

EVALUATION OF TWO SWAB TRANSPORT SYSTEMS FOR THE RECOVERY OF SIXTEEN BACTERIAL SPECIES OF CLINICAL SIGNIFICANCE

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ABSTRACT

One of the crucial factors affecting the laboratory's capability of detecting pathogens is the efficacy of the clinical specimen transport system in maintaining organism viability until the specimen is transported and fully processed. A total of 960 Amies charcoal-free swabs, Copan Venturi Transystem 108C (COP, Copan Diagnostics, Inc., Corona, California) and the newly-modified Starswab SP130X (STR, Starplex Scientific, Etobicoke, Ontario, Canada) were dipped in either 3×10^5 CFU/L (10^5) or 3×10^8 CFU/L (10^8) suspensions of 16 ATCC and clinical strains. Triplicate swabs per organism held for 0, 6, 24, 48 and 72h, were plated and discarded, and their growths were graded. In the 10^8 -seeded swabs, both COP and STR supported up to 72h the viability of 9 common and fastidious aerobes and 3 anaerobes; but STR failed to support viability at 48h for *Fusobacterium necrophorum* (Fn), *Neisseria gonorrhoeae* (Ng), *Peptostreptococcus anaerobius* (Pa), and *Prevotella melaninogenica* (Pm). In the 10^5 -seeded swabs, both COP and STR sustained 7 aerobic and 2 anaerobic organisms up to 72h; however, STR failed to maintain viability for Ng at 24h, Fn, Pa, and Pm at 48h, and *Clostridium perfringens* and *Haemophilus influenzae* at 72h, whereas COP only failed to sustain viability of *Streptococcus pneumoniae* after 24h. We conclude that while both COP and STR provided excellent support for viability of most organisms for up to 72h, COP was more likely to sustain viability of most fastidious aerobes and most anaerobes for longer durations than STR.

INTRODUCTION

One of the crucial factors that directly affect the clinical laboratory's ability to detect and identify pathogens in patient specimens is the efficacy of the specimen transport system in maintaining organism viability until the specimen is transported and fully processed. Although a specimen should be transported to the laboratory as soon as possible, unexpected delays have demonstrated the need for an adequate specimen transport system that would enable the laboratory to return accurate and relevant test results in a timely manner.

This study was conducted to evaluate two Amies charcoal-free swab transport systems, Copan Venturi Transystem 108C (COP, Copan Diagnostics, Inc., Corona, California, USA) and the newly-modified Starswab SP 130X (STR, Starplex Scientific, Etobicoke, Ontario, Canada) for their abilities to maintain for up to 72 hours the viability of sixteen organisms, 10 common and fastidious aerobes, and 6 anaerobes. The selection of each of the 16 bacterial species for this evaluation was associated with the importance of its recoverability from patient swab specimens in the clinical laboratory. It is crucial for an accurate evaluation of such a basic requirement for culture that not only fastidious organisms be tested, but also commonly-investigated pathogens, such as those found in wound swabs and in rectal swabs. We simulated actual clinical laboratory practice by testing the swab recovery of these organisms in two different suspensions, at concentrations of 3×10^5 CFU/L (10^5) and 3×10^8 CFU/L (10^8). Test swabs were also processed in the same manner as clinical specimens are routinely handled in the laboratory.

METHODS

1. PREPARATION OF BACTERIAL WORKING SUSPENSIONS: Pure cultures were prepared from stock ATCC strains and pre-identified clinical isolates, which were subcultured 3 times prior to commencing the evaluation to ensure strain purity and stability. The selected aerobes comprised *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Haemophilus influenzae* ATCC 19418, *Neisseria gonorrhoeae* ATCC 49226, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella heidelberg* (clinical isolate), *Shigella sonnei* (clinical isolate), *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 6305, and *Streptococcus pyogenes* ATCC 19615. Anaerobic strains comprised *Bacteroides fragilis* ATCC 25285, *B. thetaiotamicron* ATCC 29741, *Clostridium perfringens* ATCC 13124, *Fusobacterium necrophorum* ATCC 25286, *Peptostreptococcus anaerobius* ATCC 27337, and *Prevotella melaninogenica* ATCC 25845. Suspensions of test organisms were prepared using sterile trypticase soy broth to yield 10^5 and 10^8 test concentrations.

2. PREPARATION OF REFERENCE SUSPENSION, REFERENCE PLATES, AND SWAB-TIP INOCULA:

A 3×10^3 CFU/L (10^3) reference suspension was prepared for each test organism. A 0.1 mL of the suspension was pipetted to prepare reference plates in duplicate, using the spread plate method. Following incubation, colony count was performed on each of the reference plates. Inoculum size was calculated as the concentration of working solution (average number of colonies recovered on reference plate x dilution factor used x 10) x 0.2 (amount of suspension absorbed by swab tip in mL).

3. INOCULATION OF SWAB TRANSPORT MEDIA AND PLATES:

A total of 960 (480 COP and 480 STR) swabs were inoculated in parallel, with both swab systems equally tested in both 10^5 and 10^8 concentrations of each bacterial species. The swab was removed from its device and dipped in the suspension for 3-4 seconds. It was then re-inserted into the tube of the transport medium, followed by inoculation of a pre-labelled agar plate, by rolling the swab evenly onto the agar area of the primary inoculum. Triplicate swabs per organism held at room temperature for 0, 6, 24, 48 and 72h, were plated, then discarded. The IsoPlater (Vista Laboratories) was used to streak inoculated plates, which were subsequently incubated under appropriate atmospheric conditions at 36°C.

4. EFFECT OF SUCCESSIVE INOCULATION ON BACTERIAL RECOVERY:

After the first plate was inoculated, the same swab was used to inoculate two successive pre-numbered plates without reinsertion of the swab back into its transport device. This part of the study was limited to five organisms representing bacteria commonly seen in mixed growths; we studied *Neisseria gonorrhoeae*, *Salmonella heidelberg*, *Shigella sonnei*, *Staphylococcus aureus*, and *Streptococcus pyogenes*.

5. EVALUATION OF GROWTH:

Growth was graded by dividing the plate into 4 quadrants and examining growth in each. Wherever an organism failed to be recovered within 24 hours in all triplicate plates of either COP or STR or both, the procedure was entirely repeated. The recovery of each organism at each concentration tested from either COP or STR was calculated as an Average Growth Value obtained for each set of triplicates. Growth was graded using the following numeric codes (adapted from reference 5).

- 0: No growth
- 1: 1-50 colonies in 1st quadrant (primary inoculum).
- 2: >50 colonies in 1st quadrant.
- 3: Growth in 1st and 2nd quadrants.
- 4: Growth in 1st, 2nd, and 3rd quadrants.
- 5: Growth in 1st, 2nd, 3rd, and 4th quadrants.

RESULTS AND DISCUSSION

The results of this work are of direct relevance to the clinical microbiology laboratory, because failure of the swab transport system to retain viability of the infectious agent may result in misdiagnosis, inappropriate therapy, and inadequate control of infection transmission. However, maintaining viability should be accomplished without enhancing bacterial multiplication in the swab, because clinical swabs often yield mixed bacteria that may mask pathogens with slower metabolic rates. As a result, both COP and STR use inorganic phosphate in their formulations of Amies, a non-proliferative transport medium.

Our findings indicate that bacterial yield from swabs inoculated with 10⁵ concentration of the common aerobes increased as the swabs were held for longer durations, whereas that of fastidious organisms declined rapidly after an initial log phase of activity (Table 1). In contrast, the yields at the 10⁸ concentration were constant for most aerobes, except for two fastidious organisms, *Neisseria gonorrhoeae* and *Streptococcus pneumoniae*. At both concentrations, anaerobe yields also gradually declined, except for the bacteroides (Table 2), demonstrating the fragility of most fastidious aerobes and most anaerobes. These findings are in agreement with those of other investigators.

When multiple plates were successively inoculated by the same swab without reinserting the swab into its device between the inoculations, there was no significant loss of recovery attributable to the order at which the plate was inoculated.

While this study graded growth as a measure of the viability over a period of 72 hours, the key test of performance was whether the swab was able to yield growth even in small quantities after prolonged intervals. The ability to sustain viability was particularly crucial for fastidious and anaerobic organisms, especially at the lower concentration. For this reason the failure of COP to sustain the viability of 10⁵ *Streptococcus pneumoniae* at 48h (i.e., after 24h), and STR to sustain that of *Neisseria gonorrhoeae* at 24 and 48h, in 10⁵ and 10⁸ concentrations, respectively, is significant.

With the increasing reluctance against using different systems for transporting anaerobes, and because the isolation of an infectious anaerobic organism is directly related to the selection of appropriate antibiotic therapy, it is important that a multi-purpose swab system be capable of maintaining anaerobe viability for a reasonable time. Both COP and STR maintained well the viability of anaerobes for the first 24h, but STR was unable to yield any growth at 48h and beyond, of three anaerobes (*Fusobacterium necrophorum*, *Peptostreptococcus anaerobius*, and *Prevotella melaninogenica*), as well as *Clostridium perfringens* in its lower concentration at 72h.

Although both COP and STR appeared to provide equivalent swab absorbancy, STR often provided more luxurious growth where it was able to maintain organism viability. On the other hand, its ample fibrous material may have accounted for organism entanglement and reduced release, resulting in loss of recovery. While both COP and STR swab tips were made of rayon, a characteristic supporting good absorption of organisms from the sample, the complexity of the finished product may have added another variable in the evaluation. Furthermore, only COP contained oxygen scavengers through flushed nitrogen gas, which may have enhanced the survivability of certain organisms for longer durations, by reducing free oxygen in the tube.

We hope that this study will stimulate further research within the industry and the scientific community to continuously improve swab transport systems in the future.

CONCLUSION

Both COP and STR provide excellent support for viability of most organisms for up to 72h. Both swab transport systems are easy to use and to batch during specimen processing. COP is more likely to sustain viability of most fastidious aerobes and most anaerobes for longer durations than STR.

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Table 1. Comparison of the Average Growth Values* of common and fastidious aerobes recovered from COP and STR in two concentrations at different holding periods.

Organism	Concentration	IS**	0h		6h		24h		48h		72h	
			Copan	STR	Copan	STR	Copan	STR	Copan	STR	Copan	STR
<i>Enterococcus faecalis</i>	10 ⁵	0.26x10 ⁵	2.0	2.7	2.3	3.0	3.3	4.7	4.7	4.7	5.0	5.0
	10 ⁸	0.26x10 ⁸	5.0	5.0	5.0	5.0	4.7	5.0	5.0	5.0	5.0	5.0
<i>Escherichia coli</i>	10 ⁵	0.30x10 ⁵	2.0	2.3	2.3	2.7	4.7	5.0	5.0	5.0	5.0	5.0
	10 ⁸	0.30x10 ⁸	4.7	5.0	4.7	5.0	4.7	5.0	5.0	5.0	5.0	5.0
<i>Haemophilus influenzae</i>	10 ⁵	0.41x10 ⁵	2.0	2.0	2.0	2.0	1.7	1.3	1.3	0.7	1.3	0
	10 ⁸	0.41x10 ⁸	4.7	4.7	4.7	5.0	5.0	5.0	4.7	4.3	5.0	5.0
<i>Neisseria gonorrhoeae</i>	10 ⁵	0.24x10 ⁵	2.3	2.7	3.3	3.7	1.7	0	1.3	0	1.3	0
	10 ⁸	0.24x10 ⁸	5.0	5.0	5.0	5.0	5.0	4.7	4.3	0	4.3	0
<i>Pseudomonas aeruginosa</i>	10 ⁵	0.27x10 ⁵	1.7	2.7	2.3	2.7	2.3	3.7	3.0	4.7	4.0	5.0
	10 ⁸	0.27x10 ⁸	5.0	5.0	5.0	5.0	4.3	5.0	5.0	5.0	5.0	5.0
<i>Salmonella heidelberg</i>	10 ⁵	0.24x10 ⁵	1.3	2.0	2.0	2.3	5.0	5.0	5.0	5.0	5.0	5.0
	10 ⁸	0.24x10 ⁸	4.7	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.7	5.0
<i>Shigella sonnei</i>	10 ⁵	0.20x10 ⁵	1.0	2.0	5.0	5.0	5.0	5.0	5.0	5.0	4.3	5.0
	10 ⁸	0.20x10 ⁸	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.7	5.0
<i>Staphylococcus aureus</i>	10 ⁵	0.18x10 ⁵	2.0	3.0	2.3	4.0	3.7	5.0	4.3	4.7	5.0	5.0
	10 ⁸	0.18x10 ⁸	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
<i>Streptococcus pneumoniae</i>	10 ⁵	0.20x10 ⁵	1.0	1.7	1.3	2.7	1.0	2.0	0	1.3	0	1.3
	10 ⁸	0.20x10 ⁸	4.0	4.3	5.0	5.0	3.3	5.0	2.0	4.3	1.0	3.0
<i>Streptococcus pyogenes</i>	10 ⁵	0.15x10 ⁵	1.0	2.0	3.0	3.3	4.0	4.0	3.3	3.0	4.7	3.7
	10 ⁸	0.15x10 ⁸	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.3	5.0	5.0

* The Average Growth Value per triplicate set was calculated by dividing the total of graded growths by 3 (for grading codes, see METHODS, no. 5)

**IS: inoculum size (for calculation see METHODS, no. 2)

Table 2. Comparison of the Average Growth Values* of anaerobic organisms recovered from COP and STR in two concentrations at different holding periods.

Organism	Concentration	IS**	0h		6h		24h		48h		72h	
			Copan	STR	Copan	STR	Copan	STR	Copan	STR	Copan	STR
<i>Bacteroides fragilis</i>	10 ⁵	0.42x10 ⁵	2.0	2.3	2.0	2.3	2.0	2.7	2.0	2.0	2.0	2.0
	10 ⁸	0.42x10 ⁸	5.0	5.0	4.3	4.7	4.7	4.7	4.3	4.3	4.7	4.7
<i>Bacteroides thetaiotamicron</i>	10 ⁵	0.09x10 ⁵	2.0	2.0	2.0	2.0	1.7	4.0	2.7	4.7	4.0	4.0
	10 ⁸	0.09x10 ⁸	4.7	5.0	4.3	4.7	5.0	5.0	5.0	5.0	4.7	4.7
<i>Clostridium perfringens</i>	10 ⁵	0.07x10 ⁵	1.0	1.3	1.0	0.3	1.0	0.7	1.0	0.3	0.7	0
	10 ⁸	0.07x10 ⁸	4.3	4.7	3.0	2.7	4.7	5.0	5.0	5.0	2.3	2.7
<i>Fusobacterium necrophorum</i>	10 ⁵	0.16x10 ⁵	1.0	1.0	1.0	1.0	0.3	0.3	0.3	0	0.3	0
	10 ⁸	0.16x10 ⁸	2.3	2.3	3.3	3.7	2.3	2.0	2.3	0	1.3	0
<i>Peptostreptococcus anaerobius</i>	10 ⁵	0.20x10 ⁵	2.0	2.0	1.7	1.3	1.3	0.3	0.3	0	0.3	0
	10 ⁸	0.20x10 ⁸	4.0	4.0	4.0	3.7	5.0	0.3	3.3	0	1.3	0
<i>Prevotella melaninogenica</i>	10 ⁵	0.14x10 ⁵	2.0	1.3	1.7	0.3	1.3	0.3	1.0	0	0.3	0
	10 ⁸	0.14x10 ⁸	4.7	4.7	3.7	2.7	4.7	0.3	3.0	0	1.0	0

* The Average Growth Value per triplicate set was calculated by dividing the total of graded growths by 3 (for grading codes, see METHODS, no. 5)

**IS: inoculum size (for calculation see METHODS, no. 2)

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