lawn and incubated in the appropriate atmosphere – Aerobically at 37^{O}C for *Staphylococcus aureus* and Beta haemolytic streptococcus, and CO_2 at 37^{O}C for *Haemophilus influenzae*.
The plates read and colonies counted manually at 24 and 48 hours.

Results:
Conclusion:
The initial results showed that recovery was seen for all the organism/dilution/temperature
combinations at 24 hours, and for most at 48 hours, and that Sigma Transwab ® would be a suitable transport device for these organisms.
Further studies will be done and data presented for other organisms using more fastidious organisms, and a comparison of results obtained with standard transport swab devices (Eurotubo, Spain and Technical Service Consultants, UK). .

Σ-Transwab®

Eurotubo & TSC

e-Swab

Introduction

The inexorable increase in the number of specimens requiring to be processed in clinical microbiology laboratories in recent years is leading to the introduction of numerous automated systems for the initial processing of specimens including swabs. Most of these new systems are set up to process liquid samples, rather than the gel media commonly used with transport swabs. To facilitate this a number of liquid medium based transport swabs are becoming available, including the Sigma Transwab from Medical Wire. This device uses a polyurethane foam tipped swab for the collection of the patient specimen. This is then broken into a vial containing 1 ml liquid Amies medium (with no charcoal) for transport to the laboratory.

The purpose of the present study was to assess the performance of this new device with equivalent gel-based transport swabs (Technical Services Consultants and Eurotubo) available in the UK market. The study used the principles of the CLSI standard M40-A for the assessment of microbiology transport devices for three important pathogens, which were Neisseria gonorrhoeae, Haemophilus influenzae, and Streptococcus pneumoniae. In the course of the work some examples of another liquid based device (Copan e-swab) became available and were included for some of the test points.

Methods

The preliminary study used the principles of CLSI's M40-A standard, inoculating the swabs with specified dilutions of target organisms, and holding at either room temperature (RT) or 4^{O}C. (See abstract for organisms and results)

For the main study the organisms used were:

Neisseria gonorrhoea NCTC 12009
Streptococcus pneumoniae NCTC 12977
Haemophilus influenzae NCTC 12699

Fresh overnight cultures taken from a non-selective media were used in each case.

The swabs for used for comparison were :

Probact™ (Technical Service Consultants, UK)
Sigma Transwab® (Medical Wire & Equipment, UK)
Eurotubo (Deltalab, Spain)
e-swab (Copan, Italy)

A McFarland standard 0.5 was made in sterile 0.85% saline from a fresh overnight culture of each of the organisms used. (0.5 McFarland's standard = 1.5 x 10^8 CFU)

Tenfold dilutions were made for the viability studies from the 0.5 McFarland's standard. 100µl of these dilutions were lawned on to the appropriate agar plate to ensure the organism was viable from the saline dilution and used as the Growth Control.

Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} (equivalent to 1.5 x 107, 106, 105 cfu/ml respectively) were used to inoculate the swabs with 100µl (0.1ml) using 3x 1/2 tubes. 3 swabs were inoculated for each organism/dilution/time combination

The swab was placed in the 100µl inoculum for 10 seconds and then returned to the transport medium.

Swabs were kept at room temperature or at 4^{O}C for the following holding times:

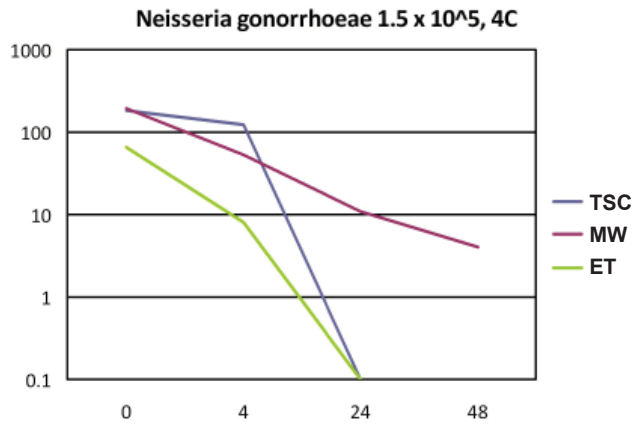
5-15 minutes (Zero Time), 4 hours, 24 hours, and 48 hours

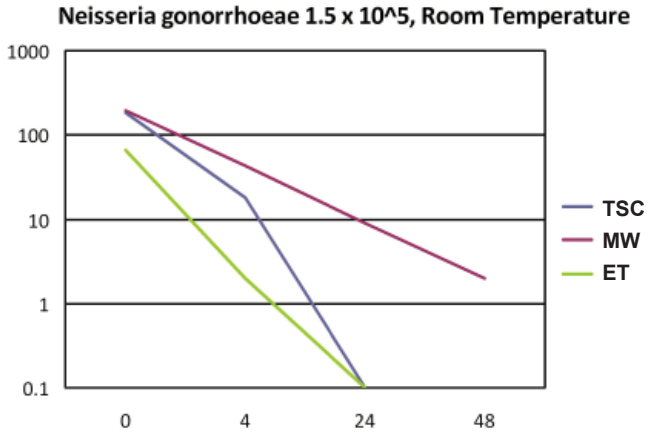
On completion of the holding time, the swab was removed from its medium and inoculated on to a chocolate blood agar and incubated under CO2 at 37^{O}C. The plates read and colonies counted manually at 24 and 48 hours. The average for the 3 swabs for each organism/dilution/time combination is shown.

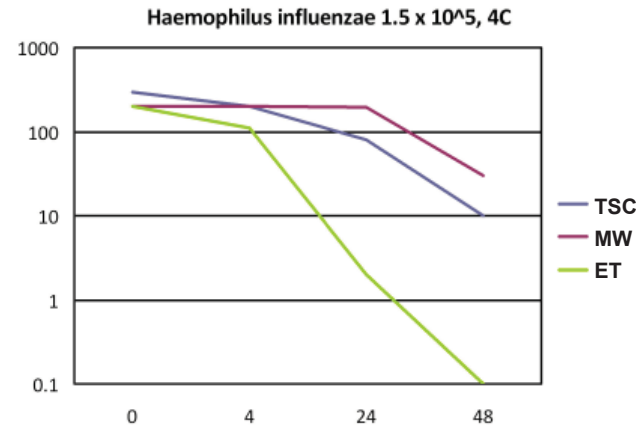
CLSI M40A recommends that the dilution to be used is that which has a zero time reading of between 30 and 300cfu on the plate. In this experiment the 10^{-2} dilution (inoculum 1.5 x 10^5 cfu/ml) was the one which most consistently met this criterion.

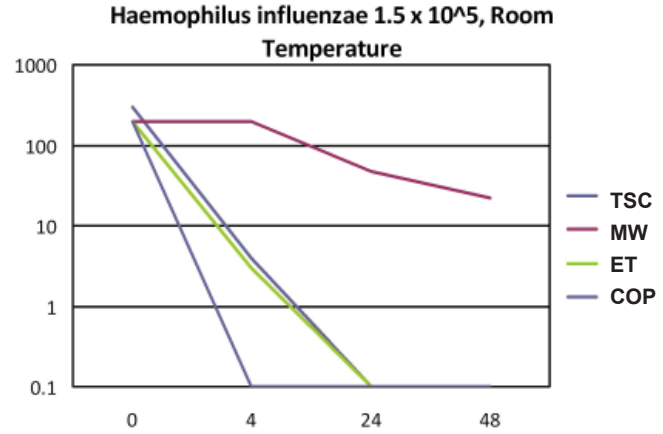
Results

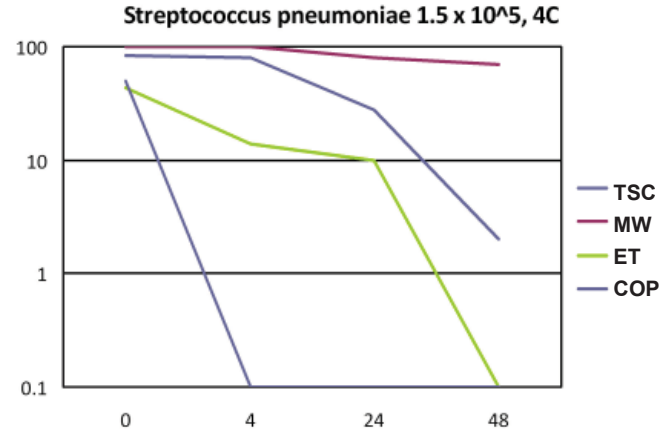
Holding Temperature	**4C**					**Room Temperature**			
Holding Time (hours)	**0**	**4**	**24**	**48**		**0**	**4**	**24**	**48**
Neisseria gonorrhoeae									
TSC	**185**	**124**	**0**	**0**		**185**	**18**	**0**	**0**
MW	**194**	**53**	**11**	**4**		**194**	**43**	**9**	**2**
Eurotubo	**66**	**8**	**0**	**0**		**66**	**2**	**0**	**0**
Haemophilus influenzae									
TSC	**296**	**200**	**80**	**10**		**296**	**4**	**0**	**0**
MW	**200**	**200**	**196**	**30**		**200**	**200**	**48**	**22**
Eurotubo	**200**	**110**	**2**	**0**		**200**	**3**	**0**	**0**
Copan						**200**	**0**	**0**	**0**
Streptococcus pneumoniae									
TSC	**84**	**80**	**28**	**2**		**84**	**36**	**5**	**0**
MW	**100**	**100**	**80**	**70**		**100**	**100**	**50**	**14**
Eurotubo	**44**	**14**	**10**	**0**		**44**	**8**	**0**	**0**
Copan	**50**	**0**	**0**	**0**		**50**	**0**	**0**	**0**

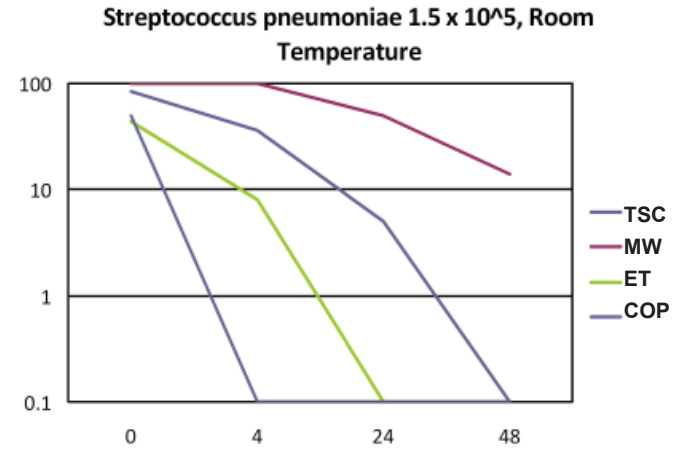












Discussion & Conclusion

Neisseria gonorrhoeae, Haemophilus influenzae, and Streptococcus pneumoniae are significant pathogens, although the incidence of haemophilus and streptococcus are being reduced by vaccines in developed countries. They are also notoriously fastidious and can be difficult to recover if specimens are not collected and handled correctly. In the present study the performance of a new type of transport device, the Σ-Transwab® using a charcoal free liquid Amies was compared with that of another liquid based product (e-swab®, Copan) and two transport swab devices (Technical Services Consultants and Eurotubo) which use conventional charcoal-free Amies gel. It is normally assumed that the agar in semi-solid medium acts as a protectant for fastidious organisms by slowing down the diffusion of oxygen.

According to CLSI M40-A, Swab-Elution Method, to be considered acceptable, "there shall be no more than a 3 log10 (1 x 103 ± 10%) decline in CFU between the zero-time CFU count and the CFU of the swabs that were stored".

Based on this criterion, for the three organisms tested, Σ-Transwab®, and only Σ-Transwab® complied with the standard, both at room temperature (20-25^{O}C) and refrigerated temperatures. For Haemophilus influenzae and Streptococcus pneumoniae the decline was of less than 1 log10, over 48 hours, and for Neisseria gonorrhoeae the decine was within 2 log10. In addition the Σ-Transwab® significantly outperformed the gel-medium products (Technical Services Consultants and Eurotubo), and the other liquid medium product (e-swab®,Copan).

On the basis of this study Σ-Transwab® has been shown to be a suitable transport device for the collection and transport of Neisseria gonorrhoeae, Haemophilus influenzae, and Streptococcus pneumoniae. In addition the preliminary study showed similar satisfactory recovery of Staphylococcus aureus and Beta-haemolytic streptococcus, and without overgrowth.

Reference

CLSI M40-A, 2003, *Quality Control of Microbiological Transport Systems; Approved Standard* , Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA