

## Comparison of Two Transport Systems for Recovery of Aerobic and Anaerobic Bacteria from Abscesses

ITZHAK BROOK

*Departments of Pediatrics and Surgery, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814*

Received 3 June 1987/Accepted 13 July 1987

**An evaluation of two commercially available swab transport systems, Port-A-Cul (PAC; BBL Microbiology Systems, Cockeysville, Md.) and Anaerobic Specimen Collector (ASC; Becton Dickinson Vacutainer Systems, Rutherford, N.J.), in the recovery of organisms from clinical specimens was done. Fifteen abscesses were drained, and swabs of their contents were placed in the transport systems until they were inoculated for detection of aerobic and anaerobic bacteria. The swabs were plated immediately after collection and after delays of 4, 24, 48, and 72 h. A total of 70 bacterial isolates, 47 anaerobes and 23 aerobes, were recovered from specimens inoculated immediately after collection. The survival of anaerobic bacteria was better in the PAC system than in the ASC system. This was evident as the length of delay in cultivation was extended. At 4 h, 46 anaerobic isolates were recovered in the PAC system, compared with 39 in the ASC system ( $P < 0.1$ ). At 24 h, 45 isolates were recovered in the PAC system and 26 isolates were recovered in ASC ( $P < 0.001$ ); at 48 h, 40 were recovered in PAC and 15 were recovered in ASC; and at 72 h, 32 were recovered in PAC and 6 were recovered in ASC. There were no differences between the systems in the recovery of aerobic bacteria. These data demonstrate the usefulness of the PAC system in the recovery of anaerobes and the need for quality control of all transport systems for anaerobic bacteria.**

The protection of anaerobic bacteria from exposure to oxygen and drying during the transport of clinical specimens to the microbiology laboratory is a critical step in the recovery of these organisms. Various transport media have been used to overcome these problems (4, 5, 7, 9-11). Although aspirated material is more suitable for transportation (2, 5), swabs are often used. The present study was designed to compare the efficacy and clinical usefulness of two commercially available swab transport systems for anaerobic bacteria, Port-A-Cul (PAC; lots C7CGLJ and G60ZFZ; BBL Microbiology Systems, Cockeysville, Md.) and Anaerobic Specimen Collector (ASC; lots 6G642 and 5G644; Becton Dickinson Vacutainer Systems, Rutherford, N.J.). I embarked on this study after I observed a significant increase in the recovery of anaerobic bacteria in clinical specimens when the PAC transport system replaced the ASC transport system.

The 15 specimens included in the study were obtained from abscesses that were surgically drained from pediatric and adult patients (age range, 11 to 52 years; mean, 26 years). These included intra-abdominal (6 specimens), rectal (3 specimens), peritonsillar (3 specimens), retropharyngeal (2 specimens), and pelvic (1 specimen) abscesses. Antimicrobial agents were administered to eight patients before specimen collection. Each abscess was drained by a syringe, and the contents of the abscess was taken immediately to the microbiology laboratory, where the pus was placed in a sterile plastic container. Each abscess content was simultaneously processed by the two transport systems, by dipping the swab into the pus and immediately placing it into the appropriate tube. Five units of each system were simultaneously inoculated for processing immediately after collection and after 4, 24, 48, and 72 h. They were retained until processing at a room temperature of 23 to 26°C.

The anaerobic conditions within the transport systems were achieved as follows. For PAC, the swab was introduced into a semisolid medium that contains reducing substances which help to maintain oxidation-reduction poten-

tial. The ASC contains 10% hydrogen in an anaerobic gas mixture, into which the swab is dropped.

At each processing time, the swabs from each transport system were plated on media that support the growth of aerobic and anaerobic bacteria. Sheep blood (5%), chocolate, and MacConkey agar plates were inoculated for the isolation of aerobic organisms. The plates were incubated at 37°C aerobically (MacConkey) and under 5% CO<sub>2</sub> (blood and chocolate) and examined at 24 to 48 h. To isolate the anaerobes, the specimens were plated on prereduced vitamin K<sub>1</sub>-enriched brucella blood agar, on an anaerobic blood agar plate containing kanamycin and vancomycin, on an anaerobic blood plate containing phenylethyl alcohol, and into enriched thioglycolate broth (12). These media were incubated in anaerobic jars at 37°C and examined at 48 and 96 h. The thioglycolate broth was incubated for 14 days. Bacteria were identified by conventional methods (8, 12). Statistical analysis was done with the Student *t* test.

All 15 abscesses yielded bacterial growth. Microorganisms potentially susceptible to the antimicrobial agents given were recovered from the abscesses in the eight patients treated with antimicrobial agents. Thirteen specimens yielded a mixed aerobic-anaerobic flora, whereas two yielded only anaerobes. A total of 70 bacterial strains, 47 anaerobes and 23 aerobes or facultative organisms, were recovered from specimens cultured immediately after collection. This accounts for averages of 3.1 anaerobic and 1.5 aerobic bacteria per abscess (a total of 4.6 isolates per abscess). The types of organisms were similar to those reported in previous clinical studies of anaerobic infections which used optimal bacteriological techniques (1-3). The predominant anaerobic isolates (Table 1) were *Peptostreptococcus* sp., *Bacteroides fragilis* group, *Bacteroides melaninogenicus* group, *Clostridium* sp., and *Fusobacterium* sp. The major aerobic or facultative bacteria were *Streptococcus* sp., *Staphylococcus aureus*, and *Escherichia coli*.

TABLE 1. Bacteria isolated from 15 abscesses at each sampling interval

Organisms	No. of isolates from indicated system at following time:								
	0 h	4 h		24 h		48 h		72 h	
		PAC	ASC	PAC	ASC	PAC	ASC	PAC	ASC
<b>Anaerobic</b>									
<b>Gram positive</b>									
<i>Peptostreptococcus</i> sp.	14	14	11	14	8	12	5	10	4
<i>Veillonella</i> sp.	3	3	2	2	2	2	0	1	0
<i>Eubacterium</i> sp.	4	4	4	4	2	3	0	3	0
<i>Clostridium</i> sp.	5	5	5	5	3	5	2	3	0
<i>Propionibacterium</i> sp.	3	3	2	3	2	3	1	1	1
<b>Gram negative</b>									
<i>Bacteroides</i> sp.	3	3	2	3	0	2	1	2	0
<i>B. fragilis</i> group	6	6	5	6	4	6	3	5	1
<i>B. melaninogenicus</i> group	5	5	5	5	3	4	2	4	0
<i>Fusobacterium</i> sp.	4	3	3	3	2	3	1	3	0
All anaerobes	47	46	39	45	26 <sup>a</sup>	40	15 <sup>a</sup>	32	6 <sup>a</sup>
<b>Aerobic and facultative</b>									
<b>Gram positive</b>									
<i>Streptococcus</i> sp.	7	7	7	7	7	6	7	6	6
<i>Staphylococcus aureus</i>	5	5	5	5	5	5	4	5	5
<b>Gram negative</b>									
<i>Escherichia coli</i>	4	4	4	4	3	3	3	3	2
<i>Klebsiella pneumoniae</i>	2	2	2	2	2	2	2	2	2
<i>Pseudomonas aeruginosa</i>	3	3	3	2	3	1	3	1	2
<i>Proteus</i> sp.	1	1	1	1	1	1	1	1	0
<i>Enterobacter</i> sp.	1	1	1	1	0	1	0	1	0
All aerobes	23	23	23	22	21	19	20	19	17
All organisms	70	69	62	67	47	59	35	51	23

<sup>a</sup> Highly significant statistical difference compared with PAC ( $P < 0.001$ ).

Good survival of aerobic and facultative bacteria was noted in both PAC and ASC for the first 48 h. Comparison of the recovery rates of the anaerobic bacteria demonstrated the superiority of the PAC system over the ASC system. This was especially evident as the length of delay in cultivation was extended. The organisms that did not survive in the ASC transport system were members of all species represented.

Observation of the colony morphological characteristics of the anaerobic isolates demonstrated that even after 4 h of delay, the growth on solid media after inoculation from the ASC system was scant and the colonies were smaller than those observed on solid media after inoculation from the PAC system.

Anaerobic bacteria are important clinical isolates in abscesses and wounds (2). They are predominant in infections in and around the oral and rectal areas (1, 3) and in intra-abdominal and pelvic infections (2). Although surgical drainage is of prime importance, the ability to recover these bacteria ensures the proper selection of antimicrobial agents for the therapy of these infections. Swabs specimens are generally maintained in a transport system until they reach the laboratory and are often delayed overnight if they arrive after the laboratory is closed. The survival of anaerobes in transport systems is therefore of prime importance.

Several transport systems are available. The efficacies of these systems were demonstrated in several studies (6, 9-11). Many of these studies used stock strains of anaerobic bacteria to simulate clinical specimens, and the numbers of organisms absorbed in each swab were quite large (6, 7, 10). Although the efficacy of ASC in the recovery of anaerobic bacteria from clinical specimen was previously evaluated

(9), most specimens were processed after only 3 h of delay. However, delays in cultivation in the clinical setting may exceed this length of time.

Our data clearly demonstrate the superiority of the PAC system over the ASC system. Although both systems were shown to be effective in preserving simulated specimens, their efficacies in sustaining the viability of anaerobes in clinical samples are the crucial test. The inferior efficacy of ASC compared with PAC in our study, despite previous positive reports (6, 9), may have been a result of the increased moisture present in the PAC system or variations in the quality of the PAC system. This report reiterates the need for constant quality control of all transport systems for anaerobic bacteria. It also highlights the need to test the efficacies of these systems in maintaining the viability of anaerobic bacteria in samples collected from clinical specimens.

The present study illustrates the potential deficiency of a swab system in preserving the viability of clinical specimens. Although a properly transported aspirate is recommended, a swab may be the only feasible vehicle. In these instances, transport kit preservation efficacy should be an issue, especially if delay is likely.

I am grateful for the laboratory assistance of Thom Williams and Thom Elliot and the secretarial assistance of Hayati Atan.

#### LITERATURE CITED

1. Brook, I. 1981. Aerobic and anaerobic bacteriology of peritonitis abscess in children. *Acta Paediatr. Scand.* **70**:831-835.
2. Brook, I. 1983. Anaerobic infections in childhood. A textbook. G. K. Hall Medical Publisher, Boston.

3. **Brook, I., and S. M. Finegold.** 1981. Aerobic and anaerobic bacteriology of cutaneous abscesses in children. *Pediatrics* **67**:891-895.
4. **Chow, A. W., P. J. Cunningham, and L. B. Guze.** 1976. Survival of anaerobic and aerobic bacteria in a nonsupportive gassed transport system. *J. Clin. Microbiol.* **3**:128-132.
5. **Hallander, H. O., A. Flodström, and K. Holmberg.** 1975. Influence of the collection and transport of specimens on the recovery of bacteria from peritonsillar abscesses. *J. Clin. Microbiol.* **2**:504-509.
6. **Hill, G. B.** 1978. Effects of storage in an anaerobic transport system on bacteria in known polymicrobial mixtures and in clinical specimens. *J. Clin. Microbiol.* **8**:680-688.
7. **Justen, T., A. M. Jensen, and S. Hoffmann.** 1983. The survival of anaerobic bacteria at 4°C and 22°C on swabs in three transport systems. *Acta Pathol. Microbiol. Immunol. Scand.* **91**:17-22.
8. **Lennette, E. H., A. Balows, W. Hausler, Jr., and H. J. Shadomy (ed.).** 1985. *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
9. **McConville, J. H., R. F. Timmons, and S. L. Hansen.** 1979. Comparison of three transport systems for recovery of aerobes and anaerobes from wounds. *Am. J. Clin. Pathol.* **72**:968-971.
10. **Mena, E., F. S. Thompson, A. Y. Armfield, V. R. Dowell, Jr., and D. J. Reinhardt.** 1978. Evaluation of Port-A-Cul transport system for protection of anaerobic bacteria. *J. Clin. Microbiol.* **8**:28-35.
11. **Smith, G. L. F., C. G. Cumming, and P. W. Ross.** 1986. Survival of Gram-positive anaerobic cocci on swabs and their isolation from mouth and vagina. *J. Clin. Pathol.* **39**:93-98.
12. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1980. *Bacteriology manual*, 4th ed. Star Publishing Co., Belmont, Calif.