



Comparison of the New Starplex StarSwab II™ and the New Copan Vi-Pak Amies Agar Gel Collection and Transport Swabs with BBL™ Port-A-Cul™ for the Maintenance of Anaerobic and Fastidious Aerobic Organisms

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ABSTRACT

Of utmost importance when evaluating patient clinical samples for infectious agents is specimen transport to the clinical laboratory in as near its original state as possible. In this study we compared Starplex StarSwab II™ (Starplex Scientific, Ontario, Canada), and Copan V-Pak Amies Agar Gel transport swabs (Copan Diagnostics Inc., Corona, California) to BBL™ Port-A-Cul™ (Becton Dickinson Microbiology Systems, Sparks, Maryland) for the survival of anaerobic and fastidious aerobic bacteria. The Copan Vi-Pak system has been modified by nitrogen gas flushing to keep an ideal low Eh condition, and to prevent oxidation of the transport media. The survival of 13 different microorganisms (9 anaerobic [*Clostridium perfringens*, *Peptostreptococcus anaerobius*, *Propionibacterium acnes*, *Eubacterium lentum*, *Prevotella bivia*, *Prevotella melaninogenica*, *Bacteroides fragilis*, *Fusobacterium nucleatum*, and *Fusobacterium necrophorum*] and 4 aerobic [*Haemophilus influenzae*, *Streptococcus milleri*, *Neisseria gonorrhoeae*, and *Streptococcus pneumoniae*]) at 0, 6, 24, and 48 hours post inoculation and storage at room temperature was evaluated. Serial dilutions were used to quantify the number of viable organisms on sheep blood agar or chocolate plates. The recovery of all organisms by the three swabs was similar at time point 0. However, Copan Vi-Pak Amies Agar Gel transport out-performed the two other transport swabs after the 0-hour time point. While there was no dramatic reduction in the number of viable organisms in the Copan Vi-Pak system after 6 h incubation, a 2–4 log₁₀ reduction was observed for *P. anaerobius*, *P. bivia*, *F. nucleatum*, and *F. necrophorum* after 6 & 24 hours incubation in the other two swab systems' transport media. Moreover, Copan Vi-Pak system maintained the viability of *H. influenzae* and *N. gonorrhoeae* organisms for up to 24–48 hours whereas there was a 3–5 log₁₀ reduction using the two other swabs. In conclusion, Copan Vi-Pak system out-performed the two other swabs evaluated by maintaining the viability of both anaerobic and fastidious aerobic organisms for a time period long enough to transport the specimens to the clinical microbiology laboratory or to an off-site reference laboratory.

INTRODUCTION

The successful isolation of anaerobes largely depends on adequate specimen collection and transport to the clinical microbiology laboratory. During specimen transport, protection of the anaero-

bic bacteria from drying and exposure to oxygen are critical steps in the recovery of these organisms. It is well established that tissue biopsy, aspirates of fluids and exudates from suspected infected sites are superior specimens over samples collected on swabs (1). Tissues and aspirate specimens, if collected and transported properly, can provide adequate sample volume for aerobic and anaerobic cultures. However, because of the ease of using swabs (i.e. in the operating room), clinical microbiology laboratories still get patients' specimens on swabs (2). With the advent of technology, swabs transport systems utilizing semi-solid medium have been developed for transporting patient samples for anaerobic cultures. Moreover, these swabs have been shown to protect both anaerobic and fastidious aerobic organisms (3).

In this study we compared the new Starplex StarSwab II™ (Starplex Scientific, Ontario, Canada), and Copan Vi-Pak Amies Agar Gel transport swabs (Copan Diagnostic Inc., Corona, California) to BBL™ Port-A-Cul™ (Becton Dickinson Microbiology Systems, Sparks, Maryland) for the survival of anaerobic, facultative anaerobic and fastidious aerobic bacteria.

MATERIALS AND METHODS

BACTERIAL STRAIN

The survival of the following strains in the three swab transport systems was evaluated:

- *Clostridium perfringens* American Type Culture Collection [ATCC] 13124
- *Eubacterium lentum* ATCC 43055
- *Peptostreptococcus anaerobius* ATCC 27337
- *Propionibacterium acnes* ATCC 11827
- *Prevotella bivia* (clinical isolate)
- *Prevotella melaninogenica* ATCC 15930
- *Bacteroides fragilis* ATCC 25285
- *Fusobacterium nucleatum* ATCC 25586
- *Fusobacterium necrophorum* ATCC 25286
- “*Streptococcus milleri*” group (clinical isolate)
- *Haemophilus influenzae* ATCC 10211
- *Neisseria gonorrhoeae* ATCC 43069
- *Streptococcus pneumoniae* ATCC 49619)

CULTURE SWABS

The following swabs were evaluated during the study:

Copan Vi-Pak Amies Agar Gel transport swab (catalog #108 C) is Amies culture swab that has been flushed with Nitrogen gas to maintain optimal Eh potential of the gel medium. The gel medium is a protective agar gel that contains scavengers to eliminate dissolved oxygen, superoxide and free radicals.

Starplex StarSwab II™ Amies gel without charcoal (catalog #SP130X). The gel medium is non-nutritive, highly reductive due to the presence of sodium thioglycollate, and buffered phosphate.

BBL™ Port-A-Cul™ (catalog #43 21607) medium remains moist in a long column of a solid medium that is made of a balanced formula of reducing agents and resazurin in buffered isotonic agar base.

EXPERIMENTAL DESIGN

The anaerobic bacterial strains were grown on 5% reduced sheep blood agar at 37° C for 48 hours in the Bactron anaerobic chamber.

The facultative anaerobic and the aerobic organisms were grown on 5% sheep blood agar at 37°C in a 5% CO₂ incubator for 24 hours. With the exception to the *Clostridium* species, 0.5 McFarland (3 x 10⁸/ml organism) of each of the organisms was made in sterile saline solution. 1.0 McFarland was prepared for the *Clostridium*, species. 1:10 dilution (3 x 10⁷/ml organism) of each of the organisms was made in saline and 100 µl (3 x 10⁶ organism) was used to inoculate each of the swabs evaluated in duplicate. The survival of each of the organisms on each of the swabs at room temperature was evaluated at 0, 6, 24, and 48 hours. At each of the time points, the viable organisms on the swabs were recovered in 1 ml saline and 1:10, 1:100, and 1:1000 serial dilutions were made in saline. In duplicate, 100µl samples were used to quantify the organisms in each of the dilutions on 5% sheep blood agar. The organisms were spread with a plate spreader and were incubated at 37°C in the appropriate incubator.

DATA ANALYSIS

Bacterial recovery was determined by counting the colonies recovered in each of the dilutions. The number of organisms recovered is expressed as an average of duplicate samples evaluated.

RESULTS

Table 1
Recovery of Anaerobic Gram Positive Organisms

SWAB SYSTEM	ORGANISMS EVALUATED															
	<i>C. perfringens</i>				<i>E. lentum</i>				<i>P. acnes</i>				<i>P. anaerobius</i>			
	0h	6h	24h	48h	0h	6h	24h	48h	0h	6h	24h	48h	0h	6h	24h	48h
Copan Vi-Pak Amies	1x10 ⁵	8x10 ⁴	4x10 ⁴	1x10 ⁴	1x10 ⁵	6x10 ⁵	2x10 ⁵	6x10 ⁴	5x10 ⁵	2x10 ⁵	2x10 ⁵	3x10 ⁵	3x10 ⁵	6x10 ³	200	10
Starplex StarSwab II™	1x10 ⁵	8x10 ⁴	2x10 ³	0	2x10 ⁶	5x10 ⁵	3x10 ⁵	1x10 ⁴	4x10 ⁵	4x10 ⁵	1x10 ⁵	2x10 ⁵	2x10 ⁵	10	0	0
BBL™ Port-A-Cul™	2x10 ⁵	6x10 ³	1x10 ³	0	8x10 ⁵	5x10 ⁵	2x10 ⁵	7x10 ⁴	4x10 ⁵	4x10 ⁵	1x10 ⁴	1x10 ⁴	9x10 ⁴	20	0	0

Table 2
Recovery of Anaerobic Gram Negative Organisms

SWAB SYSTEM	ORGANISMS EVALUATED																			
	<i>P. bivia</i>				<i>P. melaninogenica</i>				<i>B. fragilis</i>				<i>F. nucleatum</i>				<i>F. necrophorum</i>			
	0h	6h	24h	48h	0h	6h	24h	48h	0h	6h	24h	48h	0h	6h	24h	48h	0h	6h	24h	48h
Copan Vi-Pak Amies	3x10 ⁵	2x10 ⁷	7x10 ³	0	6x10 ⁷	2x10 ⁵	2x10 ⁷	55	2x10 ⁵	2x10 ⁷	2x10 ³	1x10 ⁵	4x10 ⁷	1x10 ⁷	0	0	3x10 ⁷	2x10 ³	3x10 ³	200
Starplex StarSwab II™	2x10 ⁵	0	0	0	3x10 ³	3x10 ³	0	0	5x10 ⁵	3x10 ³	2x10 ³	4x10 ⁴	3x10 ³	3x10 ³	0	0	1x10 ⁵	7x10 ³	0	0
BBL™ Port-A-Cul™	2x10 ⁵	5x10 ³	500	500	5x10 ³	1x10 ⁴	1x10 ⁴	430	3x10 ⁵	8x10 ⁴	5x10 ³	4x10 ³	3x10 ³	1x10 ³	0	0	3x10 ³	1x10 ³	200	0

Table 3
Recovery of Anaerobic/Facultative Anaerobic Organisms

SWAB SYSTEM	ORGANISMS EVALUATED															
	<i>H. influenzae</i>				<i>N. gonorrhoeae</i>				<i>S. pneumoniae</i>				"S. milleri" group			
	0h	6h	24h	48h	0h	6h	24h	48h	0h	6h	24h	48h	0h	6h	24h	48h
Copan Vi-Pak Amies	1x10 ⁶	5x10 ³	4x10 ³	2x10 ⁵	2x10 ⁵	2x10 ⁵	1x10 ⁴	150	1x10 ⁵	6x10 ³	2x10 ⁴	4x10 ³	4x10 ³	2x10 ³	2x10 ³	1x10 ⁵
Starplex StarSwab II™	4x10 ⁵	3x10 ⁶	200	0	5x10 ⁴	4x10 ⁷	40	0	2x10 ⁵	2x10 ⁷	2x10 ⁷	5x10 ⁵	3x10 ⁷	2x10 ⁷	2x10 ⁷	3x10 ⁷
BBL™ Port-A-Cul™	1x10 ⁶	2x10 ⁷	1x10 ³	2x10 ⁷	5x10 ⁷	1x10 ³	600	0	1x10 ⁵	9x10 ³	8x10 ³	2x10 ³	2x10 ³	8x10 ³	2x10 ³	2x10 ³

DISCUSSION

Diagnosis of anaerobic infections is best achieved by submitting an aspirate of the infected tissue in a transport system that protects the organism from exposure to O₂. However, swab systems validated for maintaining the viability of anaerobic organisms are being utilized for transporting patient samples for anaerobic cultures. In this study we evaluated three different swab transport systems, Starplex StarSwab II™, Copan Vi-Pak Amies Agar Gel transport swabs and BBL™ Port-A-Cul™, for maintaining the viability of different anaerobic organisms. In addition, we challenged these swab systems with maintaining the viability of fastidious aerobic organisms.

When evaluating such swab systems, many factors have to be taken into account. These include the ability of the system to maintain the viability of different anaerobic organisms for an average of 24–48 hours. This is especially important when the patient sample has to be transported to an off-site reference laboratory. In this study Copan Vi-Pak Amies Agar Gel transport swabs outperformed the other two swabs systems. The Copan Vi-Pak swabs have been flushed with Nitrogen gas to maintain optimal Eh potential of the gel medium.

The Copan Vi-Pak swabs maintained the viability of the anaerobic gram positive organisms, *Clostridium perfringens*, *Eubacterium lentum*, and *Propionibacterium acnes*, for up to 48 hours without major loss of viability. However, the other two swab systems did not support *C. perfringens* viability past 24 hours at RT. The fastidious gram positive anaerobic organisms, *P. anaerobius*, did not survive past 0h incubation in the Starplex StarSwab II™, and the BBL™ Port-A-Cul™ swab system. However, it was recovered from the Copan Vi-Pak swabs after 6 hours incubation at RT.

Copan Vi-Pak swabs also outperformed the other two swabs in maintaining the viability of anaerobic gram negative organisms. Copan Vi-Pak swabs maintained the viability of *P. bivia* and *F. necrophorum* past 6 hours incubation in BBL™ Port-A-Cul™ swab. All three swabs supported the viability of *F. nucleatum* for 6 hours at RT.

The Copan Vi-Pak swabs further supported the viability of the fastidious aerobic organisms for 24–48 hours. Starplex StarSwab II™ did not support the viability of *N. gonorrhoeae* and *H. influenzae* past 6 hours incubation at RT while the BBL™ Port-A-Cul™ swabs has 3 log₁₀ reduction in maintaining the viability of *H. influenzae* after 48 hours incubation at RT.

Other factors that should be taken in consideration are the ease of use, whether the swabs are leak-proof, cost effectiveness, and durability. Copan Vi-Pak swabs and Starplex StarSwab II™ are easy to use, not expensive, leak-proof and do not break if accidentally dropped. On the other hand, the BBL™ Port-A-Cul™ swab system needs manipulation of the wooden swab to make it fit the tube, is breakable if dropped, and more expensive than the other two swab systems.

Finally, BBL™ Port-A-Cul™ swab system can be used as a transport system for transporting tissue samples and fluids. This needs to be further evaluated for the Copan Vi-Pak swab system

CONCLUSIONS

1. Copan Vi-Pak swabs outperformed the other two swabs evaluated for maintaining the viability of anaerobic gram positive and gram negative organisms, and fastidious aerobic organisms.
2. Copan Vi-Pak swabs' Nitrogen gas flushing appears to help in maintaining optimal Eh potential of the gel medium, thus supporting the viability of anaerobic organisms.
3. Copan Vi-Pak swabs are 1/3 – 1/2 the price of other swab systems, easy to use and unbreakable.
4. We rank Copan Vi-Pak swabs as the best of the three evaluated, followed by BBL™ Port-A-Cul™ swab, and lastly, the Starplex StarSwab II™ swabs system.

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