Evaluation of a Flocked Nylon Swab Transport System (Copan ESwab) for Maintaining Viability of Anaerobes

S. D. Allen*, J. N. Kedra, and J. A. Siders

Indiana University School of Medicine, Clarian Pathology Laboratory, Indianapolis, IN

INDIANA UNIVERSITY
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE
School of Medicine

INDIANA UNIVERSITY
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School of Medicine

ABSTRACT

Background: The recently introduced ESwab transport system incorporates a flocked nylon swab for sample collection and liquid Amies transport medium. The system potentially collects more material and releases a higher percentage of microorganisms than spun fiber swabs with agar transport media. Data for this system are currently lacking relative to anaerobes. The object of this study was to perform a quantitative evaluation of the ESwab for maintaining viability of selected clinically important anaerobic bacteria. For comparison, the BBL Port-A-Cul (PAC) agar tube transport system with polyester swabs was tested.

Methods: Anaerobic bacteria selected for this ongoing study were 11 ATCC strains and 14 recent clinical isolates. Quantitative viability studies were performed in triplicate at both controlled room temperature (RT) and refrigerator temperature (4C) at the same time. For each organism/swab device combination, viable counts were performed at zero (0) time, 24 h and 48 h according to the CLSI-40A quantitative elution method.

Results: Initial 0-time counts for 9 anaerobes studied thus far were nearly 1 log (base 10) higher for the ESwab than for the PAC. After swabs were held 24 h at 4C compared to 0-time, organism recovery from the ESwab was 76%, vs. 32% for the PAC (based on average CFU/ml for 9 organisms). After 48 h at 4C, recovery from ESwab was 59% and recovery from the PAC was 17% compared to 0-time. At RT compared to 0-time, the overall recovery of anaerobes from ESwabs at 24 h and 48 h was 12% and 2% respectively, compared to 17% and 9% for the PAC. At RT, survival in both systems was documented for all anaerobes tested except for *Fusobacterium nucleatum* ATCC 25586 that was non-viable at 48 h in the ESwab.

Conclusions: The results suggest the nylon flocked ESwab system with liquid Amies transport medium is suitable for maintaining viability of clinically important anaerobic bacteria during transport and storage either at 4C or RT. However, the ESwab performed better at 4C than it did at RT, though it maintained viability of most anaerobes tested at RT for 48 h.

INTRODUCTION

A key step in the diagnosis of anaerobic bacterial infections is the selection, collection and transport of specimens. This commonly involves the use of swabs with transport media. Although swab specimens are less desirable than aspirates collected by needle and syringe or tissue biopsies for anaerobic culture (4), the fact remains that commercially available swab transport devices are used widely in healthcare facilities for this purpose. In many healthcare settings today, specimens for culture are transported to a centralized or referral laboratory for processing. Transport delays may be unavoidable. Viability of microorganisms within the samples must be maintained for extended periods of time.

The recently introduced Copan Eswab collection and transport system was designed to maintain viability of bacteria (aerobes, anaerobes, and fastidious bacteria) in clinical specimens for up to 48 hours at room temperature and refrigerator temperature. The system includes a nylon flocked swab and a polypropylene screw-cap containing 1 ml modified Liquid Amies (Figure 1). According to the manufacturer, the swab tip is sprayed with short strands of nylon fiber which bond in a perpendicular fashion (Figure 2). A sample is absorbed by strong capillary action between the fibers and remains close to the surface of the fibers. When placed into the liquid transport medium, the system is designed for the specimen to elute with less entrapment of microorganisms than occurs with other types of swabs (e.g., rayon or Dacron) with agar transport media.

The object of this study was to perform a quantitative evaluation of the ESwab for maintaining viability of selected clinically important anaerobic bacteria. For comparison, the BBL Port-A-Cul (PAC) agar tube transport system was tested. The CLSI M40-A quantitative elution method (1) was used to test a larger collection of anaerobic bacteria than had been reported on previously (2, 3).

MATERIALS

Transport Systems

- BD, Port-A-Cul catalog number 221607 (Becton Dickinson, Baltimore Maryland)
- Copan, ESwab (liquid Amies) catalog number 480C (Murietta, CA)

Media and other Supplies

- Remel CDC Anaerobe Blood Agar (AnaBa)
- Fisherbrand 12x75 mm Culture tubes, catalog number 14-961-26
- Fisherbrand L shaped spreaders, catalog number 03-392-150
- Fisherbrand Redi Tip 1-200uL pipette tips, sterilized, catalog number 02-707-500
- Eppendorf Safe-Lock Tubes, 2.0 mL catalog number 22 60 004-4
- 0.85% physiological saline (pH 6.8-7.2), sterilized

Challenge organisms
ATCC Strains*

Porphyromonas asaccharolytica	ATCC 25260
Porphyromonas levii	ATCC 29147
Prevotella bivia	ATCC 29303
Fusobacterium nucleatum	ATCC 25586
Peptoniphilius asaccharolyticus	ATCC 14963
Propionibacterium acnes	ATCC 6919
Clostridium difficile	ATCC 9689

*Additional ATCC strains not yet tested but to be included follow:

Bacteroides fragilis	ATCC 25285
Prevotella melaninogenica	ATCC 25845
Peptostreptococcus anaerobius	ATCC 27337

MATERIALS (CONT)

Clarian Pathology Anaerobe Lab fresh clinical isolates

Bacteroides fragilis group	Body fluid
Bacteroides thetaiotaomicron	Drain site
Fusobacterium nucleatum	Wound-Lung
Fusobacterium necrophorum	PICC Line
Peptostreptococcus anaerobius	Mandible
Finegoldia magna	Rt Leg
Peptoniphilius asaccharolyticus	Placenta
Veillonella sp.	Peritoneal Fluid
Propionibacterium acnes	CSF
Eggerthella lenta	Perineal Abscess
Clostridium clostridioforme	NSI
Clostridium difficile	Wound-Groin
Clostridium difficile	Stool
Clostridium sporogones	Post-Op Wound
Clostridium sordelli	Left Arm
Clostridium perfringens	Wound
Clostridium ramosum	Peritoneal Fluid
Clostridium innocuum	Abdomen Fluid

Figure 1.

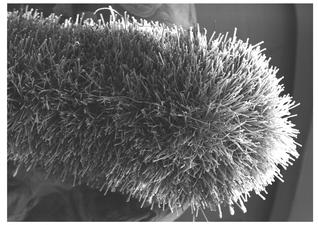

Figure 2.

METHODS

1. All bacteria were transferred to AnaBa at least three times before being used as inocula.
2. Colonies were transferred to 3ml of pre-reduced 0.85% sterile saline to achieve a turbidity of a 0.5 McFarland standard (~1.5x10^8 CFU/ml).
3. A 10-fold dilution was made of the inoculum in 0.85% sterile saline to provide a concentration of approximately 1.5x10^7 CFU/ml.
4. 100 µl of well mixed inoculum was pipetted to each of 6 Eppendorf tubes, 3 each for Port-A-Cul (PAC) and ESwab transport systems. Three swabs from each system were placed in separate tubes and allowed to absorb for a minimum of 10 seconds.
5. Inoculated swabs were then placed in the appropriate transport system and set aside to use as the 0-time determination.
6. Twelve more swabs for each transport type were inoculated in the same manner and placed in appropriate transport systems to be held for 24 h at room and refrigerator temperatures, and for 48 h at room and refrigerator temperatures.
7. Inoculation time did not exceed 20 minutes from time of preparation of inoculum suspension until all swabs were inoculated and placed in appropriate transport systems and at required temperatures.
8. To determine colony counts for the BD PAC system the swab was removed from the transport device and placed in 1ml of 0.85% sterile saline (pH 6.8-7.2). This is the primary tube suspension. It was vortexed vigorously for 15 sec. and excess liquid expressed from the swab.
9. Three 10-fold serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) in 0.85% sterile saline (pH 6.8-7.2) were made with the resulting inocula concentrations of 10^5, 10^4 and 10^3.
10. 100 µl of each dilution, including the primary tube, was plated in duplicate with the inoculum being spread evenly across the media surface with a sterile spreader. Steps 7-9 were repeated until all swabs were diluted and plated.
11. Plates were than incubated in a Coy Anaerobic Chamber (glove box) until countable colonies were visible.
12. Colony counts for the ESwab were performed in the same manner except the primary tube suspension was the ESwab transport tube that contained 1mL of liquid Amies. All other steps were the same.
13. When visible colonies were present on all plates, the plates were counted and colony counts recorded for each dilution for each swab at each temperature.

RESULTS

Averaged colony counts (expressed as $\log_{10}$ CFU/ml) for all of the 25 anaerobes tested thus far are displayed in Figure 3. Initial 0-time counts for most anaerobes were nearly one $\log_{10}$ CFU/ml higher for the ESwab than for the Port-A-Cul (PAC). This indicates better collection and release of bacteria from the ESwabs than from the PAC swabs. In general, recovery of anaerobes was higher after ESwabs were held at refrigerator temperature for 24-48 h compared to results for ESwabs held at room temperature, although most strains survived at both temperatures. Also at refrigerator temperature, ESwabs yielded higher quantitative recoveries of most anaerobes than did PAC. At room temperature the performance of ESwabs compared to PAC was nearly equivalent after 24 hours, but less than that of PAC swabs held for 48 hours.

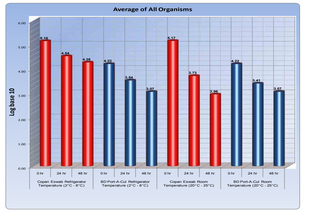

Figure 3.

Figure 4-15. Representative comparative data.

RESULTS (CONT)

Table 1. Average percent recovery of all organisms tested.

	Copan Eswab Refrigerator Temperature (2°C to 8°C)			BD Port-A-Cul Refrigerator Temperature (2°C to 8°C)			Copan Eswab Room Temperature (20°C to 25°C)			BD Port-A-Cul Room Temperature (20°C to 25°C)		
	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
Peptostreptococcus anaerobius	100	115	53	100	32	10	100	50	4	100	17	2
Peptoniphilius asaccharolyticus	100	96	130	100	23	18	100	73	5	100	6	6
Peptoniphilius asaccharolyticus , ATCC 14963	100	6	1	100	14	3	100	2	0	100	2	3
Finegoldia magna	100	88	85	100	90	35	100	91	85	100	75	71
Veillonella species	100	93	62	100	48	23	100	56	14	100	9	4
Bacteroides fragilis group	100	236	138	100	127	108	100	2	1	100	64	56
Bacteroides thetaiotaomicron	100	69	62	100	64	50	100	12	11	100	89	149
Prevotella bivia, ATCC 29303	100	7	2	100	3	0	100	0	0	100	3	0
Porphyromonas asaccharolytica, ATCC 25260	100	15	1	100	3	1	100	2	0	100	4	0
Porphyromonas levii, ATCC 29147	100	81	69	100	37	16	100	43	3	100	5	1
Fusobacterium nucleatum, ATCC 25586	100	15	7	100	5	1	100	0	0	100	10	7
Fusobacterium nucleatum	100	65	45	100	85	18	100	1	0	100	2	0
Fusobacterium necrophorum	100	53	28	100	7	10	100	35	1	100	22	9
Eggerthella lentum	100	104	79	100	24	77	100	61	36	100	122	16
Propionibacterium acnes, ATCC 6919	100	51	39	100	16	4	100	66	42	100	56	28
Propionibacterium acnes	100	72	78	100	87	95	100	43	75	100	90	71
Clostridium clostridioforme	100	0	0	100	12	0	100	0	0	100	40	68
Clostridium difficile	100	2	0	100	4	0	100	0	0	100	4	4
Clostridium difficile	100	43	27	100	74	19	100	151	10	100	93	7
Clostridium difficile , ATCC 9689	100	0	0	100	1	2	100	0	0	100	5	7
Clostridium innocuum	100	68	41	100	45	11	100	8	2	100	37	95
Clostridium perfringens	100	91	75	100	45	36	100	90	49	100	49	47
Clostridium ramosum	100	69	53	100	37	22	100	43	2	100	35	33
Clostridium sordellii	100	89	69	100	90	81	100	80	69	100	102	111
Clostridium sporogenes	100	124	117	100	171	62	100	119	132	100	92	68
AVERAGE	100	66	50	100	46	28	100	41	22	100	41	34

Table 1 demonstrates percent recoveries for each of the 25 anaerobes tested thus far in the transport systems. After swabs were held 24 h in the refrigerator compared to 0-time, organism recovery from ESwabs was 66% vs. 46% for the PAC. After 48 h in the refrigerator, recovery from ESwab was 50% and recovery from the PAC was 28% compared to 0-time. At room temperature compared to 0-time, the overall recovery of anaerobes from ESwabs at 24 h and 48 h was 41% and 22%, respectively, compared to 41% and 34% for the PAC, respectively.

At refrigerator temperature, poor or no survival was noted for *Clostridium difficile* (2 of 3 strains) and *C. clostridioforme* in both systems. *Prevotella bivia* and *Porphyromonas assacharolytica* also were problematic for both systems. A clinical isolate of *Fusobacterium nucleatum* showed much better recovery after it had been held in the refrigerator in both systems compared to room temperature for both systems. At room temperature, recoveries were better for 4/5 strains of anaerobic cocci tested with ESwab compared to PAC at 24 h, though the yields in both systems at 48 h tended to decrease. Also at room temperature, *Porphyromonas levii* survived better in ESwab for 24 h than in PAC, but its recovery in both systems at 48 h was poor. At room temperature, a strain each of the *Bacteroides fragilis* group and *B. thetaiotaomicron* were recovered much better with the PAC than with ESwab, though recoveries of both strains were excellent in both systems after they were held in the refrigerator. In addition, differences were noted in recoveries of certain *Clostridium* species (e.g., *C. clostridioforme*, and 2/3 *C. difficile* strains), at room temperature as can be seen in Table 1.

CONCLUSION

- Our findings indicate enhanced release of anaerobic bacteria from the nylon flocked ESwab compared to the rayon swab used with the PAC as indicated by higher 0-time counts for 24/25 anaerobes tested. A single exception was noted for one *B. fragilis* group strain (5.42 $\log_{10}$ CFU/ml from ESwab, but 5.52 $\log_{10}$ CFU/ml from PAC).
- The ESwab is a suitable alternative to the PAC for maintaining viability of clinically important anaerobic bacteria at refrigerator temperature or room temperature. However, the ESwab performed better at refrigerator temperature than it did at room temperature, though it maintained viability of most anaerobes tested at room temperature for 48 h.
- As a note of caution, poor or no survival was noted for certain clostridia and fastidious anaerobic gram-negative rods at refrigerator temperature and room temperature in both the ESwab and PAC systems (24-48 h). Additional data are needed to assess the survival times for these clostridia and fastidious anaerobic gram-negative rods in both systems (i.e., at times < 24 h).

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