

EVALUATION OF THE COPAN VENTURI TRANSYSTEM FOR ANAEROBES USING CLINICAL SPECIMENS

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ABSTRACT

The Copan Venturi Transystem (CVT) has been shown to maintain viability of pure cultures of anaerobic bacteria for 24h to 48h. We evaluated the ability of this system to support anaerobes in clinical specimens. Excess pus was obtained from surgery patients and placed into Port-a-cul vials for transport to the research laboratory. 0.1 ml of the pus was inoculated onto 3 CVT swabs and 3 Port-a-cul swabs and kept at room temperature. A control sample was inoculated onto supplemented brucella, PEA, LKV and BBE agars at the beginning of the experiment (time 0h). The inoculated swabs were transferred into the anaerobic chamber after 4, 24, and 48h and cultured by suspending the contents into 1 ml brucella broth, and inoculating each of the above media with 2 drops. After 5 days of incubation, the different colony types were described and growth was quantitated and compared to growth obtained at the 0 time. The ten evaluable specimens yielded 53 strains of anaerobic bacteria, including *B. fragilis* group (19), *Bilophila wadsworthia* (3), *Clostridium* sp. (13), *Eubacterium* sp. (4), *Fusobacterium* sp. (1), *Lactobacillus* sp. (1), *Porphyromonas* sp. (2), *Prevotella* sp. (5), and *Peptostreptococcus* sp. (5). After 4h in the transporters, all except one strain of a *Clostridium* sp. were recovered. After 24h, one strain each of *C. innocuum* and *P. melaninogenica* were not detected from the CVT, and one strain of *Porphyromonas* was not detected from the Port-a-cul swab. After 48h, 8 strains were not recovered from the CVT and 4 were not recovered from the Port-a-cul, especially strains that were originally present in small numbers in the specimen. These included some isolates of *Clostridium*, *F. nucleatum*, *Porphyromonas*, *Prevotella* and *Peptostreptococcus*. The two systems are similar in their abilities to maintain anaerobic bacteria.

INTRODUCTION

Protection of anaerobic bacteria from exposure to oxygen during the transport of clinical specimens to the laboratory is crucial for the survival of these organisms. We previously evaluated the Venturi Transystem (Copan Diagnostics, Corona, CA) for its ability to maintain the viability of 5 ATCC and 5 clinical strains of anaerobic bacteria and found it to be comparable to the Port-a-cul swab transport system (Becton Dickinson Microbiology Systems, Cockeysville, MD) (1). In the present study, we obtained excess pus collected at surgery from patients with infections and used it to compare the abilities of these two swab systems to maintain the viability of the isolates after 4h, 24h, and 48h. The transported samples were plated onto selective and non-selective media for isolation of aerobic and anaerobic bacteria. After incubation, the different species were enumerated and identified and compared to recovery at the 0 time.

MATERIALS

Specimens: Specimens consisting of at least 2 ml of pus were collected from patients undergoing surgical procedures and injected into a Port-a-cul vial (BD) or Anaerobic Transport System (Anaerobic Systems, Morgantown, CA) with a syringe. The vials were transported to our research laboratory for further processing, usually within 10 hours. The specimens were screened by gram-stain for the presence of multiple types of organisms suggestive of anaerobes prior to entry into the study. The specimen sources were as follows: peritoneal fluid (6), rectal abscess (2), pelvic abscess (1), and chest drainage (1)

Transport Systems: The Copan Venturi Transystem (CVT) system consists of a plastic transport tube containing Amies medium without charcoal. The plastic sheath is pinched above the semi-solid medium to prevent spillage and to reduce the surface area for oxygen diffusion. The tube is flushed with nitrogen prior to sealing with a plastic screw-cap. The tube and swab are then packaged in a gas impermeable plastic wrap that is flushed with nitrogen prior to sealing to provide an oxygen free environment.

The Port-a-cul system consists of a screw-capped glass tube with a deep column of reduced Cary-Blair semi-solid agar medium. The inoculated swab is placed deep into the agar.

METHODS

The transporter containing the pus was brought into the anaerobic chamber, and 0.1 ml was placed onto a swab. The swab was resuspended into 0.9 ml of brucella broth, and two drops were immediately inoculated onto supplemented brucella blood agar, and supplemented phenylethyl alcohol blood agar. One drop was inoculated onto each half of a biplate containing laked blood agar with kanamycin and vancomycin, and Bacteroides-bile-esculin (BBE) agar. The original pus and the swab suspension were removed from the anaerobic chamber. Two drops of the suspension were inoculated onto plates of MacConkey, TSA-blood and Rose agars for culture of aerobic and facultative organisms. This represented the "0" time culture.

Using a tuberculin syringe, 0.1 ml of the pus was inoculated onto three sets of swabs each for the CVT and Port-a-cul systems for subsequent culture at 4h, 24h and 48h. At the indicated times, the swabs were brought into the anaerobic chamber and suspended in 0.9 ml of brucella broth. Anaerobic and aerobic media were inoculated as described above. After incubation, the different colony types were quantitated as 1+ to 4+ and identified according to standard methods. If growth occurred on more than one type of media, the one with the greatest quantity was used for calculating the growth score. The growth scores of all isolates within each group were averaged.

RESULTS

The results are presented in Table 1

After 4h, one isolate of *C. subterminale* was not recovered from either system.

After 24h, one strain each of *C. innocuum* and *Pr. melaninogenica* were not recovered from the CVT system, and one strain of *Por. asaccharolytica* was not recovered from the Port-a-cul.

After 48h, the CVT did not support the viability of both strains of *Por. asaccharolytica* and one strain each of *Pr. intermedia*, *Pr. bivia*, *F. nucleatum*, and *Ps. magnus*. Port-a-cul did not support viability of *Pr. intermedia*, *F. nucleatum* and *Ps. magnus*. Some of the aerobic organisms, especially *E. coli*, appeared to grow in both systems. One strain of *Citrobacter koseri* was overgrown by *E. coli* and *K. pneumoniae* in both systems and was not recovered after 24 and 48h. One strain of *P. aeruginosa* that was initially present at 1+ growth was lost in both systems. Some of the viridans streptococci also did not survive well. Overall, at 24h, 51/53 anaerobes and 31/34 aerobes were recovered from the CVT transporter, while the Port-a-cul grew 52/53 anaerobes and 30/34 aerobes. After 48h, 44/53 anaerobes and 28/34 aerobes were recovered from the CVT transporter, while 49/53 anaerobes and 28/34 aerobes were recovered from the Port-a-cul.

DISCUSSION

Although aspirates are preferable to swab systems for anaerobic cultures, swabs are frequently used in many hospitals. In a previous study, we reported that the Copan Venturi Transport system maintained viability of 10 of 10 clinical and ATCC strains of anaerobes after 24h and 48h with only a slight decrease in colony counts. In the current study, we used pus specimens to compare the CVT with the Port-a-cul system. After 24h, the two systems were comparable in their abilities to maintain viability of most of the organisms. After 48h, some of the more fastidious strains were not recovered from either system. Some of the facultative organisms increased in numbers after 24 and 48 hours and overgrew some of the smaller colonies. Both systems are adequate for transport of clinical specimens within the time frame required for most clinical specimens to reach the clinical laboratory.

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Table 1. Percent of surviving strains and average growth scores of organisms recovered from the Copan Venturi Transystem and the Port-a cul system after 4, 24, and 48 hours.

Organism	0 Hours		4 Hours				24 Hours				48 Hours			
	Total#	Score	Copan		Port-a-cul		Copan		Port-a-cul		Copan		Port-a-cul	
			%Surv.	Score	%Surv.	Score	%Surv.	Score	%Surv.	Score	%Surv.	Score	%Surv.	Score
<i>B. fragilis</i>	2	3.5	100	3	100	3	100	3	100	3	100	3.5	100.	3.5
<i>B. thetaiotaomicron</i>	4	2.75	100	3.25	100	3.75	100	3	100	3.75	100	3.5	100	3.4
Other <i>B. fragilis</i> group	13	3.24	100	3.07	100	2.77	100	2.93	100	3.08	100	3.32	100	3.23
<i>Prevotella</i> sp.	5	2.6	100	2.2	100	2.4	80	2.2	100	2.4	40	1	80	2.2
<i>Porphyromonas</i> sp.	2	2	100	1	100	1	100	1.5	50	1.5	0	0	50	0.5
<i>Fusobacterium nucleatum</i>	1	3	100	1	100	2	100	2	100	2	0	0	0	0
<i>Bilophila wadsworthia</i>	3	2.33	100	3.33	100	2.67	100	2.33	100	2.67	100	3	100	3
<i>Peptostreptococcus</i> sp.	5	3	100	2.2	100	2.4	100	2.2	100	2.8	80	1.6	80	1.8
<i>Clostridium innocuum</i>	7	2.43	100	2.74	100	2.57	85.7	2.43	100	3.14	85.7	2.43	100	2.86
<i>Clostridium</i> sp.	6	2.33	83.3	1.83	83.3	2.33	100	2.5	100	2.83	83.3	2.5	100	2.83
<i>Eubacterium</i> sp.	4	2.75	100	2.5	100	2.25	100	2	100	2.75	100	2.75	100	2.25
<i>Lactobacillus cateniforme</i>	1	3	100	3	100	3	100	3	100	3	100	3	100	3
<i>E. coli</i>	8	2.38	100	2.13	100	2.29	100	3.5	100	3.5	100	4	100	4
Other <i>Enterobacteriaceae</i>	6	2.83	83.3	2.5	83.3	2.33	83.3	2.67	83.3	2.67	66.7	2.67	83.3	2.83
<i>Ps. aeruginosa</i>	3	1.67	100	1.33	66.7	1	66.7	2.33	66.7	2.33	66.7	2.67	66.7	2.67
<i>Commamonas</i>	1	1	100	1	100	1	100	3	100	3	100	4	100	3
<i>Enterococcus</i> sp.	4	2.25	100	1.5	100	1.25	100	2.25	100	2.25	100	2.75	100	2.75
<i>Streptococcus</i> sp.	8	2.25	87.5	2	75	2	87.5	2.63	75	2.63	75	2.38	75	2.13
<i>St. epidermidis</i>	2	3	100	1.5	100	2	100	1.5	100	1.5	100	1.5	50	1.5
<i>Corynebacterium</i> Sp.	2	1	100	1	100	1.5	100	1	100	1	50	0.5	50	0.5

Footnotes for Table 1.

Other enterics = *K. pneumoniae* (2), *E. cloacae* (1), *P. vulgaris* (1), *P. mirabilis* (1), *C. koseri* (1)
 Enterococcus = *E. faecium* (3), *E. faecalis* (1)
 Streptococcus = *S. constellatus* (4), *S. salivarius* (1), *Streptococcus* group C/G (1), *Gemella morbillorum* (1), *Aerococcus* (1)
 Corynebacterium = *C. amycolatum* (1), *C. xerosis* (1)
B. fragilis group = *B. ovatus* (2), *B. uniformis* (4), *B. vulgatus* (2), *B. distasonis* (1), *B. splanchnicus* (1), unidentified (1)
Prevotella = *P. melaninogenica* (1), *P. loeschii* (1), *P. bivia* (2), *P. intermedia* (1)
Peptostreptococcus = *P. magnus* (3), *P. anaerobius* (1), *P. micros* (1)
Clostridium sp. = *C. subterminale* (1), *C. symbiosum* (1), *C. clostridioforme* (2), unidentified (2)
Eubacterium = *E. lentum* (2), *E. limosum* (1), unidentified (1)