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Evaluation of a Novel Swab Transport System for Maintaining Viability of Anaerobes and Impact of Using Different Inoculum Broths

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ABSTRACT

Background: Swabs are often used in general practice and surgery for purposes of sampling, transporting, and maintaining viability of pathogens present. The objectives of this study were (1) to evaluate the performance of a novel multipurpose swab, the Amies Plus SP140X (Starplex Scientific Inc., Toronto, Canada) for its ability to maintain viability of anaerobes at 20-25°C (RT) and 4-6°C (FRG), and (2) to compare its performance when using different broths for inoculum preparation: phosphate buffer saline (PBS), Schaedler's broth (SB), and Fastidious Anaerobe Broth (FAB).

Methods: Using CLSI M40A Roll-Plate Method, swabs were seeded in duplicate with 10 anaerobes: 5 reference strains (*Bacteroides fragilis* [*Bf*] ATCC 25285, *Clostridium novyi* [*Cn*] ATCC 7659, *Fusobacterium nucleatum* [*Fn*] ATCC 25586, *Peptostreptococcus anaerobius* [*Pa*] ATCC 27337, *Prevotella melaninogenica* [*Pm*] ATCC 25845) and 5 clinical strains (*Actinomyces* sp. [*Ac*], *B. ureolyticus* [*Bu*], *B. vulgatus* [*Bv*], *C. perfringens* [*Cp*], *C. sordellii* [*Cs*]). Organisms were suspended in PBS, SB, and FAB and serially diluted to inoculum concentrations ranging from 10^4-10^5 CFU/ml. Swabs were stored at RT and a duplicate set at FRG, and plated on pre-reduced brucella agar at 0, 24, and 48 hr. Plates were incubated anaerobically for 48 hr. Viability was compared to the zero-hr count for each organism-broth combination at RT and FRG for each time interval.

Results: *Bf*, *Bu*, *Bv* and *Cp* were adequately recovered from swabs regardless of time interval, storage temperature, or inoculum broth. Higher CFU counts were obtained by swabs seeded with FAB or SB suspensions than by those with PBS, and by swabs stored in FRG compared to those in RT. With PBS, there was no recovery in RT at 24 hr of *Cn*, *Fn*, *Pa*, or *Pm*, or at 48 hr of *Ac* or *Cs*, but they were viable up to 48 hr in FRG, except for *Fn*, which survived for 24 hr only in swabs seeded with FAB or SB suspensions and stored in FRG.

Conclusions: SP140X is capable of maintaining viability of anaerobes for at least 24 hr when tested in FAB or SB and stored in FRG. PBS is suboptimal to FAB and SB for supporting anaerobe viability. Swab refrigeration enhances recovery of anaerobes.

INTRODUCTION

One of the crucial steps for accurate laboratory diagnosis of infection is to ensure adequate sampling and transport of pathogens present, without critically compromising their viability. Although considered suboptimal to direct culture, swabs are often used in general practice and surgery, and have become increasingly important in view of the delay in specimen transport impacted by recent strategies of cost containment and consolidation of laboratory services.[3] As a result, there have been continuous efforts to develop and evaluate new and improved swab transport systems.[1-6]

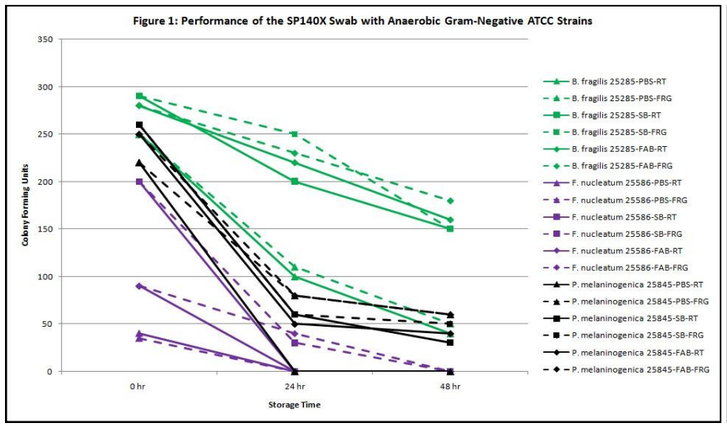
The Amies Plus SP140X (Starplex Scientific Inc., Toronto, Canada) is a novel multipurpose swab, with the advantage of reduced charcoal content that enhances its Gram stain interpretation. The objectives of this study were (1) to evaluate its ability to maintain viability of anaerobes at 20-25°C (RT) and 4-6° C (FRG), and (2) to compare its performance when using different broths for inoculum preparation, namely, phosphate buffer saline (PBS), Schaedler's broth (SB), and Fastidious Anaerobe Broth (FAB).

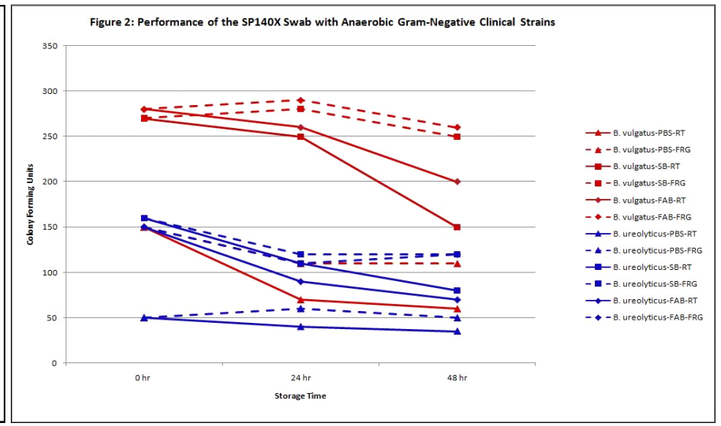
METHODS

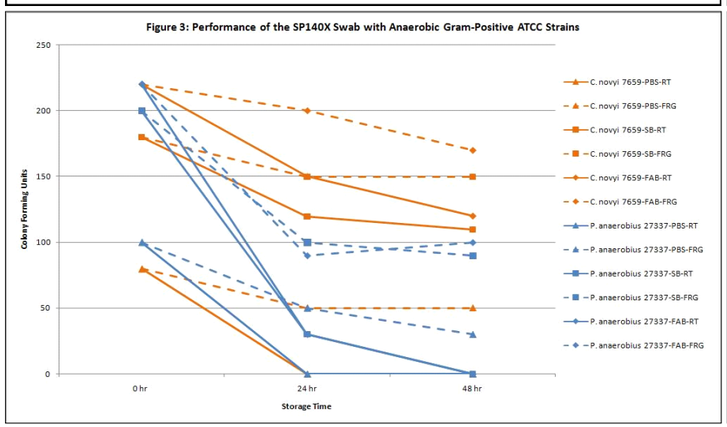
Using the CLSI M40A Roll-Plate Method, swabs were seeded in duplicate with 10 anaerobes, comprising 5 reference strains (*Bacteroides fragilis* ATCC 25285, *Clostridium novyi* ATCC 7659, *Fusobacterium nucleatum* ATCC 25586, *Peptostreptococcus anaerobius* ATCC 27337, *Prevotella melaninogenica* ATCC 25845) and 5 clinical strains (*Actinomyces* sp., *B. ureolyticus*, *B. vulgatus*, *C. perfringens*, *C. sordellii*).

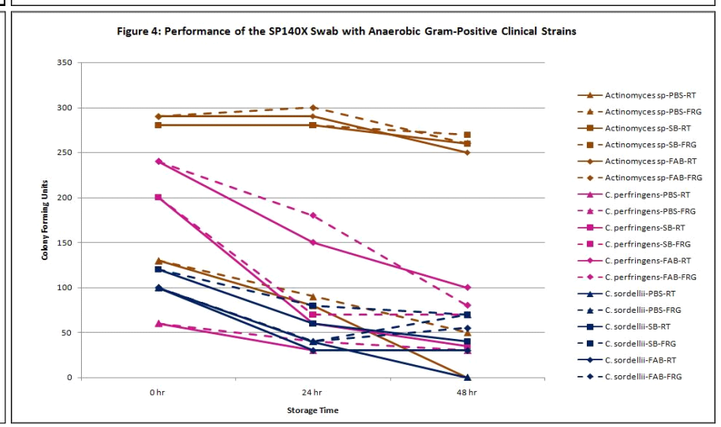
Each organism was suspended in PBS, SB, and FAB and the suspensions were serially diluted from 1.5 x 10^8 CFU/mL (0.5 McFarland Std.), into 1:10, 1:100, 1:1000, and 1:10,000 dilutions, to inoculum concentrations ranging from 10^4-10^5 CFU/mL, which were used to seed swabs. The swabs were stored in RT and a duplicate set in FRG, and were plated on pre-reduced brucella-blood agar at 0, 24, and 48 hr.

Plates were promptly incubated anaerobically and read at 48 hr. Viability was compared to the zero-hr count closest to 300 CFUs for each organism-broth combination at RT and FRG for each time interval.









PBS, phosphate buffer saline; SB, Schaedler's broth; FAB, Fastidious Anaerobe Broth; RT, 22-25°C; FRG, 4-6°C.

RESULTS & DISCUSSION

Data of colony counts were plotted by Gram-reaction and type of strain. As can be seen from the graphs, *B. fragilis* (Figure 1), *B. ureolyticus* (Figure 2), *B. vulgatus* (Figure 2), and *C. perfringens* (Figure 4) were adequately recovered from the swabs regardless of time interval, storage temperature, or inoculum broth.

Overall, higher CFU counts were obtained by swabs seeded with FAB or SB suspensions than by those obtained with PBS, and by swabs stored in FRG compared to those in RT. With PBS, there was no recovery in RT at 24 hr of *C. novyi* (Figure 3), *F. nucleatum* (Figure 1), *P. anaerobius* (Figure 3), or *P. melaninogenica* (Figure 1), or at 48 hr of *Actinomyces* sp (Figure 4) or *C. sordellii* (Figure 4), but they were viable up to 48 hr in FRG, except for *F. nucleatum* (Figure 1), which survived for 24 hr only in swabs seeded with FAB or SB suspensions and stored in FRG.

The poor recovery of *F. nucleatum* from PBS-inoculated swabs and from the other swabs stored at RT is consistent with its generally reported fastidious viability.[5,6]

Based on these data, FAB and SB appear to be superior to PBS in supporting viability of anaerobes, likely due to their enrichment with growth stimulating factors, such as cysteine, hemin, peptones, and vitamin K_1. On the other hand, the inability of the swab to sustain viability of certain organisms may be related to inoculum threshold or may be strain-dependent, as described previously.[3,4]

Our results also indicate that swab refrigeration enhances anaerobe viability. Improved survival of fastidious organisms in refrigerated swabs has been previously reported,[1] and our work extends these findings to more anaerobes.[2,7]

Limitations of the Study:

- A small number of anaerobic strains was studied. Large scale studies are needed that would employ much larger numbers of clinical strains.
- Such evaluations are often hampered by technical challenges due to the nature and size of inoculum preparation and swab absorption, that may impact the accuracy of recovery and colony counts.[3,5]
- A single lot of SP140X was used in this study. The presence of "lot-to-lot" variability of results observed by some investigators performing swab evaluations suggests that such evaluations should be conducted with several lots of the same product to ensure reliability and consistency of the data.[1]

Further studies are planned to confirm and extend the findings of this work, using a much larger sample of anaerobic organisms.

CONCLUSIONS

- The Starplex Amies Plus SP140X swab is capable of maintaining viability of anaerobes for ≥ 24 hr when tested in FAB or SB and stored in FRG.
- PBS is suboptimal to FAB and SB for supporting anaerobe viability.
- Swab refrigeration enhances recovery of anaerobes.

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