Evaluation of the New BBL™ CultureSwab Plus™ Transport Device (Becton Dickinson, Baltimore, MD) for Recovery of Anaerobes.

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ABSTRACT

BBL™ CultureSwab Plus™ (BCSP), a new transport device for anaerobic bacteria, was evaluated at room temperature for its ability to maintain the viability of 6 anaerobic ATCC reference strains (species of Bacteroides, Clostridium, Fusobacterium, Peptostreptococcus, and Porphyromonas) and 10 clinical anaerobic isolates (species of Bacteroides, Clostridium, Eubacterium, Fusobacterium, Peptostreptococcus, Prevotella, and Veillonella). Three other commonly used anaerobic transport systems, the Culturette and Port-A-Cul (Becton Dickinson) and the Starswab II (Starplex Scientific), were also evaluated simultaneously. After the 16 anaerobes were placed in the 4 transport systems, bacterial viability in each transport tube was determined at 0, 4, 24 and 48 hours. The BCSP had the highest recovery rates for the greatest number of species at various time intervals. Both the BCSP and Port-A-Cul had similar (within 1 log10) averaged colony counts for 14 of the 16 total anaerobes at all time intervals (2 Clostridium spp. were excluded due to enhanced growth in the Port-A-Cul). Compared to the BCSP, viable counts at 48 hours were 2 logs10 lower with the Culturette and Starplex systems. Clostridium difficile and Peptostreptococcus anaerobius lost viability in all transport systems at 24 and 48 hours, respectively. Fusobacterium nucleatum lost viability in all transport systems, except the BCSP, at 24 and 48 hours. Our findings suggest that the BCSP is an excellent alternative to the Port-A-Cul and other commonly used anaerobic transport systems for commonly encountered anaerobes isolated from clinical specimens.

INTRODUCTION

Swab transport devices containing various types of clinical materials are commonly received in clinical microbiology laboratories. Although swab specimens are less desirable than aspirates collected by needle and syringe or tissue biopsies for anaerobic culture (1, 7), the fact remains that commercially available swab transport devices are used widely in healthcare facilities for this purpose. Some of these systems have been available for many years and have been evaluated with varying results (2-6, 8, 9). The object of this study was to evaluate a new transport system, the BBL™ CultureSwab Plus™ (Becton Dickinson, Baltimore, MD), for its ability to maintain viability of a wide variety of anaerobic bacteria at 0, 4, 24 and 48 hours. In addition, three other anaerobic transport systems, the Culturette and Port-A-Cul (Becton Dickinson) and the Starswab II (Starplex Scientific), were evaluated simultaneously for comparison.

MATERIALS

Transport systems

- New BBL™ CultureSwab Plus™ Transport Device (BCSP; Becton Dickinson, Baltimore, MD)
- BD, Port-A-Cul catalog number 4321607 (Becton Dickinson, Baltimore, MD)
- BD, Culturette (Modified Stuart's) for aerobes and anaerobes catalog number 4360210 (Becton Dickinson, Baltimore, MD)
- Starplex Starswab II (Modified Amies clear) catalog number SP130X (Starplex Scientific, Etobicoke, Ontario, Canada)

MEDIA AND OTHER SUPPLIES

- Remel CDC Anaerobe Blood Agar (AnaBa)
- In house Buffered Gelatin Diluent (BGD)
- 12x75 mm Culture Tubes
- Marsh Biomedical Products Spreader for surface streaking catalog number SP05

ATCC Reference Strains

Bacteroides fragilis (25285)
Porphyromonas levii (29147)
Fusobacterium nucleatum (25586)
Clostridium perfringens (13124)
Clostridium difficile (9689)
Peptostreptococcus anaerobius (27337)

Indiana University Hospital Anaerobe Lab fresh clinical isolates

Bacteroides thetaiotaomicron (57308D)
Clostridium clostridioforme (57565A)
C. innocuum (57956D)
C. ramosum (57997C)
Eubacterium lentum (58173D)
Fusobacterium mortiferum (57428A)
F. necrophorum (57494A)
Peptostreptococcus micros (57837C)
P. tannerae (57645E)
Veillonella species (57535B)

METHODS:

1. All organisms used were transferred to Remel CDC Anaerobe blood agar (AnaBa) at least 3 times before using in the study.
2. Organisms were grown on AnaBa for 24h after which a swab was used to transfer colonies into pre-reduced BGD to achieve a turbidity of a 0.5 McFarland standard (~$1.5x10^8$ CFU/ml).
3. Dilutions were then made to approximate 10^6 CFU/ml.
4. Duplicate swabs from each transport system were inoculated by dipping them vertically into the diluted BGD for 5-10 s. Swabs were removed and drained for 2 s and then placed into the transport media/system.
5. Four times were studied: 0, 4h, 18-24h, and ~ 48h. Swabs were placed into each of the 4 transport media and allowed to sit at room temperature until the given time for plating.
6. Swabs were taken out of the transport system and placed into a tube containing 0.9 ml of pre-reduced BGD, vortexed and rung out and discarded.
7. For each tube of BGD, serial 2-fold dilutions were made (in duplicate). Each tube was mixed and 0.1ml was transferred into 0.9 ml of reduced BGD for a total of 4 dilutions.
8. 0.1 ml of each dilution was inoculated onto AnaBa and spread onto the plate using a disposable hockey stick.
9. Plates were incubated for 48 h at 35C or until there was sufficient growth (i.e. C. perfringens colonies were counted after overnight incubation while Porphyromonas levii colonies were counted after 3 days incubation). Plates counted had between 30 to 300 colonies whenever possible.
10. Colony counts were recorded (a total of 4 counts for each organism/transport system combination) and an average colony count was determined.
11. Averaged colony counts (expressed as log10 CFU/ml) for each of the 16 anaerobes tested were added together and then averaged for each time interval and for each transport system used. Colony counts for 2 isolates (C. clostridioforme and C. ramosum) were excluded because each isolate exhibited increased growth in the Port-a-cul system. The final values obtained gave an indication of the overall combined viability of the 14 anaerobes tested in each transport system.
12. Percent recovery for each organism/transport system combination was calculated by dividing the average colony count for each time interval (0, 4h, 18-24h, and 48h) by the initial colony count at time 0.

RESULTS

Averaged colony counts (expressed as log10 CFU/ml) for each of the 6 ATCC reference strains and 10 clinical anaerobes are displayed in graphs 1-16.

Averaged colony counts (expressed as log10 CFU/ml) for each of 14 of the 16 anaerobes tested (6 ATCC reference strains and 8 clinical isolates) are listed in Table 1. Both the BCSP and Port-A-Cul had similar (within 1 log10) averaged colony counts for 14 of the 16 total anaerobes at all time intervals (2 Clostridium spp. were excluded due to enhanced growth in the Port-A-Cul). Compared to the BCSP, overall viable colony counts at 48 hours were 1 and 2 logs10 lower, respectively, for the Culturette and Starplex systems.

Percent recovery for each of the 16 anaerobes tested in each transport system is listed in Table 2. The BCSP had the highest recovery rates for the greatest number of species at various time intervals (when 2 Clostridium spp. were excluded due to enhanced growth in the Port-A-Cul). Clostridium difficile and Peptostreptococcus anaerobius lost viability in all transport systems at 24 and 48 hours, respectively. Fusobacterium nucleatum lost viability in all transport systems, except the BCSP, at 24 and 48 hours.

Table 1. Average Colony Counts (log base 10 CFU/ML)

TIME 0	Culturette	Port-A-Cul	Starplex	BCSP
B fragilis ATCC 25285	8.55	7.4	7.72	7.41
B. thetaiotaomicron IU 57308	7.99	7.21	7.27	7.23
F. mortiferum IU 57428	6.51	6.32	5.86	7.03
F. necrophorum IU 57494	7.54	6.55	7.05	7.21
F. nucleatum ATCC	6.28	4.63	5.98	5.36
P. levii ATCC 29147	8.35	6.35	7.92	7.56
P. tannerae IU	8.53	6.7	7.84	7.25
E. lentum IU 58173	8.51	7.72	8.09	7.68
C. clostridioforme IU 57565	7.54	6.24	6.86	6.68
C. difficile ATCC 9689	6.42	5.4	5.61	5.5
C. innocuum IU 57956	8.02	7.05	7.59	7.23
C. perfringens ATCC 13124	8.0	6.79	7.45	7.25
C. ramosum IU 57997	7.81	6.93	7.59	7.3
P. anaerobius ATCC 27337	7.47	6.2	7.26	6.9
P. micros IU 57837	8.02	6.64	7.12	7.02
Veillonella spp IU 57535	8.77	8.22	8.43	7.97
Average	7.77	6.65	7.23	7.04

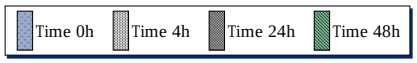
TIME 4h	Culturette	Port-A-Cul	Starplex	BCSP
B fragilis ATCC 25285	8.26	7.27	7.79	7.39
B. thetaiotaomicron IU 57308	8.04	7.3	7.69	7.18
F. mortiferum IU 57428	6.45	5.82	6.52	6.8
F. necrophorum IU 57494	7.58	6.81	7.06	7.34
F. nucleatum ATCC	5.33	**0**	4.1	4.74
P. levii ATCC 29147	7.46	5.65	6.47	7.84
P. tannerae IU	7.42	6.15	7.72	7.4
E. lentum IU 58173	8.28	7.35	7.97	7.51
C. clostridioforme IU 57565	6.01	5.7	4.92	6.55
C. difficile ATCC 9689	5.32	3.7	3.4	4.95
C. innocuum IU 57956	8.21	7.18	7.71	7.34
C. perfringens ATCC 13124	7.77	6.14	7.14	7.01
C. ramosum IU 57997	7.73	6.77	7.46	7.04
P. anaerobius ATCC 27337	7.44	4.83	6.51	7.05
P. micros IU 57837	7.97	7.01	7.45	7.02
Veillonella spp IU 57535	8.67	6.91	7.69	7.73
Average	7.37	5.91	6.73	6.93

TIME 24	Culturette	Port-A-Cul	Starplex	BCSP
B fragilis ATCC 25285	8.18	7.62	7.59	7.55
B. thetaiotaomicron IU 57308	7.93	7.82	7.7	7.65
F. mortiferum IU 57428	6.39	5.86	**0**	6.73
F. necrophorum IU 57494	6.06	6.34	3.7	6.72
F. nucleatum ATCC	**0**	**0**	**0**	3.4
P. levii ATCC 29147	4.4	5.4	4.4	6.3
P. tannerae IU	6.94	6.38	4.9	7.87
E. lentum IU 58173	7.84	6.86	7.16	6.98
C. clostridioforme IU 57565	**0**	7.55	**0**	6.87
C. difficile ATCC 9689	**0**	**0**	**0**	**0**
C. innocuum IU 57956	7.25	7.33	6.7	6.81
C. perfringens ATCC 13124	7.15	4.57	7.03	6.16
C. ramosum IU 57997	6.56	7.32	5.32	7.16
P. anaerobius ATCC 27337	4.1	4.54	3.4	6.11
P. micros IU 57837	7.44	6.04	6.94	6.97
Veillonella spp IU 57535	8.16	5.31	4.0	7.36
Average	5.53	5.56	4.30	6.29

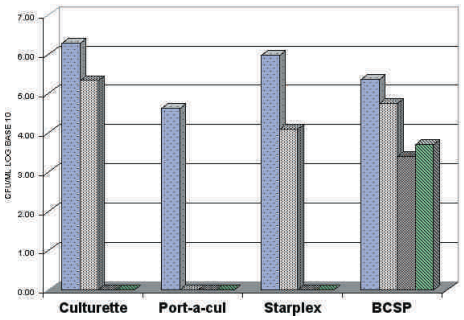
TIME 48	Culturette	Port-A-Cul	Starplex	BCSP
B fragilis ATCC 25285	7.7	7.58	7.37	7.7
B. thetaiotaomicron IU 57308	7.46	8.19	7.2	7.54
F. mortiferum IU 57428	3.4	6.04	**0**	5.14
F. necrophorum IU 57494	**0**	5.95	**0**	5.46
F. nucleatum ATCC	**0**	**0**	**0**	3.7
P. levii ATCC 29147	**0**	5.1	**0**	5.36
P. tannerae IU	4.48	6.2	**0**	6.95
E. lentum IU 58173	6.9	7.5	7.18	6.61
C. clostridioforme IU 57565	**0**	7.98	**0**	5.96
C. difficile ATCC 9689	**0**	**0**	**0**	**0**
C. innocuum IU 57956	5.53	7.13	**0**	5.86
C. perfringens ATCC 13124	6.84	4.1	6.53	5.31
C. ramosum IU 57997	6.03	8.18	4	6.74
P. anaerobius ATCC 27337	**0**	**0**	**0**	**0**
P. micros IU 57837	7.33	6.28	7.01	7.22
Veillonella spp IU 57535	7.69	6.11	6.67	7.02
Average	3.96	5.4	2.87	5.41

Table 2. Percent recovery (CFU/ML)

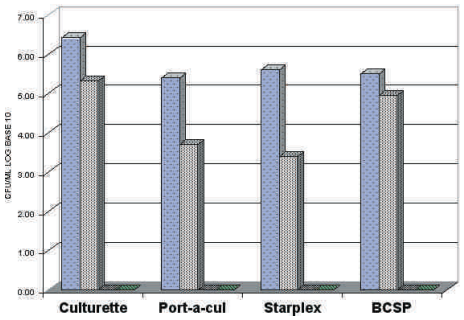
Organism	Culturette				Port-a-cul				Starplex				BCSP			
	0 h	4 h	24 h	48 h	0 h	4 h	24 h	48 h	0 h	4 h	24 h	48 h	0 h	4 h	24 h	48 h
Bacteroides fragilis ATCC 25285	100	50	42	14	100	74	168	152	100	117	74	45	100	94	138	196
Bacteroides thetaiotaomicron IUMC 58173D	100	113	87	30	100	123	402	951	100	265	272	86	100	90	261	205
Fusobacterium mortiferum IUMC 57428A	100	89	75	0.1	100	32	34	53	100	461	**0**	**0**	100	60	51	1
Fusobacterium necrophorum IUMC 57494A	100	109	3.3	**0**	100	182	61	25	100	102	0.04	**0**	100	136	32	2
Fusobacterium nucleatum ATCC 25586	100	11	**0**	**0**	100	**0**	**0**	**0**	100	1.3	**0**	**0**	100	24	1	2
Porphyromonas levii ATCC 29147	100	13	0.01	**0**	100	20	11	6	100	4	0.03	**0**	100	191	5	1
Prevotella tannerae IUMC 57645E	100	8	3	0.01	100	28	48	32	100	75	0.12	**0**	100	140	412	50
Eubacterium lentum IUMC 58173D	100	59	22	3	100	42	14	59	100	75	12	12	100	68	20	8
Clostridium clostridioforme IUMC 57565A	100	3	**0**	**0**	100	29	2069	5532	100	1	**0**	**0**	100	75	156	19
Clostridium difficile ATCC 9689	100	8	**0**	**0**	100	2	**0**	**0**	100	1	**0**	**0**	100	29	**0**	**0**
Clostridium innocuum IUMC 57956D	100	155	17	0.3	100	135	189	119	100	132	13	**0**	100	130	39	4
Clostridium perfringens ATCC 13124 (CP)	100	58	14	7	100	22	1	0.2	100	49	38	12	100	58	8	1
Clostridium ramosum IUMC 57997C	100	82	6	2	100	70	247	1788	100	75	1	0.02	100	55	72	28
Peptostreptococcus anaerobius ATCC 27337	100	94	0.04	**0**	100	4	2	**0**	100	18	0.01	**0**	100	142	16	**0**
Peptostreptococcus micros IUMC 57837C	100	89	27	21	100	236	25	44	100	211	65	77	100	100	90	158
Veillonella species IUMC 57535B	100	79	25	8	100	5	0.1	1	100	18	0.004	2	100	58	25	11



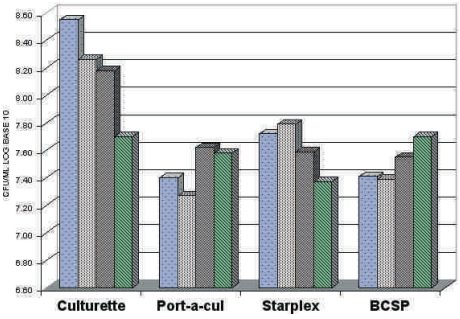
Fusobacterium nucleatum **(ATCC 25586)**



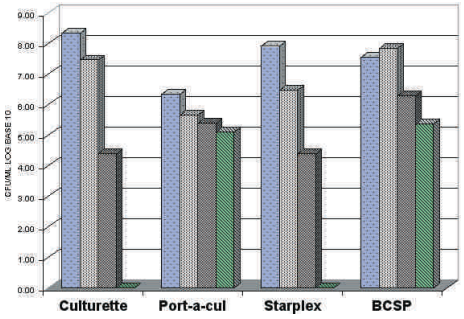
Clostridium difficile **(ATCC 9689)**



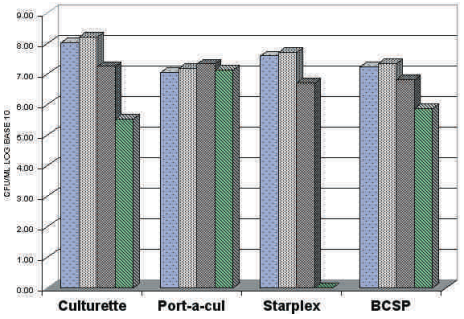
Bacteroides fragilis **(ATCC 25285)**



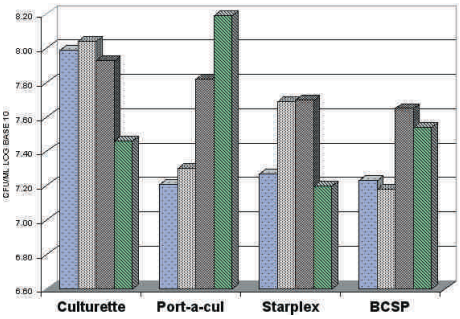
Porphyromonas levii **(ATCC 29147)**



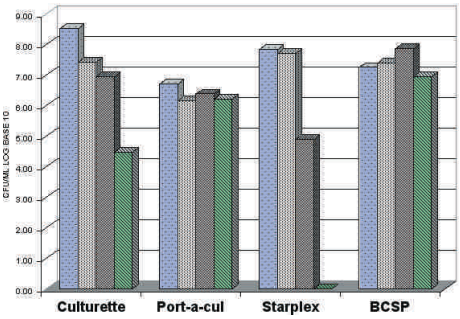
Clostridium innocuum **(IU 57956D)**



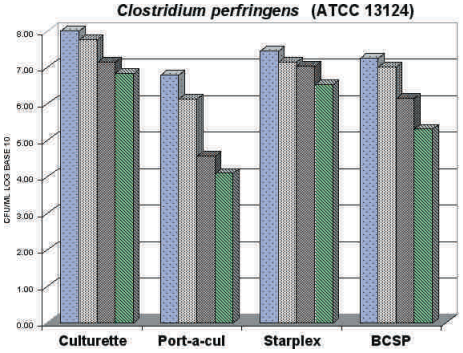
Bacteroides thetaiotaomicron **(IU 58173)**



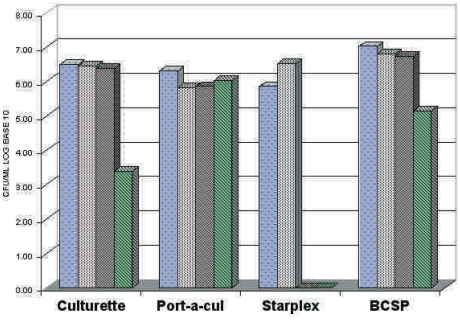
Prevotella tannerae **(IU 57645E)**



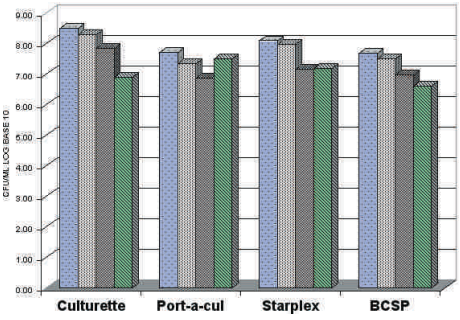
Clostridium perfringens **(ATCC 13124)**



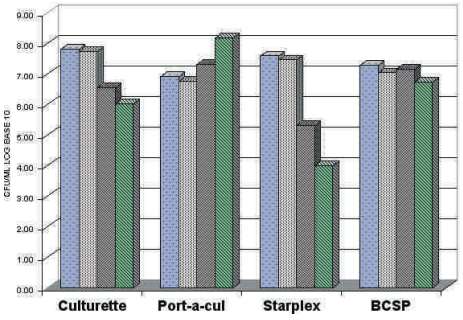
Fusobacterium mortiferum **(IU 57428A)**



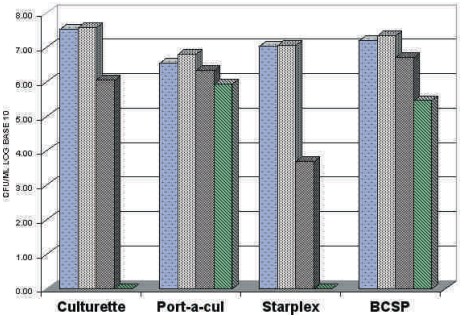
Eubacterium lentum **(IU 58173D)**



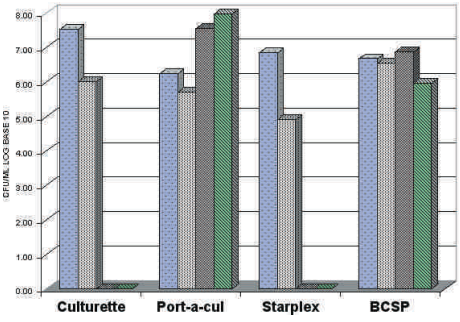
Clostridium ramosum **(IU 57997)**



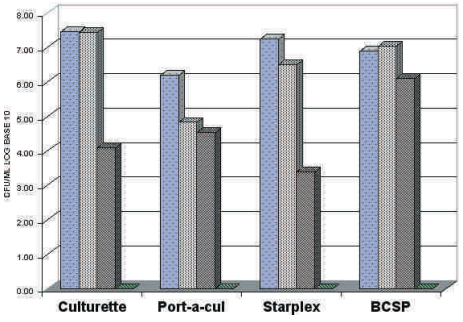
Fusobacterium necrophorum **(IU 57494A)**

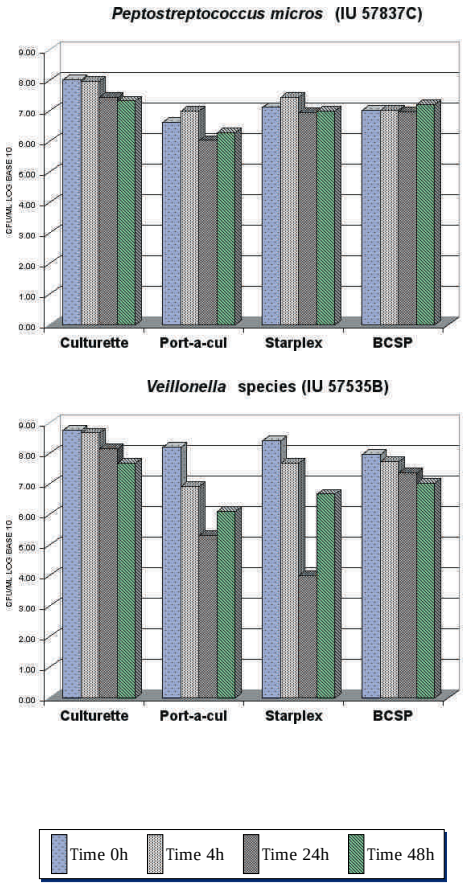


Clostridium clostridioforme **(IU 57565A)**



Peptostreptococcus anaerobius **(ATCC 27337)**





CONCLUSION

Our findings suggest that the BCSP is an excellent alternative to the Port-A-Cul and other commonly used anaerobic transport systems for commonly encountered anaerobes isolated from clinical specimens.

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