

Comparison of Bacterial Survival in Two Transport Systems Stored at Room and Refrigerator Temperatures

A. Robinson, M. L. Gruver; Sacred Heart Medical Center, Spokane, WA.

ABSTRACT

Optimization of specimen integrity and organism preservation during transport is essential for the production of clinically relevant results. The advent of centralized labs has heightened this concern. The following select aerobic and anaerobic isolates were evaluated for survival after incubation at room (RT) and refrigerator (4°) temperatures in the BBL CultureSwab Plus* (CS) and Starplex Starswab II (SS): *Neisseria gonorrhoeae* (GC) ATCC 43069 alone and combined with *Pseudomonas aeruginosa* (PSA) ATCC 27053, *Hemophilus influenzae* (Hflu) ATCC 10211, *Streptococcus pneumoniae* (Pneumo) ATCC 6305 alone and combined with *Escherichia coli* (E.coli) ATCC 25922, *Fusobacterium nucleatum* (Fuso) ATCC 25586, and *Peptostreptococcus anaerobius* (Pepto) ATCC 27337. Multiple swabs of each transport system were inoculated with 0.1 mL of 10⁷ CFU/mL of each bacterium and held at RT and 4°C. Serial dilutions and colony counts, in duplicate, were performed at 0, 6, 24, and 48 h. The percent recovery, as compared to 0 h, was determined for each time interval. For GC alone, CS was superior to SS at RT but equivalent at 4°. For GC mixed with PSA, more GC were recovered from CS than SS at both RT and 4°, with fewer GC isolated from the mixture with SS compared to GC alone. For Hflu, CS was superior to SS at both temperatures, with greater recovery from SS at 4°. Pneumo alone survived better at 4° from both swabs, with greater survival at 24 and 48 h in SS. For Pneumo mixed with Ecoli, more Pneumo were recovered from SS at 6 and 24 h, with both swabs showing decreased survival of Pneumo from the mixture at 48 h compared with the pure Pneumo. More Fuso were recovered at RT for both swabs, with greater survival in CS at 6 h. Survival of Pepto was markedly enhanced at 4° from both systems, with greater recovery from CS. PSA and Ecoli showed rapid growth at RT to >300% of the original inoculum in CS and SS; overgrowth was controlled at 4°. In summary, optimal recovery of organisms was at 4°, with the exception of Fuso. Except for Pneumo, the bacteria tested showed greater survival in the BBL CultureSwab Plus* compared to Starplex Starswab II, regardless of temperature.

INTRODUCTION

The optimization of specimen integrity and organism preservation during transport is essential for the production of clinically relevant information. The development of centralized labs in many communities and the acquisition of local labs by regional or national private labs have contributed to lengthy specimen transport delays that compromise the integrity of specimens submitted for bacterial culture. Recently, the long held dogma that specimens for bacterial culture should be held at room rather than refrigerator temperature has been challenged. During specimen transport at elevated temperatures, a particular concern is the overgrowth of fastidious organisms by more robust bacteria, such as *Escherichia coli* or *Pseudomonas aeruginosa*.

The purpose of this study was to evaluate select fastidious aerobic and anaerobic isolates, both alone and in combination with non-fastidious bacteria, for survival after incubation at room and refrigerator temperatures in the BBL CultureSwab Plus* and Starplex Starswab II. The organisms included in the comparison were *Fusobacterium nucleatum*, *Hemophilus influenzae*, *Peptostreptococcus anaerobius*, *Neisseria gonorrhoeae* alone and combined with *P. aeruginosa*, and *Streptococcus pneumoniae* alone and combined with *E. coli*.

MATERIALS AND METHODS

ORGANISMS. The following ATCC strains were included in the evaluation: *Fusobacterium nucleatum* ATCC 25586, *Hemophilus influenzae* ATCC 10211, *Peptostreptococcus anaerobius* ATCC 27337, *Neisseria gonorrhoeae* ATCC 43069 alone and combined with *Pseudomonas aeruginosa* ATCC 27053, and *Streptococcus pneumoniae* ATCC 6305 alone and combined with *Escherichia coli* ATCC 25922.

TRANSPORT SYSTEMS. The BBL CultureSwab Plus (CS) (BD Diagnostics Systems, Sparks, MD)* and the Starplex Starswab II (SS) (Starplex Scientific Inc, Ontario, Canada) were included in the evaluation. Both transport systems consist of a sterile peel pouch containing a rayon tip swab and Amies agar gel medium without charcoal.

INOCULUM. Fresh 18 to 24 h growth of aerobic and anaerobic organisms was used to prepare test organism suspensions in saline equivalent to a 0.5 McFarland standard (~1.5 X 10⁸ CFU/mL). The 0.5 McFarland suspension was diluted 1:10 in saline, yielding ~10⁷ CFU/mL.

STUDY PROTOCOL. Multiple swabs of each transport system were inoculated with 0.1 mL of 10⁷ CFU/mL of each bacterial suspension and held at room (20-22C) or refrigerator (4°C) temperature. Serial dilutions and colony counts, in duplicate, were performed at 0, 6, 24, and 48 h. The percent recovery, as compared to 0 h, was determined for each time interval.

RESULTS

- Greater numbers of *Fusobacterium* spp. were recovered at room temperature (RT) than 4°C with both swab systems, with greater survival (78% versus 50%) in CS compared with SS at 6 h (Figures 1 and 2).
- For *Hemophilus influenzae*, CS provided superior organism recovery compared to SS at both temperatures. SS preserved substantially more of the initial inoculum at 4°C than at RT, especially at 24 and 48 h (Figures 3 and 4).
- For *Neisseria gonorrhoeae* alone, CS was superior to SS at RT, 59% versus 4% recovery at 6 h, but equivalent at 4°C (Figures 5 and 6). At RT, SS recovered 0% of the initial inoculum at 24 and 48 h and only 4% at 6 h.
- Survival of *Peptostreptococcus* spp. was markedly enhanced at 4°C for both systems, with greater recovery from CS (Figures 7 and 8). However, regardless of the transport temperature, SS failed to recover any organisms at 24 and 48 h.
- Streptococcus pneumoniae* alone survived better at 4°C from both swabs, with greater survival at 24 and 48 h in SS (Figures 9 and 10).
- For *N. gonorrhoeae* mixed with *Pseudomonas aeruginosa*, more *N. gonorrhoeae* were recovered from CS than SS at both RT and 4°C, with fewer *N. gonorrhoeae* (23%) isolated from the mixture with SS compared to *N. gonorrhoeae* alone (54%) (Figures 11 and 12). The overgrowth of *P. aeruginosa* was dramatically controlled in both transport systems at 4°C compared with RT, showing an organism recovery of ~ 40% to 90% versus ~ 90% to > 7000%, respectively.
- For *Streptococcus pneumoniae* mixed with *E. coli*, more *S. pneumoniae* were recovered from SS at 6 and 24 h, with both swab systems showing decreased survival of *S. pneumoniae* from the mixture at 48 h compared with the pure *S. pneumoniae* (Figures 13 and 14). The overgrowth of *E. coli* was dramatically controlled in both transport systems at 4°C compared with RT, showing an organism recovery of ~ 20% to 130% versus ~ 120% to > 9000%, respectively.

Figure 1. *Fusobacterium nucleatum* at 4°C

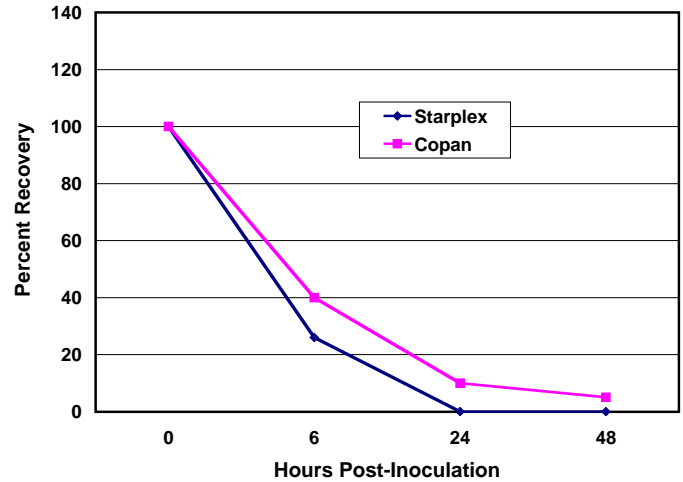


Figure 2. *Fusobacterium nucleatum* at 22°C

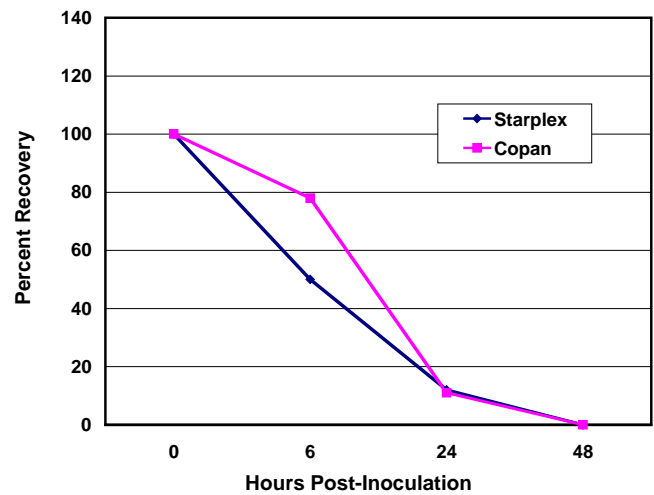


Figure 3. *Hemophilus influenzae* at 4°C

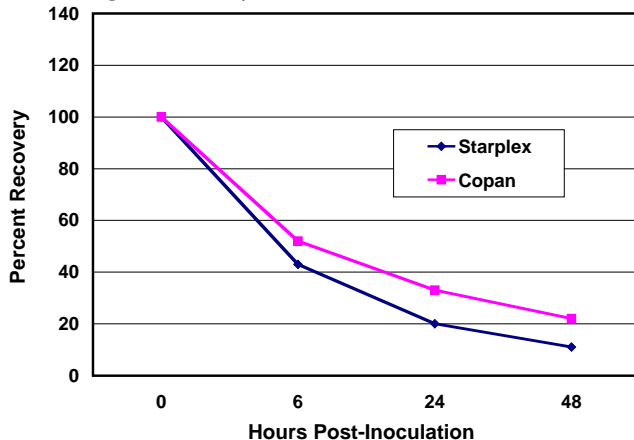


Figure 4. *Hemophilus influenzae* at 22°C

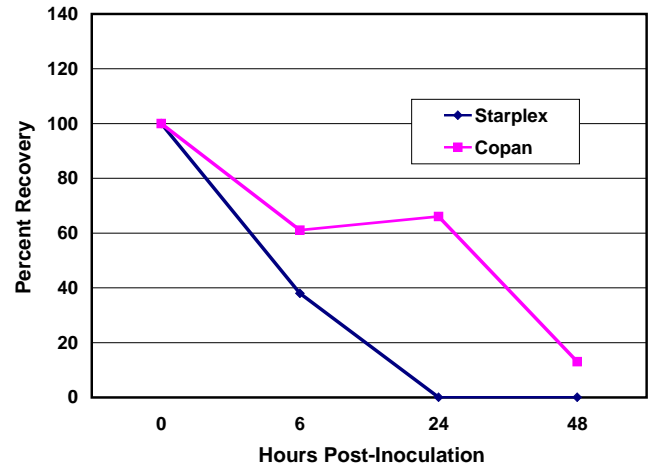


Figure 5. *Neisseria gonorrhoeae* at 4°C

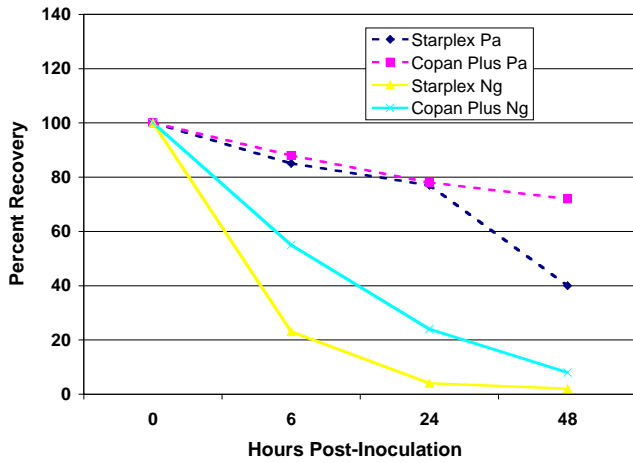


Figure 6. *Neisseria gonorrhoeae* at 22°C

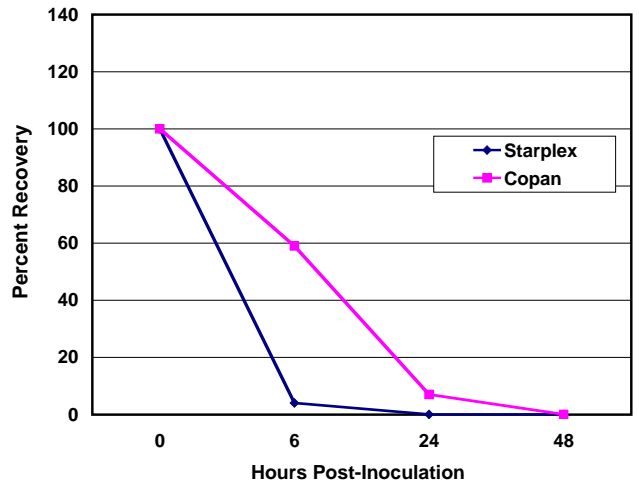


Figure 7. *Peptostreptococcus anaerobius* at 4°C

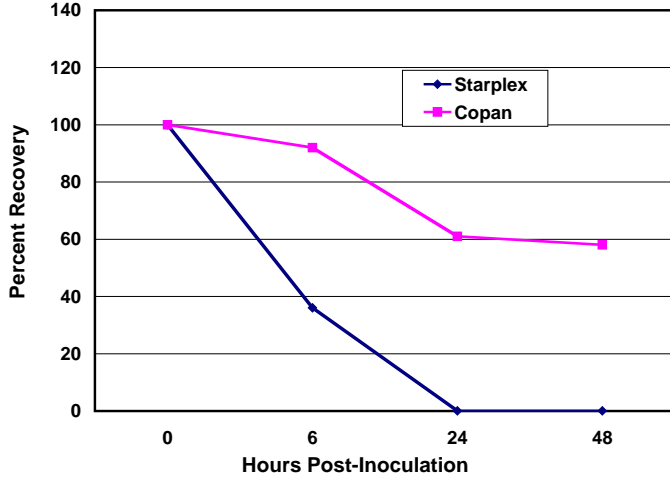


Figure 8. *Peptostreptococcus anaerobius* at 22°C

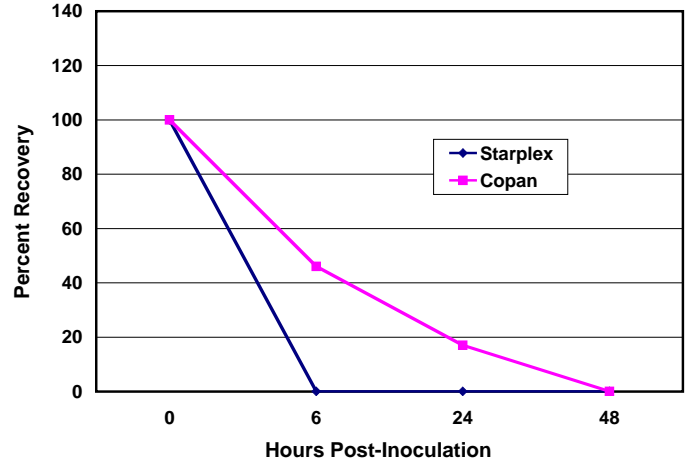


Figure 9. *Streptococcus pneumoniae* at 4°C

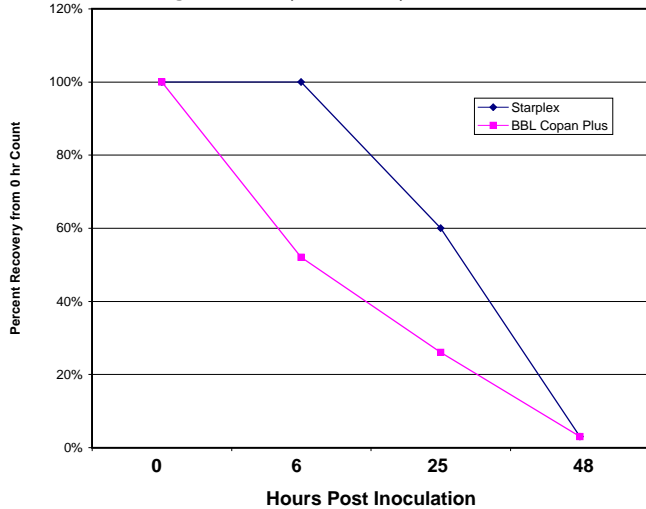


Figure 10. *Streptococcus pneumoniae* at 22°C

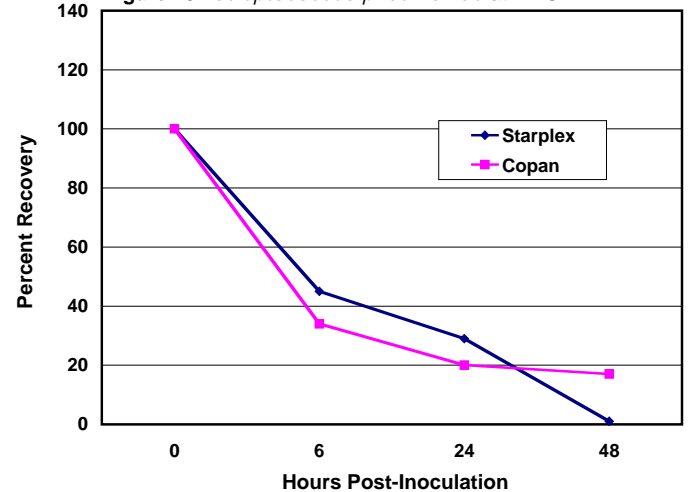


Fig. 11. *N.gonorrhoeae* combined with *P.aeruginosa* at 4°C

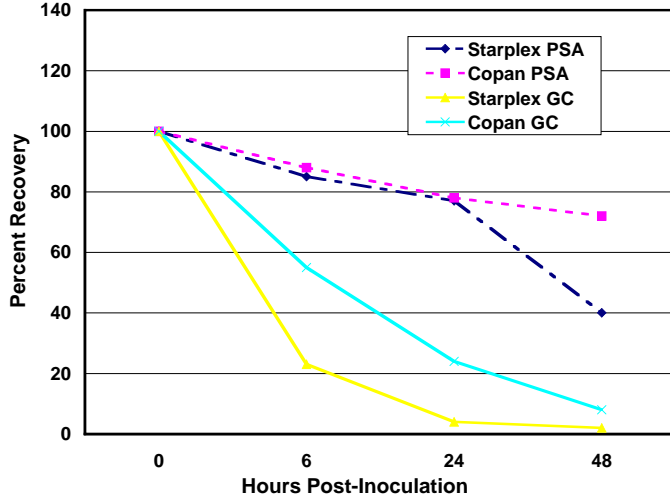


Fig. 12. *N.gonorrhoeae* combined with *P.aeruginosa* at 22°C

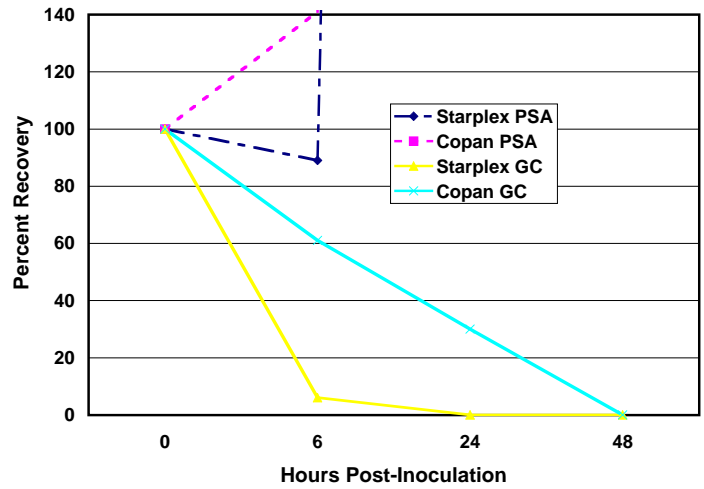


Figure 13. *S.pneumoniae* combined with *E.coli* at 4°C

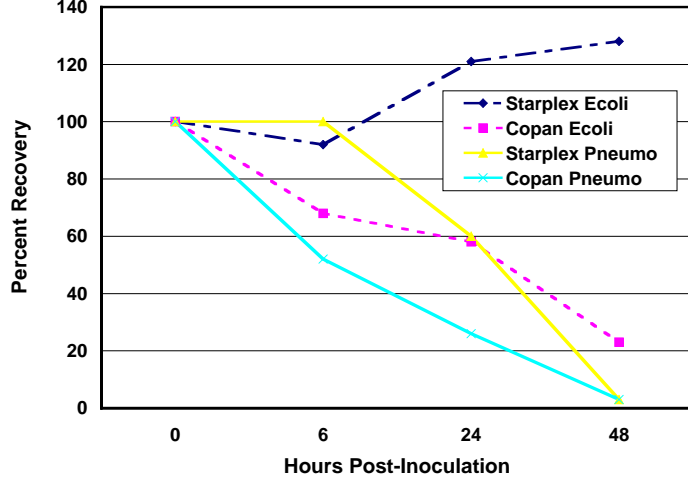
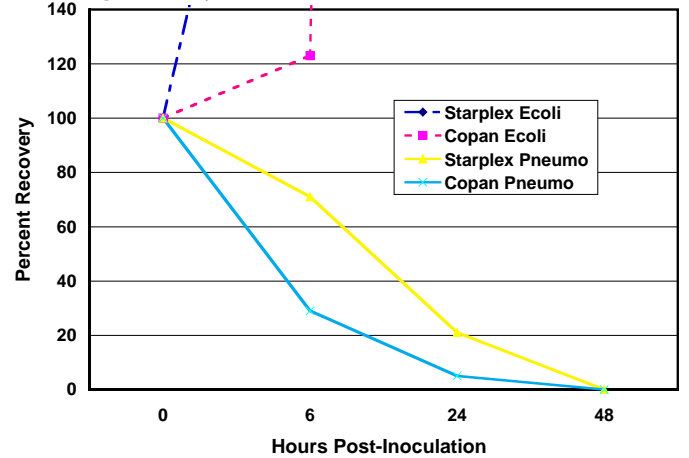


Figure 14. *S.pneumoniae* combined with *E.coli* at 22°C



SUMMARY AND CONCLUSIONS

Optimization of specimen integrity and organism preservation during transport is essential for the production of clinically relevant information. The purpose of the present study was to evaluate select fastidious aerobic and anaerobic isolates, both alone and in combination with non-fastidious bacteria, for survival after incubation at room and refrigerator temperatures in the BBL CultureSwab Plus* and the Starplex Starswab II. The observations included the following:

1. *P. aeruginosa* and *E. coli* showed rapid growth at RT to >300% of the original inoculum in CS and SS; overgrowth of these non-fastidious organisms was controlled at 4°C.
2. Recovery of the fastidious organisms was enhanced at 4°C, with the exception of *Fusobacterium* spp.
3. Except for *Streptococcus pneumoniae*, the fastidious bacteria tested showed greater survival in the BBL CultureSwab Plus* compared to the Starplex Starswab II, regardless of the transport temperature.
4. Serious consideration should be given to recommending refrigerator rather than room temperature transport of specimens for bacterial culture, including genital specimens submitted for *Neisseria gonorrhoeae* culture.

*BBL CultureSwab Plus is manufactured by Copan in Italy.