



COMPARISON OF TWO SWAB SYSTEMS USING AMIES TRANSPORT AT VARIOUS TEMPERATURES FOR THE RECOVERY OF AEROBIC AND ANAEROBIC BACTERIA

R. MOORE-NESS, R. SAUTTER; PINNACLEHEALTH LABORATORIES, HARRISBURG, PA.

ABSTRACT

We evaluated the survival of various aerobic and anaerobic bacteria using two Amies agar gel transport swabs. Copan Amies without charcoal (COP) and Starplex Starswab II (STAR) were evaluated at two storage temperatures. Organisms used to challenge the transport systems were obtained from the ATCC: *Fusobacterium nucleatum* ATCC 25586, *Haemophilus influenzae* ATCC 10211, *Neisseria gonorrhoeae* ATCC 43069, *Peptostreptococcus anaerobius* ATCC 27337, *Streptococcus pneumoniae* ATCC 6305, and *Streptococcus pyogenes* ATCC 19615. A working inoculum of 10⁷ cfu/ml was prepared for each organism tested. Standard suspensions of the organisms were inoculated into the transport systems by diluting the original suspension to 10⁻¹ and 10⁻² and placing 100µl of each dilution into sterile tubes and allowing the inoculum to completely absorb onto the swabs. Duplicate swabs of each type were inoculated with the organism suspensions to test swabs held at room temperature (20-25°C) and refrigerator temperature (~4°C) for 0, 6, 24, and 48hrs storage. Following storage at each temperature, the swabs were removed from the transport device and placed into 1 ml. of sterile saline. Each swab inside the sterile tube was vortexed to produce an eluent. Serial dilutions were performed to equal 10⁻¹ and 10⁻² of the original elutions. 100µl of the undiluted, 10⁻¹ and 10⁻² suspensions were plated to a trypticase soy agar plate, chocolate agar or CDC anaerobic blood agar and incubated at 35°C depending upon the growth parameters of the test organism. Culture plates were evaluated following 24 and 48 hrs. incubation and colony counts were generated whenever possible from plates containing between 30 and 300 cfu. The COP swab consistently outperformed the STAR swab. Each organism survived for 48 hrs using the COP swab at both temperatures. The best survival was achieved at 4°C. However, the STAR swab failed to maintain viability in the system for *F. nucleatum*, *H. influenzae*, and *P. anaerobius* at room temperature and for *H. influenzae* at 4°C. The COP swab system is a superior transport device for the organisms tested.

INTRODUCTION

The collection and transport of clinical specimens to the microbiology laboratory are essential to dispensing good quality care. Most inpatient specimens are received into the laboratory within 4 h after collection. Outpatient specimens, however, may be received in the clinical laboratory 24 h after their collection. Many laboratories have instituted an outreach effort in order to bring in more laboratory work. These specimens may be stored and transported at a variety of temperatures. Previous studies have shown that Amies medium is a superior transport media when stored at refrigerator temperature.

The purpose of this study was to compare the ability of two collection systems containing amies medium without charcoal to maintain the viability of several microorganisms at refrigerator temperature (~4°C), and 20-25°C.

Starplex Amies Agar Gel



Copan Amies Agar Gel



MATERIALS AND METHODS

Organism	Source
<i>Neisseria gonorrhoeae</i>	ATCC 43069
<i>Haemophilus influenzae</i>	ATCC 10211
<i>Streptococcus pyogenes</i>	ATCC 19615
<i>Streptococcus pneumoniae</i>	ATCC 6305
<i>Fusobacterium nucleatum</i>	ATCC 25586
<i>Peptostreptococcus anaerobius</i>	ATCC 27337

Swab products to be tested:

Copan Amies Agar Gel without Charcoal
Starplex Amies Agar Gel without Charcoal

Quantitative Vortex Method:

1. Prepare suspension of each test bacterium in saline equivalent to 0.5 McFarland Standard (approximately 1.5×10^8 CFUs per ml) using a BD Crystal Spec Nephelometer or similar equipment.

2. Dilute the 0.5 McFarland suspension 1:10 in saline to create the “inoculum suspension”.

3. Prepare 3 test swabs for each time point 0 hrs, 6hrs, 24 hrs and 48hrs and each temperature.

4. Dose each test swab with 100 μ l of the “inoculum suspension”. This can be done by pipetting 100 μ l volumes of the suspension into wells of a microtiter plate using a volumetric pipettor, then using the swab to completely carefully absorb all the liquid from well of the plate.

5. Place inoculated swabs into transport medium and hold at the specified temperature: Controlled Room Temperature 20–25°C (68°–77°F) or in a refrigerator (approx. 4°C).

6. Immediately remove each time 0 hr swab and place in tube containing 1ml of sterile saline.

7. Vortex mix the swab vigorously for 15 seconds. Thoroughly squeeze out as much liquid contents, as possible from the swab tip by pressing against the sidewalls of the tube and then discard the swab.

8. Using a volumetric pipettor remove 100 μ l of the vortex suspension and perform serial dilutions in 0.9 ml of saline to create 10^{-1} and 10^{-2} dilutions.

11. Using a volumetric pipettor remove 100 μ l from the vortex swab suspension and from each serial dilution and spread evenly over the entire surface of an appropriate culture plate. Perform this procedure in duplicate so there are two plate counts for the original vortex suspension and for each saline dilution.

12. Incubate plates in the correct atmospheric conditions for the test organism for 24 to 48 hours before examining and counting. When ever possible, count plates resulting in 30–300 colonies upon culture. Average the results for the duplicate plate cultures of each serial dilution.

13. Express CFU plate counts as a percentage of zero time colony counts.

CONCLUSIONS

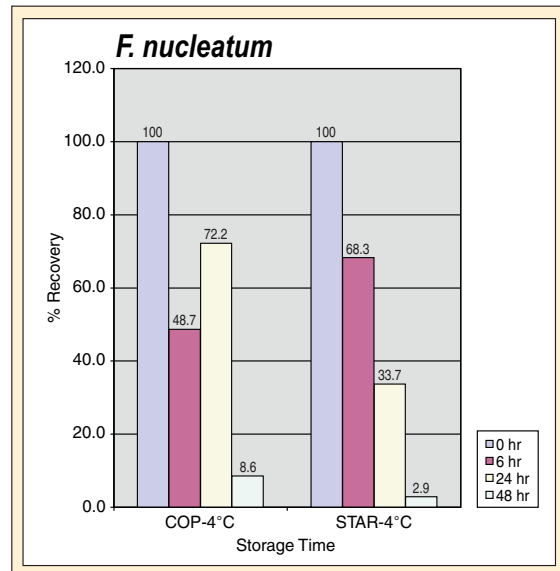
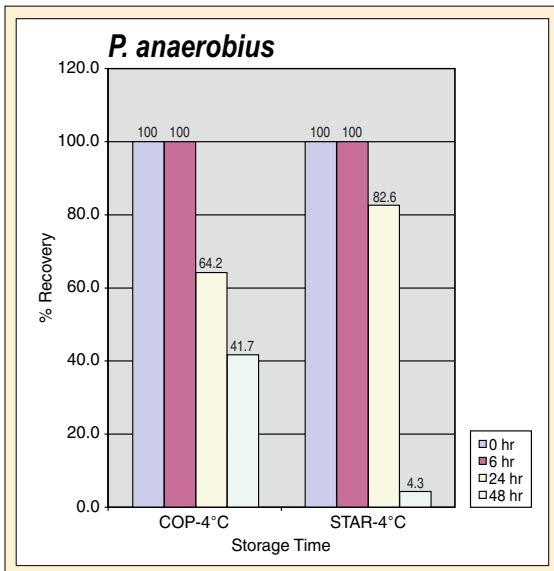
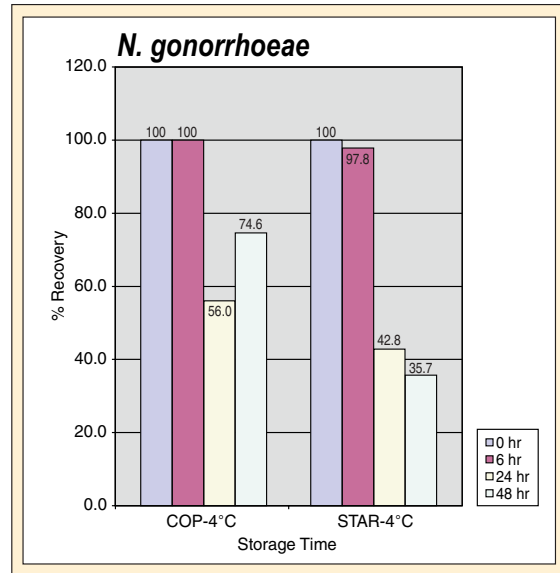
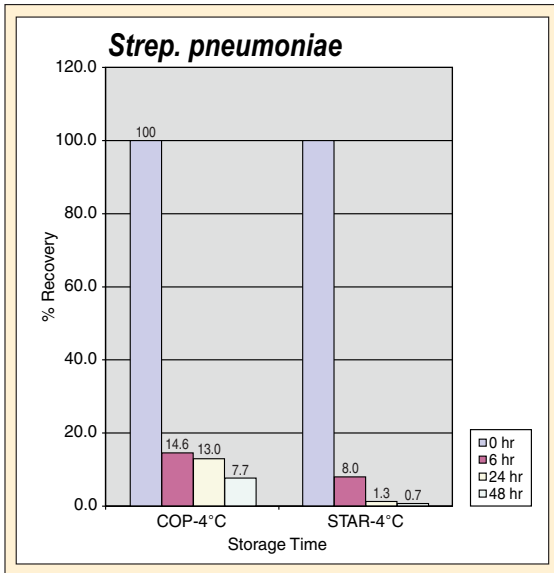
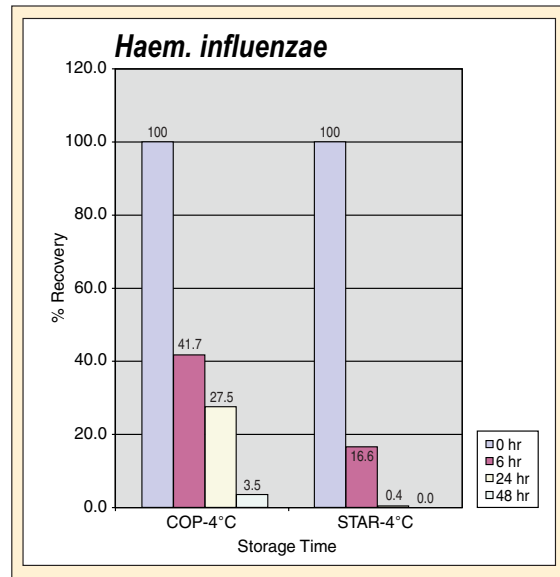
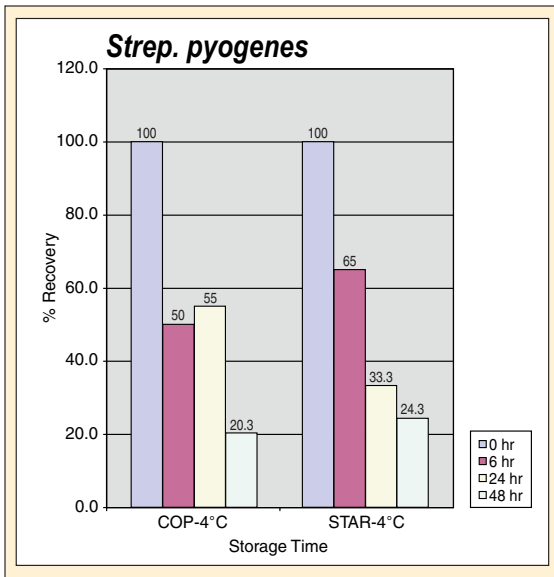
1. All organisms survived following 6 h incubation in all transport devices. However, the Starplex system failed to maintain viability of *H. influenzae* and *P. anaerobius* at 24 hrs and *F. nucleatum* at 48 hr when held at room temperature. Colony counts and percent recovery for *H. influenzae* in Starplex swabs held at -4°C were also significantly lower than Copan.
2. Previous studies have demonstrated that fastidious bacteria such as *N. gonorrhoea* survive better when held at refrigerator temperatures. Our study also demonstrated higher percentage survival of *P. anaerobes* and *F. nucleatum* at refrigerator temperature compared with room temperature.
3. The Copan system outperformed the Starplex system for each organism studied.

REFERENCES

1. Sautter, R. L., Robin Yeakle and Julie Bihl, 1985. Increased Isolation of Pathogenic Microorganisms Utilizing a saline rinse of routine culture swabs. Abst. of Annual Meeting, ASM C29I. Washington, D.C.
2. Y. Sun, T. Taylor, L. Williams, and R.L. Sautter. 1996. Comparison of bacterial viability using both the EZ Brand Collection and Transport system with the Difco Swab Transport Pack. ASM, Washington D.C. C221.
3. Sautter, R.L. and M.T. Wilson, 1988. Specimen Transport Containers are not created equal. Clin. Micro. Newsletter, Vol. 10, No. 23: 181-183.
4. Farhat SE, et. al. 2001 Efficacy of a Swab Transport System in Maintaining Viability of *Neisseria gonorrhoeae* and *Streptococcus pneumoniae*. J. Clin. Microbiol. 39:2958
5. Perry J. 1997. Assessment of Swab Transport Systems for Aerobic and Anaerobic Organism Recovery. J. Clin. Microbiol. 35:1269-1271.
6. Van Horn K, C. Toth. 2000. Comparison of Four Swab Transport Systems for the Recovery of Aerobic Microorganisms. Abst. of Gen Meeting, ASM Washington DC.
7. A. Robinson, M.L. Gruver. 2002 Comparison of Bacterial Survival in Two Transport Systems Stored at Room and Refrigerator Temperatures. Abst. of Gen Meeting, ASM Washington DC.

RESULTS

4 °C



RESULTS

Room Temperature

