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Assessment of Swab Transport Systems for Aerobic and Anaerobic Organism Recovery

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Saline suspensions of 11 aerobes and anaerobes were used to inoculate swabs from Port-A-Cul (Becton Dickinson), Culturette EZ (Becton Dickinson), and Copan Amies gel (Copan Diagnostics). Swabs were removed from transport devices at 0, 24, and 48 h postinoculation and then extracted by vortexing in 1.0 ml of saline, and organism survival was determined by quantitative plate counts. For the organisms tested, Culturette EZ allowed <1% of the original inocula to be recovered after 24 h. Port-A-Cul was only slightly better. Recovery was best with the Copan gel-containing system. Agar gel swab systems may be useful for multipurpose transport devices.

A variety of specimens arrive in the microbiology laboratory daily in swab transport devices. Evidence suggests that swab specimens are inferior to fluid specimens when wounds, exudates, and drainage are collected, especially for anaerobic culture (4). Regardless, the availability and ease of swab collection result in the receipt of many such specimens.

Cost containment issues have intensified the search for multipurpose instrumentation and consolidation of equipment and reagent use. Specimen collection and transport devices have not escaped this scrutiny. Finegold et al. reported in 1974 that maintaining aerobic, anaerobic, and facultative organisms in an anaerobic environment approaches an ideal method for transport of all bacteria (1). This multiuse approach is especially appealing in today's new order of laboratory medicine. Port-A-Cul (PAC; Becton Dickinson, Cockeysville, Md.) tubes include aerobe transportation as part of their intended use but are commonly thought to be more appropriate for the recovery of anaerobes. More recently, Culturette EZ (EZ; Becton Dickinson, Cockeysville, MD), a polyurethane foam swab device without transport medium, has been evaluated as a multipurpose culture device capable of promoting the recovery of aerobes, viruses, and some anaerobes from clinical specimens (2, 5, 7). Package insert information indicates EZ has been shown to yield results equivalent to those of commercial devices containing transport media. The expected results include the maintenance of most microorganisms for up to 48 h, with fastidious organisms such as *Neisseria gonorrhoeae* and *Streptococcus pneumoniae* recovered in 24 h or less.

I have been interested for a number of years in organism recovery from swab transport devices, including the affects of vortexing versus direct streaking and other variables inherent in swab handling (6). In the interest of cost containment and the potential for one "does-it-all" swab transport system, I evaluated PAC tubes, EZ, and our current swab system, Copan Venturi Transystem Amies gel without charcoal (CAG; Copan Diagnostics, Inc., Corona, Calif.) for their potential as both aerobic and anaerobic culture transport devices. Simplification of system inoculation and organism quantitation was aided by using single-swab versions of these products.

Previous swab evaluations performed in our laboratory indicated that anaerobes and fastidious aerobes were key organisms to assess the capability of a swab transport device to provide reasonable bacterial recovery. Seven anaerobes and four aerobes (American Type Culture Collection strains) were used in this study. A BBL Prompt device (Becton Dickinson) designed to approximate a 1.5×10^8-CFU/ml inoculum for disc diffusion susceptibility testing was used to prepare the initial organism inocula. A 1:10 dilution of each bacterial suspension was prepared as a final working volume of 5.0 ml in sterile saline. Swabs absorbed about 0.1 ml; thus, final concentrations of organisms (10^6 CFU/ml) approximated bacterial loads in collection sites such as infected wounds, which often harbor 10^6 CFU/g of pus, nasal and pharyngeal flora consisting of 10^4 CFU/ml of aerobes in addition to 10^5 CFU/ml of anaerobes, and cultures of vaginal and urethral discharge resulting from gonococcal infections that yield an average of 10^5 CFU/ml, with a range of 10^2 to 10^7 CFU/ml (3). Three swabs of each transport device were inoculated by being dipped vertically into the inoculum for 5 s, were removed and allowed to drain for 10 s, and then were placed into their respective transport devices and held at room temperature. At 0, 24, and 48 h postinoculation, organism survival was evaluated by vortexing each of the three swabs in 1.0 ml of saline for 30 s, expressing them against the tube wall prior to discarding, and then preparing duplicate plate counts with a 0.001-ml calibrated loop or 0.01- and 0.10-ml aliquots delivered by sterile pipette tips. The inoculum was dispersed over the agar surface with disposable spreaders. A total of six counts were averaged on triplicate swabs for each organism, device, and sample time studied. Plate counts at 0 and 24 h generally fell within a 10^4- to 10^5-CFU/ml range. Organism survival rates in this model were best compared by expressing recovery as a percentage of plate counts at time zero. This eliminated straight quantitation variables such as individual swab absorption rate and mechanical removal of organisms within the transport device.

Survival of individual aerobic organisms and average recovery are presented in Table 1. Previous studies in our laboratory endorsed data that certain organisms (*Streptococcus pyogenes* and *Bacteroides fragilis* in this study) increase in number during 48 h of storage at room temperature in PAC. Although survival (or growth) is impressive, overgrowth by these organisms could diminish the presence of other pathogens in lower numbers common in mixed-flora specimens. EZ promoted recovery of fewer organisms at 24 and 48 h than did PAC and CAG. Recovery is expressed as a percentage of baseline counts (time zero) and did not reflect the actual numbers recovered on culture plates. PAC and EZ supported recovery of <1% of the original inocula of *N. gonorrhoeae* and *H. influenzae*, and PAC

TABLE 1. Comparison of recovery rates of PAC, EZ, and CAG for fastidious and common aerobic organisms

Organism	% Survival from 0 h count (100%)					
	PAC		EZ		CAG	
	24 h	48 h	24 h	48 h	24 h	48 h
Neisseria gonorrhoeae	<1	0	0	0	23	6
Haemophilus influenzae	<1	0	<1	0	84	24
Streptococcus pneumoniae	18	4	<1	0	13	<1
Streptococcus pyogenes	113	129	2	<1	76	53
% Avg recovery	33	33	1	0	49	22

allowed recovery of only 18% of the initial concentration of *Streptococcus pneumoniae* after 24 h, even though both devices had time zero counts of 2.8×10^6 CFU/ml. The PAC device recovered an average of 2.5×10^4 CFU/ml at 24 h, while EZ recovered an average of only 300 CFU/ml. Although both counts represented <1% of the original inoculum at time zero, PAC numbers would have allowed a better chance of *H. influenzae* recovery upon routine culture if specimens contained $>10^6$ CFU/ml. Overall, CAG provided the best survival rate of these aerobes through 48 h.

Recovery of anaerobes was better from the agar gel-containing devices (Table 2). *B. fragilis* was not greatly affected by exposure to ambient air as are most other anaerobes; thus, EZ provided sufficient organisms to allow recovery after 48 h. EZ did not produce similar recovery for other anaerobes studied. CAG provided the best survival rates for all anaerobes over the 48-h incubation period. PAC allowed recoverable numbers of all strains for 24 h.

Agar gel formulations used in CAG (5 ml of Amies gel) and PAC (11 ml of buffered isotonic agar) protect swabs from ambient air and provide a moist, balanced pH atmosphere for microorganisms during transport and storage. The medium-free atmosphere used by EZ does not dilute the specimen with fluid or agar nor does it provide potential nutrients for organism growth or a means for mechanical scrubbing of the swab. The major difference between EZ and the other devices is the polyurethane foam swab, compared to a polyester swab on a wooden stick used in PAC and the rayon swab provided by CAG. Swab absorbency differences were significant. EZ absorbed 0.028 ml or about 25% as much as the 0.120 ml taken up during the 5-s immersion with PAC and CAG swabs. The foam swab actually appeared to be somewhat hydrophobic. A previous report on EZ used a swab inoculation scheme that required swabs to remain in contact with organism suspensions a minimum of 3 min (2). This appears to be an unrealistic time period in which to collect clinical specimens but most likely resulted in maximum absorption of fluid inoculum.

TABLE 2. Comparison of recovery rates of PAC, EZ, and CAG for anaerobic organisms

Organism	% Survival from 0 h count (100%)					
	PAC		EZ		CAG	
	24 h	48 h	24 h	48 h	24 h	48 h
Bacteroides fragilis	87	115	36	12	92	82
Peptostreptococcus anaerobius	<1	<1	1	0	17	1
Clostridium difficile	10	0	1	0	30	<1
Clostridium perfringens	8	2	<1	1	21	18
Fusobacterium nucleatum	3	0	<1	0	40	11
Eubacterium lentum	28	19	6	<1	58	54
Porphyromonas gingivalis	5	<1	0	0	12	1
% Avg recovery	20	20	7	2	39	24

Ergonomic and design features were considered in this evaluation as contributory to the overall usefulness of the transport devices. The PAC format evaluated consisted of a sterile pack containing one size D tube and a separate package of two swabs. Polyester swabs were affixed to wooden shafts and after specimen collection were used to insert the swab to within 5 mm of the bottom of the agar column. The wooden shaft then had to be broken off evenly with the lip of the tube for subsequent retrieval with forceps. Sharp, splintered ends resulted and were considered potentially hazardous, requiring special discard. The round glass tube rolled easily on smooth surfaces and was subject to breakage when falling to the floor. There may also be concerns about glass in pneumatic tube delivery systems. The CAG device was similar in size and design to many commercially available swab transport systems. A rounded bottom provided easy upright storage in most laboratory racks, and although it rolled on smooth surfaces, it was made of unbreakable polypropylene. The swab and tube cap were integral and formed a tight seal. Five milliliters of agar gel filled the tube past a narrow constriction (venturi) that provided a capsular compartment of medium that resisted cracks, bubbles, and removal of the agar gel when the swab was withdrawn. The EZ tube had a crimped bottom that prevented the tube from rolling but produced a flared end with sharp edges that did not fit in common laboratory racks and that had the potential to puncture specimen transport bags. All devices had waterproof, user-friendly peel-pouches. CAG and EZ had large preattached labels for sample and patient information. PAC had no such label, and the glass tube required a felt tip marking pen for durable labeling, an item not always available to health care personnel.

A comparative study of this nature cannot be performed with actual clinical specimens without introducing uncontrollable variables. Clinical specimens vary in viscosity and contain cellular and chemical constituents that may act as nutrients or toxins and that are often polymicrobial. These factors all have the potential to affect organism viability. Vortexing of swabs with subsequent quantitative cultures was performed to circumvent variables in swab porosity, organism entanglement in swab fibers, and mechanical transfer of microorganisms to agar surfaces. These systems were designed for direct swab inoculation of culture media, but that method did not lend itself to characterization of the potential for long-term organism survival without the introduction of variables. The presence of mucopurulent and sanguineous material would most likely aid in the protection of organisms from drying and from the deleterious effects of ambient air, a situation that would be favorable to EZ. Likewise, because the foam swab is not very absorbent, specimen material adherent to the swab surface would be more readily available for transfer to media without the dilution effect of a transport medium. Unfortunately, a laboratory model accurately reproducing clinical specimens allowing multiple sampling is not available. The survival of test organisms reported in this study may not faithfully reflect results with actual clinical material or recovery rates based on more common 2- to 4-h transport times, but it does allow comparisons of recovery rates to be made and most certainly permits insight into a system's ability to sustain organism viability, a notion taken on faith if not investigated.

In this study, agar gel transport devices performed better than the non-gel device and allowed recovery rates of 12 to 92% of the original inocula of seven anaerobes and 13 to 76%

of the original inocula of four common aerobic bacteria after 24 h. Rather than stock separate and often costly collection and transport systems for both routine aerobic and special anaerobic cultures, it would appear prudent to further evaluate the multipurpose utilization of agar gel systems with clinical studies.

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