Evaluation of Port-A-Cul Transport System for Protection of Anaerobic Bacteria

E. MENA,[1] F. S. THOMPSON,[2] A. Y. ARMFIELD,[2] V. R. DOWELL, JR.,[2] * AND D. J. REINHARDT[3]

Departmento de Microbiologia, Facultad de Medicina, Universidad de El Salvador, San Salvador, El Salvador[1]; Enterobacteriology Branch, Bacteriology Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia 30333[2]; and Biology Department, Georgia State University, Atlanta, Georgia 30303[3]

The protection of anaerobes in Port-A-Cul (PAC) transport system (Bioquest, Div. of Becton, Dickinson & Co., Cockeysville, Md.) tubes and vials was studied. Ten species of obligately anaerobic bacteria commonly isolated from clinical specimens were used to prepare simulated swab and fluid specimens in high and low concentrations. Samples in PAC tubes and vials were held for 2, 24, and 48 h at ambient temperature and in a refrigerator. In addition, samples of the simulated specimens were exposed to controlled anaerobic and aerobic conditions in vented tubes and vials, with and without PAC medium, at ambient and refrigerator temperatures. Viable bacterial colony counts from specimens in PAC tubes and vials used as recommended by the manufacturer were consistently greater than those from specimens exposed to the different controlled conditions. The protection in PAC was about equal for specimens with either high or low concentrations of bacteria. Protection of the anaerobes in PAC was more obvious with swab than with fluid specimens. Quantitative recovery of anaerobes from refrigerated PAC samples, with few exceptions, was comparable to that from PAC samples held at ambient temperature.

Exposure of clinical samples to oxygen is one of the main reasons for unsuccessful recovery of anaerobes. Although all isolates of anaerobes are not equally affected by the deleterious action of oxygen (3, 14, 17, 23), clinical samples must be cultured as soon as possible after they are collected. Delays are sometimes unavoidable, and in those instances the samples must be protected in the best possible way until they are processed. Systems currently in use for protection of bacteria in clinical specimens during transport include gassed-out rubber-stoppered tubes and vials containing oxygen-free gas (15, 21), tubes containing an anaerobic transport medium (2, 5, 12, 16, 21, 22), and transport devices which rely on chemical reactions for removal of oxygen after the specimen is collected (1, 24).

Polymicrobial infections involving obligate anaerobes are common (9, 10, 19). Ideally, a transport system should maintain the viability of all types of microorganisms in a sample without promoting multiplication. At this time, however, no transport system is available which is suitable for all types of clinical material that may harbor anaerobes. Physicians and microbiologists must select from the available transport devices those most appropriate for their particular needs.

Simulated clinical samples compare only par-

tially with actual clinical specimens that contain mucus, leukocytes, erythrocytes, immunoglobulins, complement, and other components which may protect or inhibit bacteria present. Nevertheless, the use of simulated samples permits an assessment of the approximate degree of protection that can be expected from a given transport device. The object of this study was to perform a controlled quantitative evaluation of a transport system for the protection of obligately anaerobic bacteria, using bacterial suspensions prepared in spent culture medium as simulated specimens.

MATERIALS AND METHODS

Bacteria. Stock strains of 10 species of obligately anaerobic bacteria commonly isolated from human clinical materials were chosen for this study. Bacterial strains were obtained from lyophilized stock cultures in the Anaerobe Section, Enterobacteriology Branch, Center for Disease Control, Atlanta, Ga., and included *Bacteroides distasonis, Clostridium ramosum, Peptostreptococcus anaerobius, Bifidobacterium eriksonii, Veillonella parvula, Propionibacterium acnes, Fusobacterium necrophorum, Fusobacterium nucleatum, Bacteroides fragilis,* and *Clostridium perfringens.* These species have varying degrees of sensitivity to and tolerance for oxygen (19). Cultures were studied, and their identities were confirmed as described previously (7, 15).

Culture medium filtrate. Bacterial suspensions were prepared by suspending cells to the desired turbidity in the liquid portion of spent broth cultures after filtration. A 0.1-ml portion of culture from a 24-h thioglycolate medium (BBL 0135C; Baltimore Biological Laboratory, Cockeysville, Md.) culture was used to inoculate 500 ml of Lombard-Dowell medium with 0.1% glucose (20). After 18 to 24 h of incubation under anaerobic conditions at 35°C, the bacterial suspension was filtered through a Falcon 7102 filter (0.45 μm; Falcon Plastics, Oxnard, Calif.) using negative pressure. The bacteria were discarded, and the filtrate was used as a suspending medium to prepare the simulated clinical specimens in an anaerobic box. Spent medium was used to prepare cell suspensions to avoid adding additional nutrients which could permit multiplication of the bacteria. Preliminary studies showed that growth of the anaerobes tested in Lombard-Dowell medium with 0.1% glucose was limited by some factor(s) in the medium. There was little or no increase in turbidity of the cultures after 18 to 24 h of anaerobic incubation at 35°C.

Bacterial suspensions. Cells from an 18- to 24-h Lombard-Dowell broth culture of each isolate were added to two freshly prepared 500-ml portions of spent culture filtrate to yield cell concentrations of approximately 10^6 and 10^4 colony-forming units (CFU) per ml. These were labeled high and low concentrations, respectively. The suspensions were held under anaerobic conditions until used.

Simulated clinical specimens. All manipulations, e.g., filtration, preparation of cell suspensions and dilutions, etc., were done in an anaerobic glove box. Two types of samples were used in this study, swab and fluid. The swab sample was prepared by delivering 0.1 ml of a bacterial suspension with a micropipettor (Micro-Selectapette; Clay Adams, Parsippany, N.J.) directly onto a swab. The fluid sample, represented by 1 ml of a bacterial suspension, was injected into a vial with a 5-ml disposable syringe.

PAC system. The Port-A-Cul (PAC) transport system in tubes and vials was recently developed by the Bioquest Division of Becton, Dickinson & Co., Cockeysville, Md., (I. J. Abramson, G. L. Evans, and P. Bathurst. Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C197, p. 68). It was designed to maintain the viability of aerobic, microaerophilic, facultatively anaerobic, and obligately anaerobic microorganisms in clinical specimens for laboratory examination. The medium contains reducing agents which help to maintain a low oxidation-reduction potential. It also contains resazurin indicator which turns to pink or lavender when the medium is aerated.

PAC medium in tubes was used as recommended by the manufacturer for the simulated swab samples. After a bacterial suspension was added to a swab, the swab was inserted into the depths of the medium, and the shaft of the applicator stick extending beyond the mouth of the tube was broken off. The cap of the tube was then tightened securely. The simulated fluid specimens were injected into PAC vials with a disposable 5-ml syringe and an 18-gauge needle. Care was taken to exclude air from the syringe and needle before the sample was injected into the vial.

Reference and control systems. The recovery of bacteria from swab and fluid samples held in PAC was compared with recovery of bacteria with reference and control systems, as illustrated in Fig. 1 through 3.

Anaerobic and aerobic vented tubes. Each tube contained a plastic disposable sterile swab (Falcon Plastics) carrying 0.1 ml of either high- or low-concentration bacterial cell suspension, as with the PAC medium in tubes, and 1 ml of phosphate buffer solution to provide humidity for the swabs. The swabs were suspended in the middle of the tubes so that neither the wall of the tube nor the phosphate buffer solution was in contact with the specimens. The caps were left loose enough to allow exchange of gases in the tubes. Anaerobic tubes were vented in an anaerobic glove box (7), and aerobic tubes were vented in room air (Fig. 1).

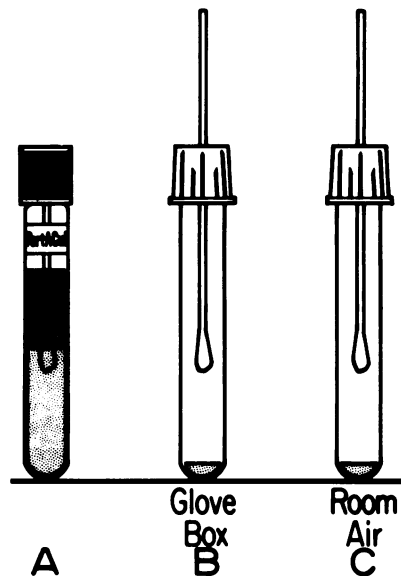**Anaerobic and aerobic vented vials.** Sterile plain vials similar to the vials used for PAC were

FIG. 1. *Swab samples. A, PAC tube used as recommended by the manufacturer; B, plain tube vented in an anaerobic glove box; C, plain tube vented in room air.*
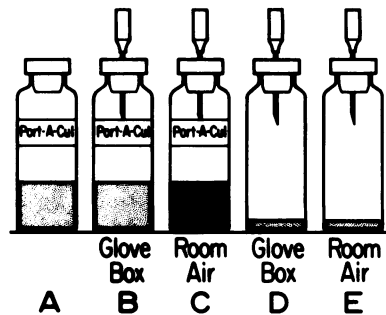

FIG. 2. *Vial samples. A, PAC used as recommended by the manufacturer; B, PAC vented in an anaerobic glove box; C, PAC vented in room air; D, plain vial vented in glove box; E, plain vial vented in room air.*

CFU/ml
Ambient Temperature

Time in Hours	0		2		24		48	
Concentration of Inoculum	H	L	H	L	H	L	H	L
	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
Swabs:								
A								
B								
C								
Vials:								
A								
B								
C								
D								
E								

FIG. 3. *Schematic representation of the protocol followed in studying the quantitative recovery of obligately anaerobic bacteria in PAC transport system, tubes, and vials. An identical protocol was followed for the tubes and vials held in the refrigerator. Abbreviations: H, high concentration of bacteria; L, low concentration of bacteria; (2), procedure was run in duplicate; A, PAC used as recommended by the manufacturer; B, vented in an anaerobic glove box; C, vented in air; D, plain vial vented in the glove box; E, plain vial vented in room air.*

inoculated with 1.0 ml each of high and low concentrations of the bacterial cell suspensions, as was done with the PAC vials, and vented with 18-gauge cotton-plugged hypodermic needles. Some of the vials were vented anaerobically in the glove box, and some were vented aerobically in room air. In addition, PAC vials with transport medium were inoculated and vented under the same conditions (Fig. 2).

Protocol. The protocol for studying the quantitative recovery of obligate anaerobes from PAC vials and tubes held at ambient and refrigerator temperatures is summarized in Fig. 3. The tubes and vials held in the refrigerator under anaerobic conditions were first placed in an open GasPak jar in an anaerobic glove box to replace the air in the container with anaerobic gas mixture (5% CO_2, 10% H_2, 85% N_2). After gassing, the lid of the jar was attached, and the jar was removed from the glove box and placed in a refrigerator at 4°C.

Quantitative cultures. At the end of each exposure time (0, 2, 24, and 48 h), the viability of the microorganisms was determined by plate counts. Swabs were aseptically removed from the tubes and placed in glass vials containing 0.9 ml of sterile buffered dilution water. Excess portions of the wooden shafts of the swabs were removed with sterile scissors. The vials were then closed and mixed (Vortex Genie mixer, set at one-third of the scale on the dial; Fisher Scientific Co., Pittsburgh, Pa.) for approximately 15 s. Next, 0.1 ml of suspension was removed from the vial with a micropipettor, and 10-fold dilutions were made. Viable counts were made of the 10^{-3}, 10^{-4}, and 10^{-5} dilutions of the high-concentration bacterial suspension and from the 10^{-2}, 10^{-3}, and 10^{-4} dilutions of the low concentrations of bacteria. Samples (0.1 ml) of the diluted cell suspensions were placed on the surface of anaerobe blood agar (8) which had been held in an anaerobic glove box for 18 to 24 h before use. The inoculum was spread on the medium with a sterile,

bent glass rod. After inoculation, the plates were incubated in an anaerobic glove box at 35°C for 4 days.

Portions (0.1 ml) of the vial samples were removed with tuberculin syringes and needles. Tenfold dilutions were prepared and plated on the surface of anaerobe blood agar as described above for swab samples.

Colony counts were performed of plates containing 20 to 400 colonies, and the number of CFU per milliliter in the samples was calculated.

RESULTS

Figures 4 through 7 represent composite (average values for high- and low-concentration samples at each point) changes in viable count with time of the 10 bacterial strains tested on
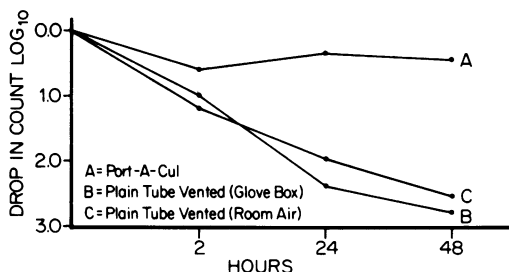

FIG. 4. *Changes in composite colony counts of swab samples of 10 obligately anaerobic bacteria at various times at ambient temperature.*
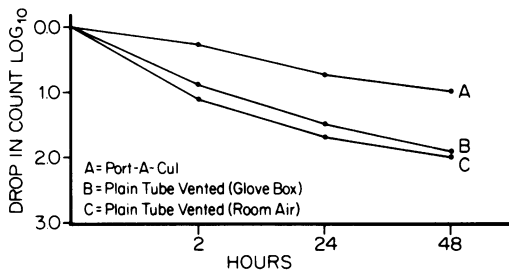
A = Port-A-Cul
B = Plain Tube Vented (Glove Box)
C = Plain Tube Vented (Room Air)

FIG. 5. *Changes in composite colony counts in swab samples of 10 obligately anaerobic bacteria with time at refrigerator temperature (4°C).*
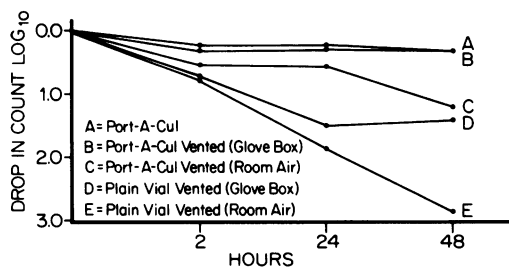
A = Port-A-Cul
B = Plain Tube Vented (Glove Box)
C = Plain Tube Vented (Room Air)

FIG. 6. *Composite log_{10} count reduction for 10 obligately anaerobic bacteria at various times from fluid samples held in PAC vials used as recommended by the manufacturer and under controlled conditions at ambient temperature.*
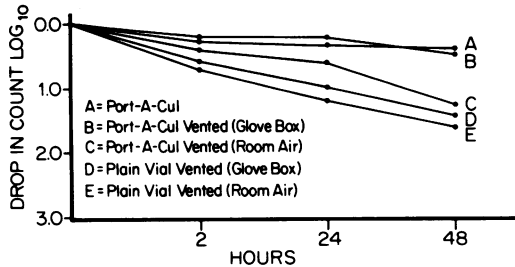
A = Port-A-Cul
B = Port-A-Cul Vented (Glove Box)
C = Port-A-Cul Vented (Room Air)
D = Plain Vial Vented (Glove Box)
E = Plain Vial Vented (Room Air)

FIG. 7. *Changes with time in composite colony counts for 10 obligately anaerobic bacteria in fluid samples held in PAC vials used as recommended by the manufacturer and under other controlled conditions at refrigerator temperature.*

swab and in fluid (vials) samples at ambient temperature (25 ± 2°C in the laboratory and 27 ± 2°C in the glove box) or refrigerated temperature (4°C). These data show that PAC used as recommended by the manufacturer protected the samples better than any of the other systems tested in the study. At ambient temperature most of the anaerobes on swabs were adequately protected by PAC for up to 48 h. There was a significant drop, however, in the viable count of *Fusobacterium nucleatum* in both the high- and the low-concentration samples in PAC after 24 h at ambient temperature. Refrigeration did not appear to enhance the protective qualities of PAC, because there were decreases in the viable counts of the bacteria after refrigeration for 48 h (Fig. 5 and 7).

The medium near the surface in the PAC tubes and vials became pink-lavender immediately after the samples were introduced, but the color soon disappeared. The depths of the medium in the PAC tubes and vials remained colorless at all times.

The overall (at both ambient and refrigerator temperatures) differences in recovery from swabs in the three different systems (tube A, PAC; tube B, plain tube vented in the glove box; and tube C, plain tube vented in room air) were considerable. A significant ($P < 0.0001$) overall difference in recovery was found between tube A and tubes B and C, but no significant difference in the recovery was found between tubes B and C. In addition, there was a significant overall difference ($P < 0.001$) in recovery between vial A (PAC) and vials D (plain vial vented in the glove box) and E (plain vial vented in room air) and between vial B (PAC vented in the glove box) and vials D and E. There was no significant difference in recovery between vials D and E.

Smaller differences were found between the PAC system and the other systems in the recovery of the anaerobes from refrigerated samples than from those maintained at ambient temper-

ature. Differences were greater for both tubes and vials at ambient temperature; the following pairs were found to be significantly different at $P < 0.01$: tube A (PAC) and tube B (plain tube vented in the glove box); tube A and tube C (plain tube vented in room air); vial A (PAC) and vial D (plain vial vented in the glove box); vial A and vial E (plain vial vented in room air). Recovery from tubes B and C and from vials D and E was not significantly different.

Overall, there was little difference in the rate of recovery of viable microorganisms from the high- and the low-concentration samples held in PAC. The rate of recovery of *P. anaerobius* from PAC, however, was less in the low-concentration sample than in the high-concentration sample at ambient temperature and at refrigerator temperature (Table 1).

The viable count of *C. ramosum* decreased one full log_{10} after 24 h of storage on swabs in PAC under refrigeration; however, similar samples showed no decrease in viable count after 24 or 48 h at ambient temperature (Table 2). The decrease in viable count of *B. fragilis* PAC swab and fluid samples was similar to that exhibited by *C. ramosum* when they were refrigerated (Table 3).

Some of the bacterial strains multiplied in the PAC tubes and vials when they were held at ambient temperature for prolonged periods. For example, the viable count of *C. ramosum* was

TABLE 1. *Changes in viable counts of P. anaerobius in swab and fluid samples held in PAC at two temperatures*

Sample	Temp (°C)	Initial bacterial concn (log_{10} CFU)	Reduction of colony count (log_{10}) after:		
			2 h	24 h	48 h
Swabs	25	6.34 (High)	1.11	1.19	2.04
		5.48 (Low)	>3.83	>3.83	>3.83
	4	6.34 (High)	0.45	1.19	1.35
		5.48 (Low)	1.60	>3.48	>3.48
Vials	25	6.60 (High)	0.30	0.40	0.32
		5.74 (Low)	2.21	2.51	2.34
	4	6.60 (High)	0.03	0.45	0.09
		5.74 (Low)	2.34	2.08	1.86

TABLE 2. *Changes in viable cell counts of C. ramosum samples on swabs held in PAC at two temperatures*

Temp (°C)	Initial bacterial concn (log_{10} CFU)	Reduction of colony count (log_{10}) after:		
		2 h	24 h	48 h
25 (Ambient)	5.00	0.43	0.00	0.00
4 (Refrigerator)	5.00	0.37	>2.00	>2.00
25 (Ambient)	3.68	1.68	0.04	0.00
4 (Refrigerator)	3.68	0.09	>1.68	>1.68

TABLE 3. *Changes in viable cell counts of B. fragilis held in PAC as swab and fluid samples at two temperatures*

Sample	Temp (°C)	Initial bacterial concn ($\log_{10}$ CFU)	Decrease in colony count ($\log_{10}$) after[a]:		
			2 h	24 h	48 h
Swabs	25 (Ambient)	6.38 (High)	0.38	0.00	0.00
	4 (Refrigerator)	6.38 (High)	0.80	1.48	1.27
	25 (Ambient)	4.34 (Low)	0.11	0.00	0.00
	4 (Refrigerator)	4.34 (Low)	0.19	0.76	1.00
Vials (fluid)	25 (Ambient)	6.72 (High)	0.32	0.00	0.00
	4 (Refrigerator)	6.72 (High)	0.00	0.82	1.15
	25 (Ambient)	4.86 (Low)	0.22	0.48	0.00
	4 (Refrigerator)	4.86 (Low)	0.32	0.82	1.03

[a] Colony count ($\log_{10}$) reduction in relation to initial concentration.

2.7 $\log_{10}$ higher than the initial count after 48 h at ambient temperature. Samples of *B. eriksonii* on swabs held in PAC at ambient temperature increased about 1 $\log_{10}$ after 48 h of storage. The viable count of *B. fragilis* also increased at ambient temperature (Fig. 8 and 9), but it decreased some when the PAC samples were refrigerated (Fig. 10 and 11).

DISCUSSION

Unless proper precautionary measures are taken during collection, transport, and laboratory processing, pronounced changes can occur in the microbial population of a clinical specimen (6, 10). Sensitivity to molecular oxygen causes some obligate anaerobes to die rapidly upon exposure to air. In clinical samples obligate anaerobes can also be overgrown by facultative anaerobes unless the sample is processed rapidly after collection. Failure to take proper precautions can result in misleading data, which indirectly may be detrimental to the patient (6, 7, 10, 15, 21).

Unfortunately, knowledge of the physiology of anaerobiosis is rather limited. At present, no one factor accounts for the oxygen sensitivity of the obligately anaerobic bacteria. We do know, however, that obligate anaerobes differ in oxygen tolerance and sensitivity, as Loesche (17) showed. Loesche called strict anaerobes those which exhibited no surface growth at oxygen concentrations above 0.5% and did not grow after 60 to 100 min of exposure to air. Moderate anaerobes multiplied on the surface of solid media in oxygen concentrations as high as 3%, and viability was barely affected after 80 min of exposure to air. Most of the common and clinically important anaerobic bacteria are moderate anaerobes, as shown by the examination of various types of clinical specimens for anaerobes (3, 12, 23).
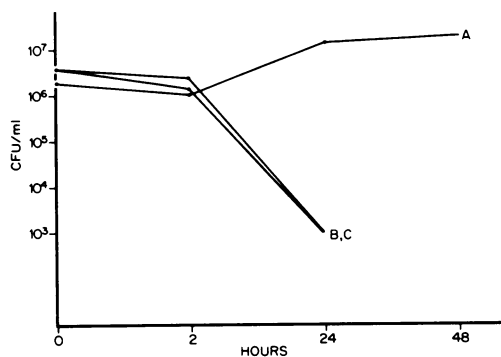

FIG. 8. *Changes in the viable count of B. fragilis swab samples at ambient temperature (25°C): (A) in PAC as recommended by the manufacturer, (B) in a plain tube vented in an anaerobic glove box, and (C) in plain tube vented in air after 2, 24, and 48 h.*
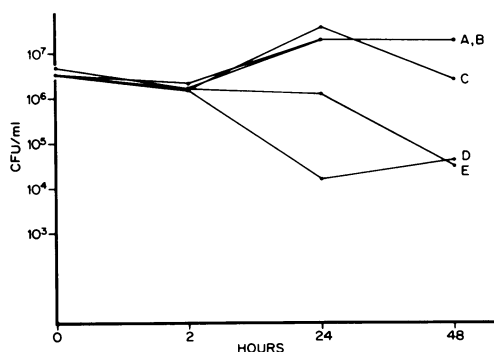

FIG. 9. *Changes in the viable count of B. fragilis samples held in vials at room temperature (25°C) after 2, 24, and 48 h in PAC used as recommended by the manufacturer (A), in PAC vented in an anaerobic glove box (B), in PAC vented in air (C), in plain vials vented in an anaerobic glove box (D), and in plain vials vented in air (E).*
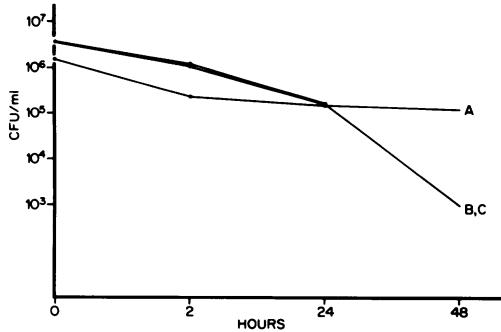
FIG. 10. *Changes in the viable count of B. fragilis swab samples held at refrigerator temperature (4°C) for 2, 24, and 48 h in PAC used as recommended by the manufacturer (A), in plain vials vented in an anaerobic glove box (B), and in plain vials vented in air (C).*
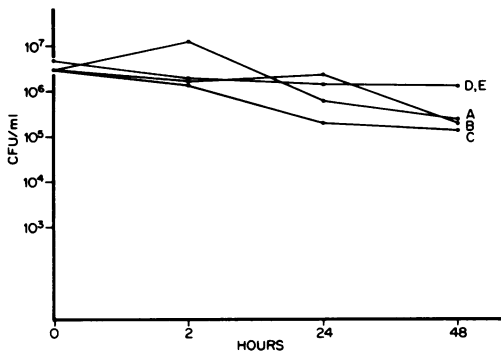

FIG. 11. *Changes with time in the viable count of B. fragilis vial samples held at refrigerator temperature (4°C) in PAC vials used as recommended by the manufacturer (A), in PAC vials vented in an anaerobic glove box (B), in PAC vials vented in air (C), in plain vials vented in an anaerobic glove box (D), and in plain vials vented in air (E).*

Because numbers and kinds of microorganisms in clinical materials vary widely, no transport device should be expected to give optimal protection for all anaerobes that may be encountered in specimens. Syed and Loesche (22) reached this conclusion after studying the survival of human dental plaque flora in various transport media. Therefore, a transport system should be selected on the basis of a quantitative evaluation which permits its values and limitations to be properly assessed.

The results of the present study indicate that the PAC transport system affords better protection to obligately anaerobic bacteria on swabs or in fluid samples than a plain tube vented in air or a vial vented within a glove box containing 85% nitrogen, 10% hydrogen, and 5% carbon

dioxide. At ambient temperature most of the obligate anaerobes on the swab samples were adequately protected by PAC for up to 48 h. However, there was a significant drop in the population of *F. nucleatum* after 24 h. This result correlates with the findings of Yrios et al. (25), who concluded that *F. nucleatum* was the only one of four anaerobic bacteria studied which showed a significant drop in the viable count after 24 h. *P. anaerobius* was another exception in swab samples held in PAC. In low-concentration samples the CFU of *P. anaerobius* per milliliter dropped sharply after 2 h of storage at ambient temperature, whereas in high-concentration samples, there was adequate protection at ambient temperature. *P. anaerobius* was the only 1 of 10 bacterial species tested that was protected better by PAC in the high-concentration sample than in the low-concentration sample. Tally et al. (23) noted that a decrease in inoculum size from 10^8 to 10^6 CFU/ml had only a minor effect on the oxygen tolerance of recently isolated anaerobic bacteria from clinical samples. The colony counts of *C. ramosum* and *B. fragilis* increased when samples were maintained in PAC at room temperature for longer than 24 h. The exact factor(s) responsible for the increase in population was not determined. This increase may have been due, however, to the presence of nutrients in the simulated clinical specimen or to growth-promoting factors in the transport medium. Chow et al. (4) reported that *B. fragilis-Staphylococcus aureus* mixtures in gassed-out tubes filled with oxygen-free carbon dioxide gradually increased in number when they were suspended in broth, but not in saline. Christian and Ederer (5) showed that *B. fragilis* in thioglycolate broth cultures added to swabs increased in number when held in three different transport media at room temperature. Also, according to Wilkins and Jimenez-Ulate (24), swabs containing anaerobes suspended in brain heart infusion broth showed growth of the microorganisms when they were held in gassed-out tubes filled with 85% nitrogen, 10% hydrogen, and 5% carbon dioxide. Gastrin et al. (11) observed growth of *C. perfringens* suspended in phosphate buffer solution with swabs held in VMG II transport at room temperature. Other workers, however, did not note proliferation in simulated samples held in transport media or in dry gassed-out tubes filled with carbon dioxide (2, 25).

Sterile saline can be inhibitory for certain bacteria (18, 25). Plain saline as well as phosphate-buffered saline, however, does not contain growth-promoting nutrients which are obviously present in media such as nutrient broth or brain

heart broth. Clinical materials may contain growth-inhibiting as well as growth-promoting factors. It also seems likely that some nutrients required for multiplication of microorganisms may be limited in clinical materials from certain types of infections, e.g., loculated abscesses.

In our study the liquid portion of spent cultures grown in Lombard-Dowell broth with 0.1% glucose (20), which is growth limiting, was used in preparing simulated specimens. Bacterial metabolic products which accumulate in the culture fluid may mimic, in a crude fashion, the bacterial products in a clinical sample such as pus from a loculated abscess.

There was no evidence in this study that the protection by PAC for bacteria was any better in refrigerated PAC swab samples than in samples at room temperature. There was some decrease in the populations of C. ramosum and B. fragilis after 24 h of refrigeration in PAC, but the recovery of viable organisms was adequate. Refrigeration can be detrimental to some anaerobes in aerated specimens (6, 7). Therefore, samples refrigerated to prevent overgrowth of aerobic or facultative bacteria should be held in gastight anaerobic containers (10). Because according to the manufacturer's instructions overgrowth of anaerobes by other microorganisms can occur in PAC at ambient temperature, the temperature range below 25°C at which specimens can be held in PAC without appreciable changes in their microbial populations should be determined. In our study recovery of bacteria from swabs held in a plain tube vented in an anaerobic glove box differed only slightly from that from swab samples held in room air. Tube B was expected to give more protection to the anaerobes than tube C (Fig. 1), but the counts of the bacteria on swabs from both tubes were significantly lower than PAC-protected swabs (tube A, Fig. 1) after 48 h of storage at ambient temperature. Desiccation of specimens on swabs is a well-recognized problem (2, 25). Moreover, the forced circulation of the atmosphere and the higher temperature inside the glove box (27 ± 2°C compared with 25 ± 2°C in the room air) possibly favored a greater water loss from swabs. Both tube B and tube C (Fig. 1) contained 1 ml of phosphate buffer solution to help maintain adequate humidity and prevent desiccation.

Studies by other workers with anaerobic gassed-out tubes have shown good protection for swab samples (24), but others have noted inadequate protection (25). Helstad et al. (13), in a study of clinical specimens carried in different transport systems, reported better recovery from swab samples held in Cary and Blair medium than from swab samples held in gassed-out tubes

filled with a gas mixture of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. The tube vented in the anaerobic glove box as used in our study is similar to the gassed-out tubes others (15, 21) have used. The experimental protocol of the present study could be modified easily so that it could be used to explore the effects of desiccation on swab samples in the glove box.

In general, fluid samples held in PAC vials were found to be as well protected as the swab samples in PAC. As with the swabs, F. nucleatum and P. anaerobius were the exceptions and decreased in count. F. nucleatum was found in a recoverable number after 48 h of storage at ambient temperature, however, and P. anaerobius was moderately affected only in the low-concentration sample. The viable counts of C. ramosum and B. fragilis decreased even less in PAC vials in the refrigerator than on refrigerated swabs in PAC. A few of the anaerobes tested could multiply in PAC vials at ambient temperature, but none did so under refrigeration—a result similar to the changes observed in swab samples discussed above. Refrigeration of clinical specimens in vials of PAC should prevent overgrowth with facultative anaerobes (and some obligate anaerobes) without appreciably decreasing the obligate anaerobe population.

The anaerobic conditions in the PAC transport system apparently were not improved by venting in an anaerobic glove box with a mixture of 85% nitrogen, 10% hydrogen, and 5% carbon dioxide. This was shown by the similarity in viable counts between anaerobically vented and unvented samples in PAC. The reducing agents in the PAC medium apparently did not maintain a sufficiently reduced environment in vials vented in air to protect obligate anaerobes. The resazurin indicator in the PAC medium turned pink to lavender except at the interface of the liquid specimen and medium in the aerated vials.

On the basis of data derived from this study it can be concluded that the PAC vials, used as recommended by the manufacturer, gave better protection to the obligate anaerobes tested than the other systems compared. However, it is not possible to predict with certainty from these data how obligate anaerobes or other microorganisms in actual clinical specimens will be protected by the PAC system.

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