Survival of Bacteria in Difco CultureSwab and Marion Culturette II Transport Systems

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The effects of the CultureSwab (Difco Laboratories, Detroit, Mich.) and Culturette II (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) transport systems on the viability of 95 clinically significant bacteria were studied. Organisms included staphylococci (8 isolates), streptococci (22 isolates), Haemophilus spp. (16 isolates), members of the family Neisseriaceae (14 isolates), Bordetella spp. (5 isolates), members of the family Enterobacteriaceae (16 isolates) and pseudomonads (14 isolates). Viability counts with both methods usually dropped by ≥90% after incubation at room temperature for periods ranging from 4 to 48 h, and statistically significant differences between the two methods were not observed. However, counts were generally higher with the Difco method, and this difference may be clinically important.

With ideal conditions, specimens from clinical material should be stained and cultured immediately after they are obtained. However, in many cases, especially in smaller laboratories without adequate night and weekend services, delay is unavoidable and transport methods are necessary for a variety of specimen types, notably throat swabs and wound and body fluid specimens (8). If a transport system is used, it should maintain viability without allowing bacterial multiplication. Several types of nonproprietary media may be used for this purpose, including Stuart, Amies with or without charcoal, and Cary-Blair media (1–6, 8–10). Specimens to be cultured for anaerobic bacteria should be transported differently from specimens to be cultured for aerobic bacteria (8). Cultures may be transported directly in media or on swabs which are then placed in media (8).

The purpose of the current study was to challenge two commercially available methods, CultureSwab (Difco Laboratories, Detroit, Mich.) and Culturette II (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.), with known concentrations of aerobic bacteria and to assess their survival after storage at room temperature for various lengths of time in an attempt to simulate clinical conditions. Culturette II was chosen over the single Marion swab because of greater use of the two-swab method at our institutions.

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Organisms tested included 22 isolates of streptococci (4 Streptococcus pyogenes, 4 S. agalactiae, 3 S. faecalis, 6 S. pneumoniae, 1 S. mitis, 1 S. salivarius, 1 S. sanguis I, 1 S. sanguis II, and 1 S. mutans), 8 isolates of staphylococci (7 Staphylococcus aureus and 1 Staphylococcus saprophyticus), 14 members of the family Neisseriaceae (5 Neisseria gonorrhoeae, 3 N. meningitidis, 1 N. lactamica, 1 N. sicca, 1 N. mucosa, and 3 Branhamella catarrhalis), 16 Haemophilus isolates (9 Haemophilus influenzae, 4 H. parainfluenzae, 2 H. parahaemolyticus, and 1 H. haemoglobiniphilus, 5 Bordetella isolates (4 Bordetella pertussis and 1 B. parapertussis), 16 members of the family Enterobacteriaceae (6 Escherichia coli, 5 Enterobacter aerogenes, 5 Klebsiella pneumoniae), and 14 Pseudomonas species (8 Pseudomonas aeruginosa, 3 P. cepacia, and 3 P. maltophilia). Of the strains listed above, 81 were recent clinical isolates, 12 were from stock strains from the American Type Culture Collection (Rockville, Md.), and 2 were from stock strains from the Centers for Disease Control (Atlanta, Ga.).

Stock cultures were frozen at −70°C in thiglycolate-glycitol (85:15 [vol/vol]) and subcultured twice on solid media before testing.

Test organisms were cultured on chocolate agar (Haemophilus spp. and members of the Neisseriaceae), charcoal agar with 10% defibrinated sheep blood (Bordetella spp.), 5% sheep blood agar (streptococci and staphylococci), or MacConkey agar plates (pseudomonads and members of the Enterobacteriaceae). All plates were obtained from BBL Microbiology Systems, Cockeysville, Md., except charcoal agar (Difco) plates which were prepared in-house according to the instructions of the manufacturer and supplemented with 10% defibrinated sheep blood (BBL). Inocula were prepared by suspending growth from plates in 1 ml of sterile saline and then diluted in saline to yield a final inoculum approximately equivalent to a 0.5 McFarland standard. Swabs were then dipped in each suspension, allowing the suspension to be absorbed completely on each swab. Volumes of approximately 0.2 and 0.3 ml were absorbed into the Difco and Marion systems, respectively. Bacterial counts at 0 h were done by plating dilutions on solid media after vortexing the swabs in 1 ml of sterile saline. Swabs were left at room temperature, and viability counts were repeated after 4, 24, and 48 h by vortexing swabs in 1 ml of sterile saline and plating dilutions of resultant suspensions. Plates were incubated for 16 to 18 h at 35°C except for Bordetella isolates, which were incubated for 5 to 7 days. Aerobic incubation was used for all organism groups except Haemophilus spp. and members of the Neisseriaceae, which were incubated in 5 to 10% CO₂.

Data were analyzed by comparing differences in colony counts between the two systems at 4, 24, and 48 h and time limits.
0. Statistical comparisons of colony counts were performed with Student's t test for paired comparisons. Testing was two tailed at the level of significance of $P = 0.05$.

Colony counts for all organism groups with the two systems are presented in Table 1. Although the Difco method generally appeared to yield higher colony counts compared with the Marion method, viability counts with both methods dropped by $\geq 90\%$ in most cases, especially after $\geq 24$ h, and differences did not attain statistical significance.

Lack of statistically significant differences between viability counts with the two methods may reflect high levels of loss of viability over the time period studied with both systems. In most cases, colony counts dropped one or more logs after $\geq 4$ h ($\geq 90\%$ inhibition), with an even greater drop at subsequent time intervals. With such conditions, statistically significant differences between the two methods were not found.

The reasons for the generally higher viability counts with the Difco versus the Marion transport methods which were used in the current study are not clear. The Difco CultureSwab consists of a rayon swab in semisolid Amies transport medium, whereas the Marion Culturette II consists of a similar swab containing the same type, but approximately double the amount, of rayon per swab (Difco swab, $0.034 \pm 0.001$ g of rayon; Marion swab, $0.059 \pm 0.001$ g of rayon [arithmetic means of 10 measurements each]), moistened by (but not immersed in) liquid Stuart medium. Reports on the comparative efficacy of various transport media differ (1–6, 8–10; F. B. Engley, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C177, p. 339). In addition, variations between products of certain manufacturers in ability to support bacterial growth have also been found (6) which complicate comparisons between published reports. Possible reasons for the improved viability with Amies versus Stuart transport media suggested in the current study may include one or more of the following factors: a reduced environment; better thermal protection; lower O$_2$ permeability with the semisolid medium; or ease of use, related to no activation step (ampoule breakage) with the Amies method. Although neither Stuart nor Amies medium is thought to be proliferative, the possibility that nutrients were present in the specimens which permitted growth and distorted proportions of organisms (7) cannot be excluded. We have no data to confirm or deny this hypothesis. However, if such growth had occurred, it would presumably have taken place with both methods with no resultant effect on data analysis.

Even though one-swab and two-swab techniques were compared with one another, we believe that the results are still valid; if anything, the Difco method should have given less favorable results compared with the Marion system because the two-swab Marion system picked up more inoculum than did the one-swab Difco method; this finding was not the case for any organism group tested. Comparisons between one-swab Difco and one-swab Marion methods should confirm the higher viability counts with the CultureSwab compared with the Culturette II methods suggested in our study.

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**LITERATURE CITED**

