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Comparison of Easy-Flow Copan Liquid Stuart's and Starplex Swab Transport Systems for Recovery of Fastidious Aerobic Bacteria

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Because samples are frequently submitted on swabs from distant sites, viability of the organism must be maintained. We compared two transport systems, a new Copan Liquid Stuart's swab with an Easy-Flow swab applicator and the Starplex Liquid Stuart's swab. The purpose of the study was to assess the release and/or recovery of organisms from the Copan system compared to that from Starplex. Triplicate swabs were seeded with 3 dilutions of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. Although the amount of the initial inoculum was the same for both transport systems, recovery by the roll-plate method at time zero was consistently increased with the Copan system (31 to 87% higher). This is the most important finding in this study. With *N. gonorrhoeae*, subsequent recoveries were similar for Copan and Starplex but poor for both systems. With *N. meningitidis* and *Haemophilus*, higher levels of recovery were clearly obtained with Copan ($P < 0.05$ to $P < 0.001$). With *Streptococcus*, subsequent recoveries for Copan and Starplex were mixed. In conclusion, Copan generally demonstrated better recovery of organisms compared to Starplex even (and especially) at time zero.

Proper transport of clinical specimens for culturing infectious agents may be the most important factor affecting the successful evaluation of these specimens. Because many samples on swabs are submitted from sites distant from clinical microbiology laboratories, it is essential that viability of the organism be maintained. While tissues and aspirates remain the specimens of choice, swabs are still commonly submitted to clinical microbiology laboratories for culture. A transport system that will maintain viability of the organism for 24 to 48 h becomes a necessity, as the need to transport these specimens a greater distance becomes a reality. Release of the bacteria from the swab also becomes an important factor. In this study, we compared two transport systems (the Copan Liquid Stuart's swab with an Easy-Flow swab applicator and the Starplex Liquid Stuart's swab) for recovery of fastidious aerobic bacteria. Although both swabs tested use liquid Stuart's as the transport medium, the Copan swab system incorporates a newly designed swab applicator that is said to improve the release of bacteria onto culture plates (5).

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MATERIALS AND METHODS

The survival of the following four fastidious aerobic bacteria in the two transport systems at two temperatures was evaluated: *Neisseria gonorrhoeae* ATCC 43069, *Neisseria meningitidis* ATCC 13090, *Haemophilus influenzae* ATCC 10211, and *Streptococcus pneumoniae* ATCC 6305.

The bacterial strains were reconstituted and grown on chocolate agar for 18 h at 37 C° to prepare them for testing. Inocula of the isolates were prepared in 0.85% physiological saline (pH 6.8 to 7.2) to a concentration of 1.5×10^8 CFU per ml, which is equivalent to a McFarland standard of 0.5. Turbidities were checked with a nephelometer. Four 1:10 dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) of the bacterial suspensions were made (2.5 ml in 25 ml of saline solution) to provide approximate final working concentrations ranging from 1.5×10^4 CFU per ml to 1.5×10^7 CFU per ml. The 10^{-2}, 10^{-3}, and 10^{-4} dilutions (1/100, 1/1,000, and 1/10,000) were used to inoculate the swabs. All swabs to be tested at the separate time intervals were inoculated with 100 μl of each organism dilution. This was done by pipetting 100-μl aliquots of the suspension into wells of a microtiter plate with a volumetric pipettor and then allowing the swab to completely absorb all the liquid from the well. Then the swabs were inserted into their respective transport devices. One set of swabs from each manufacturer was immediately planted onto chocolate agar by a standardized roll swab technique as described by NCCLS (3). Three swabbing actions were used to ensure consistent and reproducible transfer of the entire sample to the plate. These immediately inoculated plates were used to establish a time zero baseline count for each transport system. The remainder of the swabs were held for the following specified times and at specified temperatures: 6, 24, and 48 h; and controlled room temperature (RT) at 23°C and in a refrigerator at 4°C. In all, a total of six swabs for each original inoculum were prepared for each of the three time periods beyond baseline.

Each organism-dilution and time point experiment was performed in triplicate to minimize errors. The plates were incubated in 5% CO_2 for a minimum of 24 h. An average of the number of organisms recovered was expressed as a percentage of the baseline counts (counts at time zero). Plates at time zero with counts between ~100 to 350 CFU were used in the evaluation presented here. Counts of CFU were approximated and averaged for each of the three swabs for each time point and dilution.

Statistical analysis. Analysis was done by a Ph.D.-degree-level biostatician (S.V.). Fisher's exact test was used. Statistical analysis was not done at time zero to compare Starplex and Copan recoveries because no valid denominator was available for comparison.

RESULTS

Survival of *N. gonorrhoeae*, *N. meningitidis*, *H. influenzae*, and *S. pneumoniae* is shown in Tables 1 to 4, respectively. Although the initial inoculum for each strain was the same for both transport systems, recovery at time zero was consistently higher with Copan. For example, with *N. gonorrhoeae*, despite identical initial inocula from the same tube, 267 CFU were

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TABLE 1. Recovery of *N. gonorrhoeae*

Transport system (temp)	Dilution	No. (%) of colonies at h[a]:			
		0	6	24	48
Copan (RT)	1/1,000	267 (100.0)	38 (14.2)	1	0
Starplex(RT)	1/1,000	91 (100.0)	19 (20.9[b])	1	0
Copan (4°C)	1/1,000	267 (100.0)	35 (13.1)	0	0
Starplex(4°C)	1/1,000	91 (100.0)	21 (23.1[c])	1	0

[a] For values of <5 CFU, the percentage was not calculated.
[b] $P < 0.05$, comparing Copan to Starplex.
[c] $P > 0.1$, comparing Copan to Starplex (not statistically significant).

recovered at time zero with Copan compared to 91 with Starplex (Table 1). Clearly, greater recovery of both *N. meningitidis* and *H. influenzae* was obtained with Copan (Tables 2 and 3). For *S. pneumoniae*, recovery for the two systems was mixed (Table 4).

***N. gonorrhoeae*.** At time zero, recovery of *N. gonorrhoeae* was higher with Copan than with Starplex (Table 1). Survival of *N. gonorrhoeae* was roughly comparable but decreased in both systems at all times and both temperatures. When percentages of initial inoculum were considered, recovery after 6 h was slightly higher with Starplex than with Copan (23.1 versus 13.1%; $P < 0.05$).

***N. meningitidis*.** At time zero, recovery of *N. meningitidis* was higher with Copan than with Starplex (340 versus 108 CFU) (Table 2). Recovery of *N. meningitidis* was higher with Copan; at 6 h, the Copan swab maintained ~39% recovery, the Starplex maintained 12 to 13% recovery ($P < 0.0001$). At other times, the differences were also significant.

***H. influenzae*.** At time zero, recovery of *H. influenzae* was higher with Copan than with Starplex (107 versus 93 CFU) (Table 3). Recovery of *H. influenzae* was negligible with the Starplex swab after 24 and 48 h at both temperatures. After 6, 24, and 48 h at both temperatures, the recovery of *H. influenzae* was higher with Copan than with Starplex (the range of *P* values was <0.01 to <0.0001).

***S. pneumoniae*.** At time zero, recovery of *S. pneumoniae* was higher with Copan than with Starplex (192 versus 145 CFU) (Table 4). Although the two systems performed similarly, Starplex recovered a slightly higher percentage of initial inoculum of *S. pneumoniae* than Copan after 6 h at RT and at 4°C after 24 h. Recovery of *S. pneumoniae* was similar at 48 h at 4°C for both systems, with Copan recovering 37% and Starplex recovering 31%. These differences were not statistically significant.

TABLE 2. Recovery of *N. meningitides*

Transport system (temp)	Dilution	No. (%) of colonies at h[a]:			
		0	6	24	48
Copan (RT)	1/1,000	340 (100.0)	133 (39.1[b])	35 (10.3[b])	43 (12.6[b])
Starplex(RT)	1/1,000	108 (100.0)	14 (13.0)	1	0
Copan (4°C)	1/1,000	340 (100.0)	133 (39.1[b])	38 (11.2[b])	6 (1.8)
Starplex(4°C)	1/1,000	108 (100.0)	13 (12.0)	4	0

[a] For <5 CFU, the percentage was not calculated.
[b] $P < 0.0001$, comparing Copan to Starplex.

TABLE 3. Recovery of *H. influenzae*

Transport system (temp)	Dilution	No. (%) of colonies at h[a]:			
		0	6	24	48
Copan (RT)	1/10,000	107 (100.0)	78 (72.9[b])	24 (22.4[b])	8 (7.5[c])
Starplex(RT)	1/10,000	93 (100.0)	30 (32.3)	1	0
Copan (4°C)	1/10,000	107 (100.0)	103 (96.3[b])	22 (20.6[c])	25 (23.4[c])
Starplex(4°C)	1/10,000	93 (100.0)	37 (39.8)	6 (6.5)	6 (6.5)

[a] For <5 CFU, the percentage was not calculated.
[b] $P < 0.0001$, comparing Copan to Starplex.
[c] $P < 0.01$, comparing Copan to Starplex.

DISCUSSION

Although swabs may not offer the best collection method, there is often no alternative specimen available. The time period examined (6 to 48 h) encompasses the transport time for the vast majority of specimens. The Copan system had superior recovery of organisms at time zero, indicating that a release factor may have contributed to the increased levels of recovery for all the organisms, especially for *N. gonorrhoeae*, *N. meningitidis*, and *S. pneumoniae*. Although the amount of initial inoculum was the same for both transport systems, recovery at time zero was consistently superior with the Copan system (31 to 87% higher). This is the most important finding in this study; unfortunately, there is no valid way to statistically analyze the differences in recovery of organisms at time zero between Starplex and Copan. The number of CFU recovered at time zero was used as the denominator for calculating the percentage of subsequent recovery for each transport system. However, sole consideration of the percent recovery for each transport system is somewhat misleading. For instance (Table 1), 38 CFU of *N. gonorrhoeae* recovered in Copan at 6 h at RT is 14.2% of 267, the CFU count in Copan at time zero. If the Starplex recovery of 19 CFU was compared to the Copan recovery of 267 CFU at time zero, the recovery would have been 7.1, rather than 20.9% (19 of 91 CFU at time zero for Starplex), an ~14% apparent difference.

Using the roll-plate method and criteria at 24 h at both temperatures, all swabs failed for *N. gonorrhoeae*; at RT, Starplex failed at 24 h for *H. influenzae*, and Copan failed at 48 h for *S. pneumoniae*. All other swab-time-temperature combinations yielded acceptable viability numbers for the roll-plate method for the three organisms included in the M40-A standard (3). It should be noted that the direct swab–roll-plate method was used in this study, and the roll-plate method is more stringent than the swab elution method; a swab which

TABLE 4. Recovery of *S. pneumoniae*

Transport system (temp)	Dilution	No. (%) of colonies at h[a]:			
		0	6	24	48
Copan (RT)	1/100	192 (100.0)	100 (52.1)	22 (11.5[d])	1
Starplex(RT)	1/100	145 (100.0)	97 (66.9[b])	7 (4.8)	6 (4.1)
Copan (4°C)	1/100	192 (100.0)	145 (75.5[c])	67 (34.9)	71 (37.0[c])
Starplex(4°C)	1/100	145 (100.0)	107 (73.8)	81 (55.9[e])	45 (31.0)

[a] For <5 CFU, the percentage was not calculated.
[b] $P < 0.01$, comparing Copan to Starplex.
[c] $P > 0.1$, comparing Copan to Starplex (not statistically significant).
[d] $P < 0.05$, comparing Copan to Starplex.
[e] $P < 0.0001$, comparing Copan to Starplex.

fails when tested by the roll-plate method may not necessarily fail by the swab elution method.

In this study, the Copan Liquid Stuart's Transport swab and the Starplex Liquid Stuart's swab were compared for maintaining the recovery of four fastidious aerobic bacteria. In general, recovery at subsequent times was superior with the Copan system. The survival of *H. influenzae* (73% with the Copan system and 32% with the Starplex system at 6 h at RT) was clearly increased. Survival of *N. meningitidis* was also clearly increased with recovery with Copan at 39% and recovery with Starplex at 12 to 13% after 6 h ($P < 0.001$). For *N. gonorrhoeae* and *S. pneumoniae*, survival between the two systems demonstrated similar survival rates. Our finding that recovery of *S. pneumoniae* was superior at 4°C when compared to survival at higher temperatures is consistent with a previous report (1). It should be noted that the holding time recommended in the M40-A NCCLS standard is 24 h for *N. gonorrhoeae* and 48 h for *S. pneumoniae* and *H. influenzae* (3).

Our study is similar in design to one which also examined the Easy-Flow Copan swab and the Starplex swab (5). In the referenced study, fastidious anaerobes, as well as fastidious aerobes, were studied. For the aerobic organisms, our present findings were consistent with those of the previous study (5). The Copan swab demonstrated superior performance with all organisms tested, with the exception of *S. pneumoniae*, which gave similar recovery rates with both systems (5). In addition, other studies have also demonstrated improved recovery with the newly designed Copan swab (2, 4, 6).

In conclusion, the Copan swab offers a reliable collection system that can demonstrate better recovery of organisms of some of the more fastidious organisms than the Starplex system can. Copan had superior recovery of each organism at time zero. Recovery of *H. influenzae* and *N. meningitidis* was clearly increased with the Easy-Flow Copan swab; for *N. gonorrhoeae* and *S. pneumoniae*, recovery between the two systems was similar over time or mixed.

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