SURVIVAL RATE OF HAEMOPHILUS INFLUENZAE ISOLATED FROM PEDIATRIC PATIENTS IN COMMERCIAL TRANSPORT SYSTEMS

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ABSTRACT:

Background: *Haemophilus influenzae* (HI) remains a leading cause of meningitis among unvaccinated children. Studies revealed that HI accounted for 7% of identified etiologic agents of childhood community-acquired pneumonia in North America and Europe and 21% in Africa and South America. Fastidious organisms such as HI may survive only a few hours after specimen collection if placed in swab transport systems and this can be a critical component in the success of the diagnostic process. The purpose of this study was to evaluate multiple lot numbers of Amies Gel swabs from two manufacturers namely Copan, Ca, USA (**M40 Transystem**, 3 lots) and Starplex Scientific, Ont, Canada (**Starswab II**, **BactiSwab** and **Fisherfinest**, 1 lot of each) for the survival of HI over a 48hr period at room temperature (RT). **Methods:** Five HI clinical isolates from pediatric patients and one HI ATCC 10211 were tested at RT (25°C) against 3 lot numbers from both manufacturers using the Swab Elution Method described in NCCLS M40-A. A 0.5 McFarland suspension of each sample was performed in 0.85% physiological saline from a 24hr growth of the organism. Ten-fold dilutions were prepared and the swabs were inoculated in triplicate with 100µl of each sample suspension. Swabs were held at RT for 0, 6, 24 and 48hr. Duplicate plate counts were performed at 35°C after each holding time point to determine organism survival rate. **Results:** Cultures from all swab dilutions were averaged. Bacterial recovery from swabs held for 0 and 6 hr were similar for all swab lot numbers from both manufacturers. After 24hr incubation, bacterial viability from Starplex lots was dramatically lower compared to that from Copan. At the 48hr time point all three Starplex swab lots demonstrated a significant loss in viability with two of six HI strains and four HI strains failed to survive. Only Copan swabs were able to maintained viability of all six HI strains tested after 48hr at RT. **Conclusion:** Survival of HI varies between manufacturers Amies products and does not appear to be lot number dependent. Only Copan swab lots were able to comply with criteria for HI organism survival at all time points as described in NCCLS M40-A document.

INTRODUCTION:

Specimen collection and transport are considered important steps in the overall effectiveness of the Microbiology Laboratory to provide clinically relevant results. Swabs are frequently used to collect specimens, but are often considered to be a less desirable specimen collection device. Ideally swab transport system should maintain viability of microorganisms and not promote multiplication in the pre analytical phase of laboratory testing to prevent commensal flora from overgrowing. At room temperature certain organisms that survive do increase in numbers and this is a common problem, addressed in the M40-A National Committee for Clinical Laboratory Standards (NCCLS) document. A good system of quality control of swabs is important as ambient transport temperature can also vary dramatically depending on the time of the year and latitude. This problem is particularly true for Brazil because of the huge geographic size of the country and the latitude which means testing labs can be located far away from the patient and ambient temperatures are above 25ºC for many months of the year.

On the other side, *Haemophilus influenzae* (HI) remains a leading cause of meningitis among unvaccinated children. Studies revealed that HI accounted for 7% of identified etiologic agents of childhood community-acquired pneumonia in North America and Europe and 21% in Africa and South America. Fastidious organisms such as HI may survive only a few hours after specimen collection if placed in swab transport systems and this can be a critical component in the success of the diagnostic process. The purpose of this study was to evaluate multiple lot numbers of Amies gel swabs from two manufacturers namely Copan, Ca, USA (M40 Transystem, 3 lots) and Starplex Scientific, Ont, Canada (Starswab II, BactiSwab and Fisherfinest, 1 lot of each) for the survival of HI over a 48hr period at room temperature (RT), using a protocol described in a NCCLS document M40-A.

M40 Transystem

StarSwab II

BactiSwab

Fisherfinest®

METHODS:

Five HI clinical strains isolated from pediatric patients and one HI ATCC 10211 were evaluated for survival after incubation at room (25°C) temperature, using transport swabs systems from two different brands.

Bacterial strains:

1. *Haemophilus influenzae* ATCC 10211

2. *Haemophilus influenzae* H173 – clinical strain

3. *Haemophilus influenzae* H323 – clinical strain

4. *Haemophilus influenzae* H203 – clinical strain

5. *Haemophilus influenzae* H318 – clinical strain

6. *Haemophilus influenzae* H319 – clinical strain

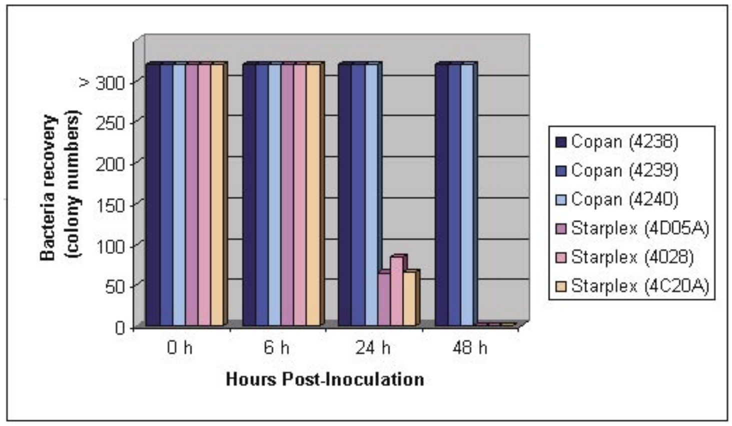
Transport swab systems:

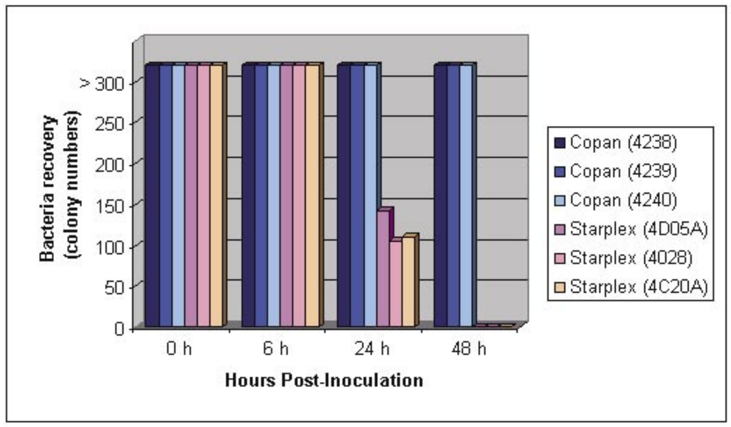
M40Transystem (Copan, CA, USA – Lots 4238, 4239, 4240), **Starswab II**, **BactiSwab** and **Fisherfinest** (Starplex Scientific, Ontario, Canada – Lots 4C20A, 4028, 4D05A, respectively). All transport systems consist of a sterile peal pouch containing a rayon tip swab and Amies agar gel medium without charcoal.

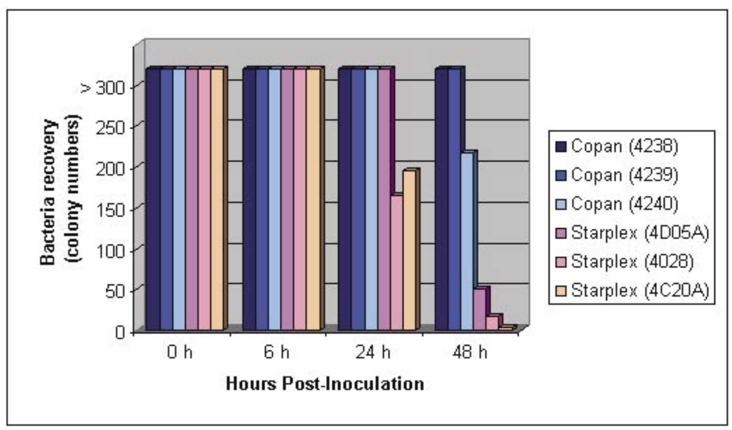
The protocol was performed using a vortex elution method:

- A 0,5 McFarland suspension in saline (85%) was prepared from an 18-24hr culture of each organism. The 0,5 suspension was diluted (1:10) in saline;
- 100ml of each organism suspension was transferred into wells of a microtiter plate using a volumetric pipette;
- Tests were performed in triplicate for each swab brand, lots and time points (0, 6, 24 and 48 hours);
- Each swab was rolled into the 100ml suspension (10 seconds) to completely absorb the inoculum and then placed into the transport device and held for the appropriate time at room temperature (25°C);
- The first swab (time point zero hour) were cultivated from within 15 minutes;
- After hold in appropriate time, all the swabs were placed in a tube with 1mL of sterile saline and vortex for 15 seconds. Serial dilutions were prepared in saline (10^{-1} and 10^{-2});
- After vortexing with tube, a 100ml of each suspension was plated in chocolate agar media. Cultures were performed in duplicate and were incubated at 35°C for 48hs;
- Counts were then performed. Average counts for 6, 24 and 48 hrs were compared to the zero hour counts for the same dilution and organism.

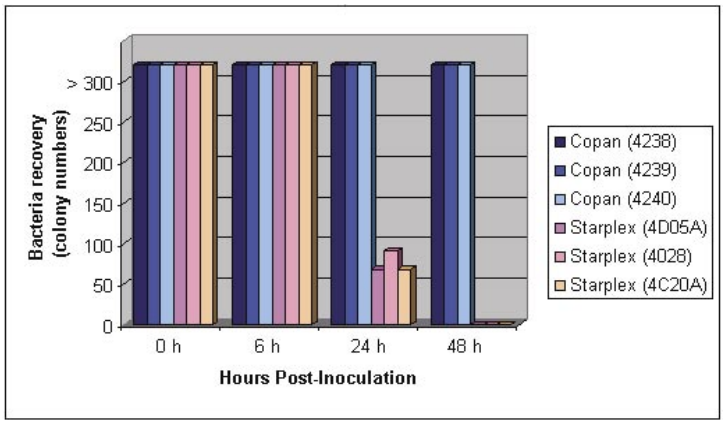
RESULTS:

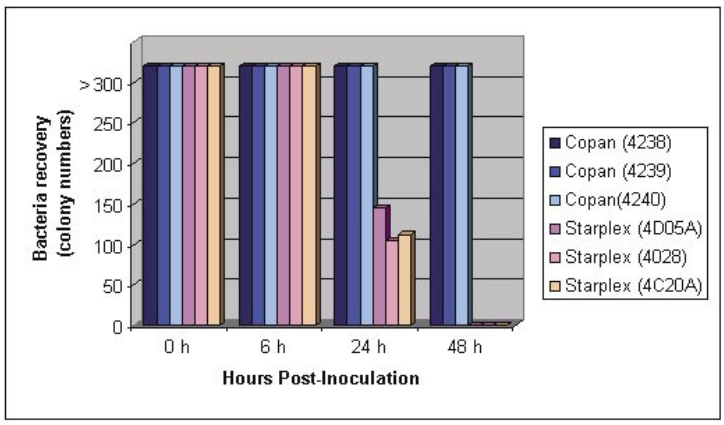


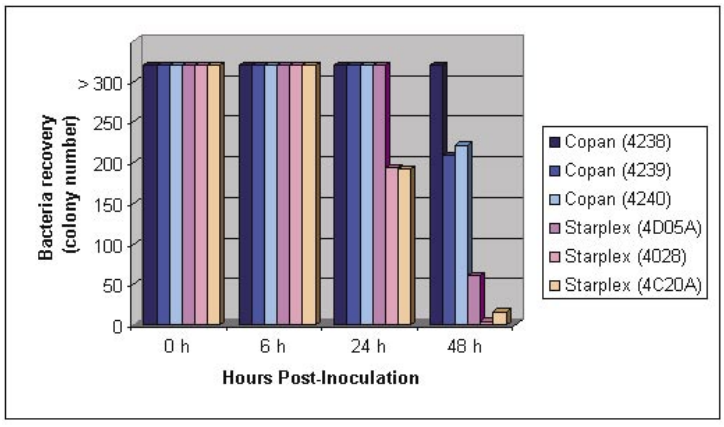




RESULTS: (continued)







CONCLUSIONS:

Bacterial recovery from swabs held for 0 and 6 hours were similar for all swab lot numbers from both manufactures.

After 24 hours incubation, bacterial viability from Starplex lots was dramatically lower compared to that from Copan.

At the 48 hours time point all three Starplex swab lots demonstrated a significant loss in viability with two of six HI strains and four HI strains failed to survive. Copan swabs were able to maintained viability of all six HI strains tested after 48 hours at room temperature (25°C).

Survival of HI varies between manufactures Amies products and does not appear to be lot number dependent.

Only Copan swab lots were able to comply with criteria for HI organisms survival at all time points as described in NCCLS M40-A document.