

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

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Received 4 August 2000/Returned for modification 29 September 2000/Accepted 20 October 2000

Of utmost importance in evaluations of clinical samples for infectious agents is proper specimen transport to the clinical laboratory. In the present study we compared three transport systems (the new Starplex StarSwab II, the new Copan Vi-Pak Amies Agar Gel collection and transport swabs, and the BBL Port-A-Cul) for survival of anaerobic and fastidious aerobic bacteria. The new Copan Vi-Pak system has been modified by nitrogen gas flushing to keep an ideal low E_h condition and to prevent oxidation of the transport medium. The Copan Vi-Pak system outperformed the other two swabs evaluated by maintaining the viabilities of both anaerobic and fastidious aerobic bacteria for 24 h for the majority of the organisms evaluated. This time period should be sufficient for transport of specimens to the clinical microbiology laboratory without compromising organism recovery.

The successful isolation of anaerobes largely depends on proper specimen collection and transport to the clinical microbiology laboratory. During specimen transport, protection of the anaerobic bacteria from desiccation and oxygen exposure is a critical step in the recovery of these organisms (1, 3, 5). It is well established that tissue biopsy specimens, aspirates of fluids, and exudates from suspected infected sites are superior to samples collected on swabs (3). 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, clinical microbiology laboratories continue to receive clinical specimens on swabs (4). Swab transport systems with semi-solid media have been developed for the transport of patient samples for anaerobic cultures. Moreover, these swabs have been shown to protect both anaerobic and fastidious aerobic organisms (5). A single transport system for the isolation of both aerobic and anaerobic organisms seems most cost-effective (4). In our reference laboratory, samples are submitted for aerobic and anaerobic cultures from all over the United States. A transport system that will maintain organism viability for 24 to 48 h while in transit is of utmost importance.

The aim of this study was to compare the performances of

the new Starplex StarSwab II (SSS) system (Starplex Scientific, Etobicoke, Ontario, Canada) and Copan Vi-Pak Amies Agar Gel collection and transport swab (CVP) system (Copan Diagnostic Inc., Corona, Calif.) to the BBL Port-A-Cul (PAC) system (Becton Dickinson Microbiology Systems, Sparks, Md.) in maintaining the viabilities of anaerobic, facultative anaerobic, and fastidious aerobic bacteria.

The survival of the following isolates in the three transport systems was evaluated: nine anaerobic strains (*Clostridium perfringens* 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), one facultatively anaerobic strain (“*Streptococcus milleri*” group [clinical isolate]), and three aerobic bacterial strains (*Haemophilus influenzae* ATCC 10211, *Neisseria gonorrhoeae* ATCC 43069, and *Streptococcus pneumoniae* ATCC 49619).

The new CVP swab is an Amies culture swab that has been flushed with nitrogen gas to maintain optimal E_h potential of the gel medium. The medium is a protective agar gel that contains scavengers that eliminate dissolved oxygen, superoxide, and free radicals. In addition, the swab container is pinched right above the gel medium in order to reduce the surface area for oxygen diffusion and to prevent the semi-solid gel from moving, thus protecting the swab tip from desiccation or O_2 exposure. SSS swab is nonnutritive and highly reductive due to the presence of sodium thioglycolate, and it is phosphate buffered. The PAC system is an

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TABLE 1. Recovery of anaerobic gram-positive organisms

Swab system	Organism	No. (%) of evaluated organisms recovered at:			
		0 h	6 h	24 h	48 h
CVP	<i>C. perfringens</i>	1 × 10 ⁵ (100)	8 × 10 ⁴ (80)	4 × 10 ⁴ (40)	1 × 10 ⁴ (10)
	<i>E. lentum</i>	1 × 10 ⁶ (100)	6 × 10 ⁵ (60)	2 × 10 ⁵ (20)	6 × 10 ⁴ (6)
	<i>P. acnes</i>	5 × 10 ⁵ (100)	2 × 10 ⁵ (40)	2 × 10 ⁵ (40)	3 × 10 ⁵ (60)
	<i>P. anaerobius</i>	3 × 10 ⁴ (100)	6 × 10 ³ (20)	200 (0.7)	10 (0.03)
SSS	<i>C. perfringens</i>	1 × 10 ⁵ (100)	8 × 10 ⁴ (80)	2 × 10 ³ (2)	0 (0)
	<i>E. lentum</i>	2 × 10 ⁶ (100)	5 × 10 ⁵ (25)	3 × 10 ⁵ (15)	1 × 10 ⁴ (0.5)
	<i>P. acnes</i>	4 × 10 ⁵ (100)	4 × 10 ⁵ (100)	1 × 10 ⁵ (25)	2 × 10 ⁵ (50)
	<i>P. anaerobius</i>	2 × 10 ⁵ (100)	10 (0.01)	0 (0)	0 (0)
PAC	<i>C. perfringens</i>	2 × 10 ⁵ (100)	6 × 10 ³ (3)	1 × 10 ³ (0.5)	0 (0)
	<i>E. lentum</i>	8 × 10 ⁵ (100)	5 × 10 ⁵ (63)	2 × 10 ⁵ (25)	7 × 10 ⁴ (9)
	<i>P. acnes</i>	4 × 10 ⁵ (100)	4 × 10 ⁴ (10)	1 × 10 ⁴ (2.5)	1 × 10 ⁴ (2.5)
	<i>P. anaerobius</i>	9 × 10 ⁴ (100)	20 (0.02)	0 (0)	0 (0)

Amies-based gel that remains moist in a long column of a solid medium that is made of a balanced formula of reducing agents in buffered isotonic agar base. PAC is the only system that incorporates resazurin as an indicator of reduced conditions.

The anaerobic bacterial strains were grown on 5% reduced Columbia sheep blood agar (SBA) at 37°C for 48 h in a Bactron IV anaerobic chamber. The facultative anaerobic organisms and the aerobic organisms were grown on 5% SBA at 37°C in a 5% CO₂ incubator for 24 h. With the exception of the *Clostridium* species, a 0.5 McFarland standard (10⁸ cells/ml) of each of the organisms was made in sterile saline solution. A 1.0 McFarland standard was prepared for *C. perfringens* due to its large cell size. A 1:10 dilution (10⁷ cells/ml) of each of the organisms was made in saline, and 100 μl (10⁶ organisms) was used to inoculate each of the swabs evaluated. The survival of the organisms on each of the swabs at room temperature was

determined at 0, 6, 24, and 48 h. At each of these time points, the viable organisms on the swabs were recovered in 1 ml of saline after vortexing of the swab for 30 s, and 1:10, 1:100, and 1:1,000 serial dilutions were made in sterile saline. In duplicate, 100-μl samples were used to quantify the organisms in each of the dilutions on 5% SBA. The organisms were spread over the agar surface with a plate spreader, and the plates were incubated at 37°C in the appropriate incubator. Bacterial recovery was determined by counting the colonies recovered in each of the dilutions. The number of organisms recovered is expressed as an average for duplicate samples evaluated and as a percentage of the baseline counts (counts at time zero).

Survival of the anaerobic gram-positive bacteria is shown in Table 1. The recovery of all of the gram-positive organisms was poor at 24 h or greater for all three swabs evaluated. Overall, the CVP swab system yielded the best

TABLE 2. Recovery of anaerobic gram-negative organisms

Swab system	Organism	No. (%) of evaluated organisms recovered at:			
		0 h	6 h	24 h	48 h
CVP	<i>P. bivia</i>	3 × 10 ⁵ (100)	2 × 10 ⁵ (67)	7 × 10 ³ (2)	0 (0)
	<i>P. melaninogenica</i>	6 × 10 ⁵ (100)	2 × 10 ⁵ (33)	2 × 10 ⁴ (3)	55 (0.01)
	<i>F. nucleatum</i>	3 × 10 ⁴ (100)	1 × 10 ⁴ (33)	0 (0)	0 (0)
	<i>F. necrophorum</i>	2 × 10 ⁵ (100)	8 × 10 ³ (4)	1 × 10 ³ (0.5)	100 (0.05)
	<i>B. fragilis</i>	4 × 10 ⁵ (100)	5 × 10 ⁵ (125)	2 × 10 ⁵ (40)	2 × 10 ⁵ (50)
SSS	<i>P. bivia</i>	2 × 10 ⁵ (100)	0 (0)	0 (0)	0 (0)
	<i>P. melaninogenica</i>	3 × 10 ⁵ (100)	3 × 10 ³ (1)	0 (0)	0 (0)
	<i>F. nucleatum</i>	1 × 10 ⁵ (100)	1 × 10 ⁵ (100)	0 (0)	0 (0)
	<i>F. necrophorum</i>	5 × 10 ⁴ (100)	4 × 10 ³ (8)	0 (0)	0 (0)
	<i>B. fragilis</i>	3 × 10 ⁶ (100)	4 × 10 ⁶ (133)	5 × 10 ⁵ (17)	5 × 10 ⁵ (17)
PAC	<i>P. bivia</i>	2 × 10 ⁵ (100)	5 × 10 ³ (3)	500 (0.3)	500 (0.3)
	<i>P. melaninogenica</i>	5 × 10 ⁵ (100)	1 × 10 ⁴ (2)	1 × 10 ⁴ (2)	430 (0.1)
	<i>F. nucleatum</i>	2 × 10 ⁵ (100)	1 × 10 ⁵ (50)	0 (0)	0 (0)
	<i>F. necrophorum</i>	2 × 10 ⁵ (100)	5 × 10 ⁴ (20)	200 (0.1)	0 (0)
	<i>B. fragilis</i>	3 × 10 ⁵ (100)	1 × 10 ⁵ (33)	1 × 10 ⁵ (33)	4 × 10 ⁵ (133)

TABLE 3. Recovery of aerobic and facultative anaerobic organisms

Swab system	Organism	No. (%) of evaluated organisms recovered at:			
		0 h	6 h	24 h	48 h
CVP	<i>H. influenzae</i>	1 × 10 ⁶ (100)	5 × 10 ⁵ (50)	4 × 10 ⁵ (40)	2 × 10 ⁵ (20)
	<i>N. gonorrhoeae</i>	2 × 10 ⁵ (100)	2 × 10 ⁵ (100)	1 × 10 ⁴ (5)	150 (0.1)
	<i>S. pneumoniae</i>	1 × 10 ⁵ (100)	6 × 10 ⁴ (60)	2 × 10 ⁴ (20)	4 × 10 ³ (4)
	" <i>S. milleri</i> " group	4 × 10 ⁵ (100)	2 × 10 ⁵ (50)	2 × 10 ⁵ (50)	1 × 10 ⁵ (25)
SSS	<i>H. influenzae</i>	4 × 10 ⁵ (100)	3 × 10 ⁴ (8)	200 (0.01)	0 (0)
	<i>N. gonorrhoeae</i>	5 × 10 ⁴ (100)	4 × 10 ³ (8)	40 (0.1)	0 (0)
	<i>S. pneumoniae</i>	2 × 10 ⁵ (100)	2 × 10 ⁴ (10)	2 × 10 ⁴ (10)	5 × 10 ³ (3)
	" <i>S. milleri</i> " group	3 × 10 ⁵ (100)	2 × 10 ⁵ (67)	2 × 10 ⁴ (6)	3 × 10 ⁴ (10)
PAC	<i>H. influenzae</i>	1 × 10 ⁶ (100)	2 × 10 ⁴ (2)	1 × 10 ⁴ (1)	2 × 10 ³ (0.2)
	<i>N. gonorrhoeae</i>	5 × 10 ⁴ (100)	1 × 10 ³ (2)	600 (1)	0 (0)
	<i>S. pneumoniae</i>	1 × 10 ⁵ (100)	9 × 10 ³ (9)	800 (1)	2 × 10 ³ (2)
	" <i>S. milleri</i> " group	2 × 10 ⁵ (100)	8 × 10 ⁴ (40)	2 × 10 ⁵ (100)	2 × 10 ⁵ (100)

recovery. The CVP swab system maintained 10% of the *C. perfringens* isolates after 48 h of incubation; however, no recovery was observed with either the SSS or the PAC system at 48 h of incubation. The CVP system also performed better at maintaining the viability of the fastidious anaerobic organism *P. anaerobius*. After 6 h of incubation, 20% of the viable organisms were recovered in the CVP system, while 0.01 and 0.02% were recovered in SSS and PAC systems, respectively. Even though few viable organisms were recovered at 24 and 48 h in the CVP system, none were recovered from the other two swabs. The CVP and PAC systems had similar performances with regard to *E. lentum*, while the CVP and SSS systems had similar performances with respect to *P. acnes*. The survivability of the organisms *P. anaerobius*, *C. perfringens*, and *E. lentum* in the PAC system is similar to that reported by Perry (4). However, the data obtained in our study cannot be compared to those of Hudspeth et al. (1) since they used a larger inoculum (4 or 5 McFarland standard) to evaluate the transport swabs. The use of large inocula may overwhelm the swab systems. In addition, on average, patient samples contain 10³ to 10⁵ organisms per ml. This is similar to the density of organisms used in this study and what others have reported from clinical samples (5, 6).

The survival of the anaerobic gram-negative bacteria was maximized with the CVP and PAC systems (Table 2). With the exception of *P. bivia*, in which 67% of the organisms were recovered after 6 h of incubation in the CVP system, similar results were obtained with the PAC system. The SSS system performed similarly with three of the organisms tested; however, the SSS system performed poorly for the recovery of *Prevotella* species. In two systems, the CVP and PAC systems, *B. fragilis* numbers increased after 48 h of incubation. This has previously been reported for PAC by Perry (4).

None of the three systems maintained the viabilities of the fastidious aerobic organisms beyond 24 h (Table 3). The CVP system seemed to be able to preserve viabilities for more than half of the inocula of *N. gonorrhoeae*, *H. influenzae*, and *S. pneumoniae* for up to 6 h, whereas there was a steep drop-off

in the other two systems. Similar to the observation for *B. fragilis*, *S. milleri* numbers increased at 48 h or longer, especially in the PAC medium.

Swab systems are not the best way to collect patient samples for either aerobic or anaerobic specimens. However, when swabs are the only choice, the new CVP swabs appear to be an acceptable choice for maintenance of the viability of aerobic and anaerobic organisms for up to 24 h. CVP swabs are less expensive than the other swabs evaluated. Indeed, CVP swabs are one-third the price of the PAC system. Another advantage to the CVP system is ease of use since no manipulations are required of the system. In contrast, with the PAC system the wooden swab must be broken after the sample is introduced to get it to fit into the transport tube. This is potentially hazardous for the person preparing the sample for transport, as the samples can splash if the wooden swab is not carefully broken. In addition, the broken wooden swab may pose a puncture hazard to a laboratorian trying to process the sample. Lastly, the CVP swabs are made of unbreakable plastic material, whereas the PAC swabs are made of glass and they are easily broken if dropped.

While none of these swab systems is ideal, based upon the viability data, cost issues, and ease of use, the CVP swabs can be recommended for use with patient specimens that are destined to be plated within 24 h of collection. On the basis of the results of the evaluation reported herein, the routine use of the other two swabs, the PAC swabs or the new SSS system, cannot be recommended at this time.

We thank Copan Diagnostics Inc. for sponsoring part of the study, Jim Kucera for critical review of the manuscript, and Ann Croft for technical support and suggestions.

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