

COMPARISON OF COPAN AMIES AGAR SWAB AND BBL PORT-A CUL SWAB FOR RECOVERY OF ANAEROBIC BACTERIA

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ABSTRACT

Specialized transport devices have been utilized for collection of clinical specimens for anaerobic culture. We compared the Copan Amies swab transport system and the BBL Port-a-cul swab transport system for recovery of 30 anaerobic bacteria. Swabs were inoculated with 100- μ l of a 10^7 CFU/ml organism suspension, incubated at room temperature for 0, 4, 24, 48h, and the swabs used to prepare three 10-fold serial dilutions in 0.9-ml saline. A 100- μ l aliquot of each dilution was inoculated to reduce anaerobic brucella blood agar and incubated in an anaerobic atmosphere for 48-96h. Colony counts were obtained and the data analyzed as percent recovery compared to the 0h (initial inoculum) growth results. The Copan swab maintained viability after 48h for 24/30 (80%) and the BBL swab for 25/30 (83%) of the anaerobes tested. Two *C. difficile* and one *F. nucleatum* were not recovered after 48h in both Copan and BBL swab systems. One *P. melaninogenica*, *B. ureolyticus*, and *P. bivia* in the Copan swab and one *P. asaccharolyticus* and *A. odontolyticus* in the BBL swab were also not recovered after 48h. After 24h, 29/30 (97%, one *C. difficile*) in the Copan swab system and 28/30 (93%, *P. asaccharolyticus* and *C. difficile*) in the BBL swab system were recovered. The Copan Amies agar swab system is comparable to the BBL Port-a-cul swab system for maintenance of viability of anaerobic bacteria.

INTRODUCTION

The recovery of anaerobic and aerobic bacteria from clinical specimens lies in proper collection and transport. It has been suggested that anaerobic transport is the ideal method of transport and maintenance of viability of aerobic, anaerobic, and facultative micro-organisms. Swab systems are a well used method for specimen transport and have become a preferred method for collection and transport due to their ease-of-use. Data has suggested that the chemical composition, structure, and design of some swabs may be inhibitory to microorganisms. The clinical specimen type may also lead to a decrease in organism viability. We compared the Copan Amies agar and the BBL Port-a-cul swab transport systems for the recovery of anaerobic bacteria. The Copan Amies agar is a modified Stuart's medium formulation and was tested for quality control with 15 anaerobes. The BBL Port-a-cul agar is a "balanced formula of reducing agents and resazurin in a buffered isotonic base" and was tested for quality control with 10 anaerobic organisms. For our tests, we expanded on the number of quality control anaerobes tested and compared the effectiveness of the two different swabs for recovery of anaerobic bacteria.

METHODS

1. The Copan Amies agar swab transport system (Copan Diagnostics, Inc., Corona, Ca) and the BBL Port-a-cul (Becton-Dickinson {BDMS}, Cockeysville, MD) were tested for maintenance of viability of 30 anaerobes, Table 1.
2. Four swabs of each type were rolled into 100 μ l of organism suspension (approximately 10^7 CFU/ml) for 5 sec. to completely absorb the inoculum.
3. All swabs were incubated at room temperature for 0, 4, 24, and 48 hours.
4. After appropriate incubation, each swab was removed from the transport tube and placed into 0.9 ml of sterile saline and mixed 10 sec. with a vortex mixer to resuspend the organisms (approximately 10^6 CFU/ml).
5. Two 10-fold serial dilutions in sterile saline were performed to achieve

tubes with approximately 10^5 and 10^4 CFU/ml.

6. 100 μ l of each of the three organism suspensions (10^4 - 10^6) for each swab were plated to anaerobic brucella blood agar (BDMS) reduced 24 hours prior to use. Duplicate plates were inoculated for each organism suspension.
7. The inoculum was spread over the entire surface of each plate with a sterile bent plastic rod (Copan).
8. Plates were incubated in an anaerobic atmosphere (AnaeroPack, Mitsubishi Gas Chemical Co., New York, NY) at 35°C for 48-96 hours.
9. Two technologists obtained colony counts for each incubation time in order to minimize any bias.
10. Results are expressed as % recovery compared to that swab-transport system's initial inoculum. (Table 1)

Table 1. Anaerobes tested (n=30)

Gram-Negatives (n=15)		Gram-Positives (n=15)	
<i>B. fragilis</i> (2)	<i>Pr. intermedia</i>	<i>C. perfringens</i>	<i>A. odontolyticus</i>
<i>B. thetaiotaomicron</i> (2)	<i>Pr. bivia</i>	<i>C. difficile</i> (2)	<i>Bifidobacterium</i>
<i>B. caccae</i>	<i>F. nucleatum</i> (2)	<i>C. innocuum</i> (2)	<i>E. lentum</i>
<i>B. distasonis</i>	<i>F. necrophorum</i>	<i>C. ramosum</i>	<i>P. anaerobius</i> (2)
<i>B. ureolyticus</i>	<i>Viellonella</i>	<i>C. sordellii</i>	<i>P. micros</i>
<i>Pr. melaninogenica</i> (2)		<i>C. tertium</i>	<i>P. asaccharolyticus</i>

All isolates are single strains tested except where noted.

RESULTS

1. All 7 *Bacteroides* species were recovered in BBL after 48h while 6/7 were recovered in Copan. *B. ureolyticus* was only recovered in Copan (97% reduction) after 24h and not after 48h.
2. One *F. nucleatum* was only recovered after 24h in both BBL (97% reduction) and Copan (99% reduction) while the other two fusobacteria were recovered after 48h in both swab systems (*F. necrophorum* >99.9% reduction in Copan and one *F. nucleatum* 99.7% reduction in BBL).
3. All 4 *Prevotella* species were recovered in BBL after 48h while one *P. melaninogenica* and one *P. bivia* were only recovered in Copan after 24h.
4. Two *C. difficile* were not recovered by either swab system after 48h. One strain was recovered in BBL (99.6% reduction) but not in Copan after 24h while the other strain was recovered in Copan (99.7% reduction) but not in BBL after 24h. The other 6 clostridia were recovered in both systems after 48h although there was a 99% reduction in BBL for the *C. perfringens*.
5. All 4 *Peptostreptococcus* species were recovered in Copan after 48h. *P. asaccharolyticus* was only recovered in BBL (87% reduction) after 4h.
6. One *A. odontolyticus* was recovered in Copan (99.8% reduction) after 48h but only recovered in BBL (99.1% reduction) after 24h.
7. Lower initial counts in BBL Port-a-cul were observed for some anaerobes, presumably the organisms were trapped in the agar. (Photo A, Table 2, Figures 1-4)

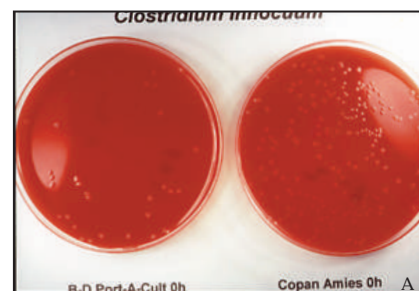


Table 2

Organism	BBL 4h	BBL 24h	BBL 48h	Copan 4h	Copan 24h	Copan 48h
<i>B. fragilis</i>	100	42	570	114	59	61
<i>B. fragilis</i>	70	52	88	138	68	32
<i>B. thetaiotaomicron</i>	77	91	165	97	132	116
<i>B. thetaiotaomicron</i>	100	200	910	100	87	154
<i>B. caccae</i>	104	105	620	67	58	45
<i>B. distasonis</i>	100	103	200	120	60	78
<i>F. nucleatum</i>	10	2	0.3	49	7	5
<i>F. necrophorum</i>	15	7	7	110	18	0.08
<i>P. melaninogenica</i>	32	1	0.5	88	14	2
<i>P. intermedia</i>	88	115	108	104	120	130
<i>C. perfringens</i>	100	62	1	51	40	20
<i>C. tertium</i>	46	26	9	87	12	8
<i>C. innocuum</i>	122	76	60	140	35	134
<i>C. innocuum</i>	51	60	93	91	17	17
<i>C. ramosum</i>	63	23	8	89	61	40
<i>C. sordellii</i>	37	90	52	23	42	93
<i>P. anaerobius</i>	76	65	48	109	34	93
<i>P. anaerobius</i>	82	127	98	106	96	93
<i>P. micros</i>	23	19	8	42	12	5
<i>Veillonella</i> sp.	35	0.8	1.4	68	47	7
<i>E. lentum</i>	49	30	6	135	46	12
<i>Bifidobacterium</i> sp.	48	100	80	208	237	222

^aNumbers are in % recovery after specified room temperature incubation time.

Fig. 1

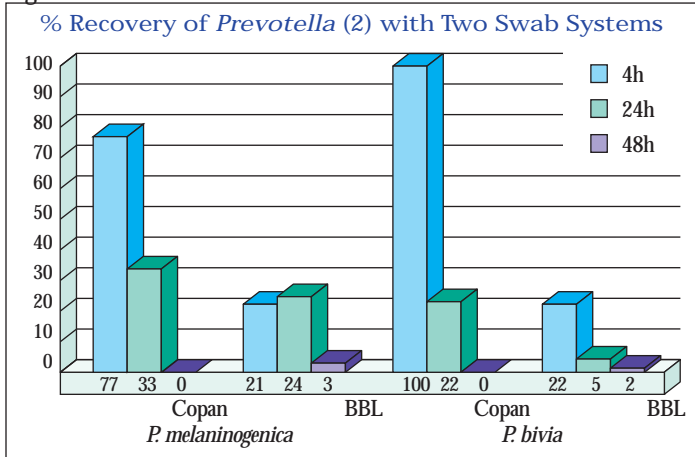


Fig. 2

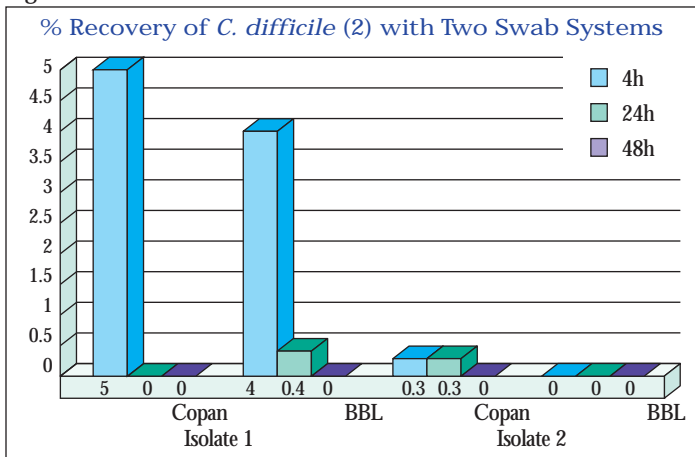


Fig. 3

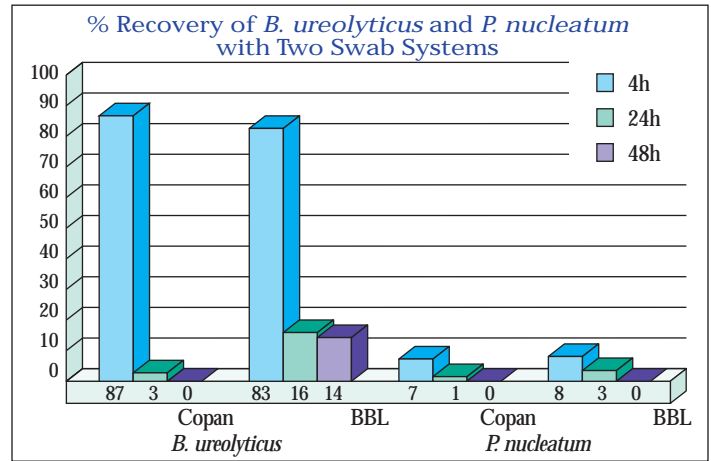
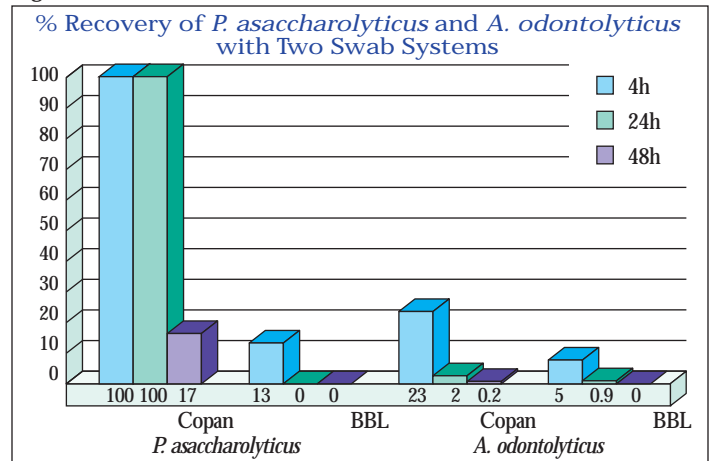


Fig. 4



OBSERVATIONS

- Copan Amies Agar Swab Transport System**
1. When breaking, the swab shaft may splinter.
 2. Only the one swab system was tested. A two swab system is available.
 3. the Amies agar has no anaerobic indicator system.
 4. Agar not filled to constriction in transport tube. (this has been corrected by Copan).
 5. Easy-to-use swab system. Place swab into gel.
 6. Swabs readily absorb the 100 µl inoculum.
 7. Swab has convenient holding knob.
 8. Expiration dates of approximately 2 years.
 9. Has label for patient demographics.
- Port-a-cul Amies Agar Swab Transport System**
1. The wooden handle swab doesn't fit completely into the tube. After specimen collection, the wooden swab must be broken after placing it into the Amies agar tube.
 2. Forceps or another device must be used to remove the broken swab from the agar.
 3. There is no label provided for patient information.
 4. The Amies agar clings to the swab tip (although this could be a plus since organisms would stay with the swab).
 5. Easy to open over-wrap with two swabs in system.
 6. The swab polyester tip readily absorbed the 100 µl of inoculum.
 7. The Amies agar has an anaerobic indicator.
 8. Expiration is approximately one year.

SUMMARY AND CONCLUSIONS

1. The Copan Amies agar swab transport system without charcoal is comparable to the BBL Port-a-cul Amies agar swab transport system for maintenance of viability of most anaerobic bacteria.
2. Neither system was able to maintain *C. difficile* for longer than 24h.
3. All anaerobes tested, except one *C. difficile* were recovered from the Copan Amies agar after 24h.
4. Immediate transport of all specimens to the microbiology laboratory is still recommended.