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Efficiency of a Transport Medium for the Recovery of Aerobic and Anaerobic Bacteria from Applicator Swabs

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The survival of four aerobic and four anaerobic pathogens was evaluated quantitatively on cotton swabs and calcium alginate swabs stored in dry tubes as compared with swabs stored in Amies Transport Medium without charcoal. Survival of the pathogens was markedly improved when stored in Amies Transport Medium, although there was considerable loss of viability after a few hours of storage.

Many clinical specimens are conveniently collected on applicator swabs (2) and then transported to the laboratory, often with a delay of several hours. Unfortunately, certain bacterial pathogens are notoriously incapable of surviving prolonged exposure on swabs. Since cotton itself may contain toxic materials, less toxic synthetic fibers such as calcium alginate may be used (2). Desiccation is another important factor leading to the death of bacteria on applicator swabs.

A number of transport media have been devised to improve the survival of pathogens on swabs and to prevent overgrowth of commensals which may be present in the specimens. Amies modification of Stuart's medium (1) represents one such medium; it is non-nutritive, it is buffered with phosphates, and it provides a reduced environment because of the presence of sodium thioglycolate and a small amount of agar. The present study was undertaken to measure quantitatively the efficiency of this medium for recovering common aerobic or anaerobic bacterial pathogens.

MATERIALS AND METHODS

The quantitative technique described by Bartlett and Hughes (3) was adapted for this study. Briefly, colonies from an actively growing agar plate culture were suspended in a small volume of 0.9% NaCl buffered to *p*H 7.2 with NaH_2PO_4 and Na_2PO_4. Tenfold dilutions were prepared in the buffered saline, and for each dilution a row of 0.050-ml drops was laid out on the inside of a sterile plastic petri plate lid. As quickly as possible, a cotton or calcium alginate swab was used to take up one of the 0.050-ml drops. The charged swabs were then stored either in dry sterile tubes or in freshly prepared Amies Transport Medium without charcoal (Difco). The transport medium was prepared in screw-cap tubes (13 by 100 mm), each filled with approximately 8.0 ml, and was held at room temperature for no longer than 24 hr before use. After 0, 2, 4, and 24 hr in the light at room temperature, a set of swabs was cultured by streaking the entire surface of either blood-agar or chocolate-agar, and the plates were incubated in candle jars or anaerobic jars (Gas-Pak, Bio-Quest). The highest dilution which gave a countable number of colonies was multiplied by the mean number of colonies observed on triplicate plates, thus providing an estimate of the approximate number of viable cells recoverable from each type of swab.

The kinds of bacteria that were examined included recent clinical isolates of *Streptococcus pyogenes*, *S. pneumoniae*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Bacteroides fragilis*, *B. melaninogenicus*, a *Peptostreptococcus* sp., and vegetative cells of *Clostridium sordellii*.

RESULTS

Initial tests with *Escherichia coli* indicated that about 60% of the bacteria picked up by a swab could be recovered by streaking the entire surface of an agar plate immediately after the drop was picked up; about 40% of the bacteria remained on or in the fibers of the moist swab. Calcium alginate was much less absorbent, and more material remained on the outer portion of the swab where it was somewhat more readily recovered by streaking the sur-

face of an agar plate; however, at the same time, the bacteria were exposed to the deleterious effects of desiccation which occurred when the swabs were allowed to stand. Use of a transport medium results in mechanical removal of approximately 4% of the bacteria from cotton swabs and as many as 44% from the less absorbent calcium alginate swabs. The care with which swabs are inserted and removed from the medium is a determinant of such losses.

The results of quantitative studies with eight fastidious or anaerobic bacterial pathogens are summarized in Table 1. These common pathogens survived for a limited time on dry cotton or calcium alginate swabs, but storage in the transport medium markedly improved the recovery of all of the strains that were tested. After a few hours in the transport medium, there was a definite decrease in the number of recoverable bacteria. Those swabs which were charged with a very small number of bacteria failed to yield any growth if held in the transport medium; however, after a few hours in dry tubes, even more swabs were negative. No attempt was made to determine whether moistening the dry swabs before streaking the agar medium would improve the recovery.

DISCUSSION

Several factors contribute to the death of bacteria on applicator swabs. Toxic materials in the cotton fibers could play a role, but our data and those of Ellner and Ellner (4) demonstrate that the use of fibers other than cotton does not greatly enhance the survival of many bacterial pathogens. The amount of moisture retained by different types of fibers seems to be a more critical variable which influences the ability of a bacterium to survive on swabs held

TABLE 1. *Recovery of viable bacteria from cotton and calcium alginate swabs held at room temperature in Amies Transport Medium without charcoal and in dry tubes*

Organism	Time held (hr)	Approx no. of colony-forming units recovered from stored swabs			
		Cotton swab		Calcium alginate swab	
		Dry tube	Amies medium	Dry tube	Amies medium
Streptococcus pyogenes	0	3×10^6	—	3×10^6	—
	2	1×10^4	1×10^6	5×10^4	2×10^5
	4	1×10^3	9×10^5	2×10^4	2×10^5
	24	0	1×10^6	0	7×10^4
S. pneumoniae	0	1×10^5	—	4×10^5	—
	2	0	2×10^4	5×10^2	3×10^4
	4	0	2×10^4	0	6×10^3
	24	0	9×10^3	0	4×10^2
Haemophilus influenzae	0	3×10^6	—	4×10^6	—
	2	0	5×10^5	2×10^3	2×10^5
	4	0	3×10^5	0	2×10^4
	24	0	2×10^5	0	2×10^3
Neisseria gonorrhoeae	0	7×10^5	—	1×10^6	—
	2	2×10^2	1×10^4	0	5×10^3
	4	0	3×10^3	0	2×10^3
	24	0	10	0	0
Clostridium sordellii	0	2×10^7	—	2×10^7	—
	2	9×10^5	1×10^6	8×10^4	3×10^6
	4	0	1×10^5	0	3×10^6
	24	0	5×10^4	0	2×10^6
Bacteroides fragilis	0	9×10^6	—	7×10^6	—
	2	1×10^6	9×10^6	7×10^6	8×10^6
	4	7×10^4	3×10^6	5×10^6	6×10^6
	24	3×10^3	1×10^5	0	2×10^2
B. melaninogenicus	0	5×10^4	—	4×10^4	—
	2	1×10^3	2×10^4	3×10^2	2×10^3
	4	8×10^2	5×10^2	50	3×10^2
	24	0	4×10^2	0	20
Peptostreptococcus sp.	0	5×10^4	—	4×10^4	—
	2	0	7×10^3	0	13
	4	0	4×10^3	0	0
	24	0	2×10^2	0	0

in dry tubes. Desiccation is one of the most important factors leading to the death of the bacteria, and this occurs rather rapidly on swabs prepared with materials such as calcium alginate.

Charcoal should be added to Amies Transport Medium to help neutralize materials which are toxic to pathogens such as *N. gonorrhoeae* (5, 6). To prepare such a medium, the tubes must be turned several times while the agar is solidifying to maintain a uniform distribution of charcoal particles. Omission of this component greatly simplifies the preparation of the medium without completely eliminating its effectiveness for the recovery of many bacterial pathogens.

Brief exposure to atmospheric oxygen can be lethal to some anaerobes but not to others. For examples the *Peptostreptococcus* sp. was very sensitive to brief exposures to air, whereas *B. fragilis* was extremely resistant in this respect. In general, the anaerobes tended to survive better in the fibers of cotton swabs than on the surface of calcium alginate swabs. The anaerobes survived much better when placed in the reduced environment of the freshly prepared transport medium, but even in such a medium the more sensitive anaerobes survived for only a short period, if at all.

It must be recognized that the viable-cell counts recorded in this report are only approximations. However, these estimates permit assessment of the relative efficiency of the holding medium as compared with dry swabs. The swabs were charged with a suspension of bacteria in a buffered saline, but in clinical specimens the bacteria may be protected by mucus, pus, blood, or other body fluids which, at the same time, may also exert inhibitory activity. Consequently, bacteria may survive in some clinical specimens better than in others, and their survival may differ from that observed under artificial, controlled experimental conditions. However, experience in our clinical laboratory supports the conclusion that all specimens which must be collected on applicator swabs should be carried to the laboratory in a transport medium if they cannot be inoculated directly onto the appropriate nutrient media. Even in a transport medium, swabs cannot be held for excessive periods, but must be processed as soon as possible. The only major disadvantage to the use of a semisolid transport medium is the unsuitability of the specimen for the preparation of stained smears. A second swab transported in a dry tube can be used for this purpose, providing excessive drying does not occur. Optimally, clinical specimens should be processed immediately, but, when a delay is unavoidable, a transport medium provides the best compromise. Although this approach gives suboptimal results, it is clearly better than using no transport medium at all.

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