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Recovery of *Haemophilus influenzae* (HI) Using the New ESwab™ Transport System

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ABSTRACT:

Background: A new patented Flocked Swab with Liquid Amies, Copan, CA, USA (ESwab™) was designed to improve the sensitivity of the traditional transport swab systems. In this new swab the organism inoculum theoretically is released into 1ml of Amies Liquid and after that it should be possible to perform different tests on the collected sample. The purpose of this study was to evaluate the ability of ESwab to support viability of HI and produce identical growth recoveries when multiple plate culture repetition protocols are conducted. A Quadrant One (P.1) seed and Kirby-Bauer (P.2) protocols were used.

Methods: A 0.5 McFarland suspension of HI ATCC 10211 strain was performed in 0.85% physiological saline from a 24h growth organism. For P.1 a 1/50,000 dilution of the bacteria was used and for P.2 the dilution was 1/10,000. Regular Transystem swabs, as well as ESwabs (Copan, USA) were inoculated with 100µl of the strain suspension. Swabs were held at 4°C for 0h, 24h, 48h and 1 week. On P.1, just one quadrant of a Chocolate Agar Plate (CAP) was seed, using first a regular Transystem swab and second, an ESwab. A third action was done by dropping 100µl from the ESwab medium in the first quadrant of a CAP and spread it, after liquid evaporating. These three steps were repeated 10 times, using the same swab and 10 different plates for each swab time holding point. On P.2, the same steps were performed, but instead of a one quadrant, a whole CAP was seeded. Bacterial survival was evaluated after 48h incubation at 35°C.

Results: HI was recovered from all ESwabs, at all holding time points and inoculation methods. No significant difference was observed on bacterial recovery rates when comparing the two different ESwab plate inoculation methods. None of the Transystem swabs using P.1 and P.2 maintained viability for HI after 1 week at 4°C and for time points 0h, 24h and 48h the number of colonies recovered from ESwabs was superior.

Conclusion: The new ESwab™ proved to be an excellent system to recovery HI viability. It provides a homogeneous sample suspension enabling multiple replicate plate inoculations with a high degree of reproducibility and consistency of organism transfer.

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. 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.

A new patented Flocked Swab with Liquid Amies, Copan, CA, USA (ESwab™) was designed to improve the sensitivity of the traditional transport swab systems. In this new swab the organism inoculum theoretically is released into 1ml of Amies Liquid and after that it should be possible to perform different tests on the collected sample. The purpose of this study was to evaluate the ability of ESwab™ to support viability of HI and produce identical growth recoveries when multiple plate culture repetition protocols are conducted. A Quadrant One (P.1) seed and Kirby-Bauer (P.2) protocols were used.

METHODS:

BACTERIA SUSPENSION:

0.5 McFarland suspension of *Haemophilus influenzae* ATCC 10211 in 0.85% physiological saline from a 24h growth organism.

SWABS:

- Transystem swab – Copan, USA
- Eswab™ – Copan, USA

PROTOCOL 1 (P.1):

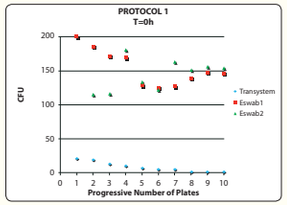
- Dilution of the bacteria: 1/50,000.
- One transystem swab and two Eswabs were used.
- Each swab was inoculated with 100µl of the organism suspension;
- Swabs were held at 4°C for 0h, 24h, 48h and 1 week;
- After hold in appropriate time, the first quadrant of a Chocolate Agar Plate (CAP) was seeded using first the regular transystem swab and second, an Eswab.
- A third action was done by dropping 100µl from the ESwab medium in the first quadrant of a CAP and spread it, after liquid evaporating.
- These three steps were repeated 10 times, using the same swab and 10 different plates for each swab time holding point

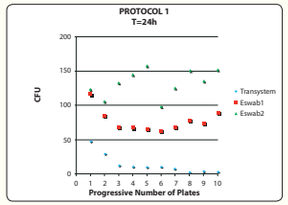
PROTOCOL 2 (P.2):

- Dilution of the bacteria: 1/10,000.
- One transystem swab and two Eswabs were used.
- Each swab was inoculated with 100µl of the organism suspension;
- Swabs were held at 4°C for 0h, 24h, 48h and 1 week;
- After hold in appropriate time, a whole Chocolate Agar Plate (CAP) was seeded using first a regular transystem swab and second, an Eswab.
- A third action was done by dropping 100µl from the ESwab medium in the first quadrant of a CAP and spread it, after liquid evaporating.
- These three steps were repeated 10 times, using the same swab and 10 different plates for each swab time holding point

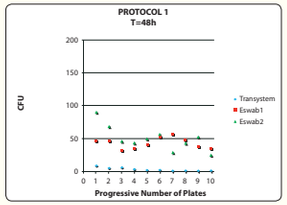
RESULTS:

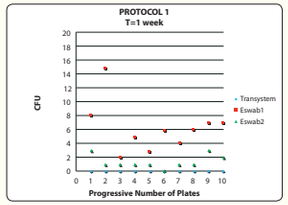
***Haemophilus influenzae* ATCC 10211**





***Haemophilus influenzae* ATCC 10211**





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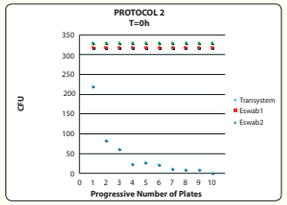

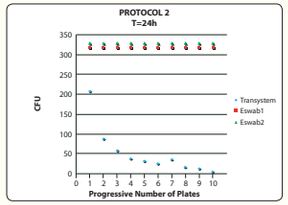

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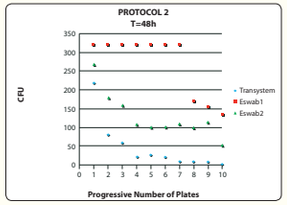

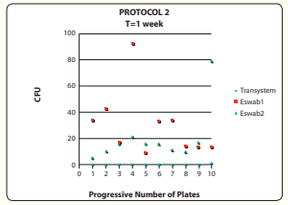

CONCLUSIONS:

1. *Haemophilus influenzae* was recovered from all ESwabs™, at all holding time points and inoculation methods.
2. No significant difference was observed on bacterial recovery rates when comparing the two different ESwab™ plate inoculation methods.
3. None of the Transystem swabs using protocol 1 and protocol 2 maintained viability for *H. influenzae* after 1 week at 4°C.
4. For time points 0h, 24h and 48h the number of colonies recovered from ESwabs™ was superior than from the Transystem swabs.
5. The new ESwab™ proved to be an excellent system to recovery *H. influenzae* viability. It provides a homogeneous sample suspension enabling multiple replicate plate inoculations with a high degree of reproducibility and consistency of organism transfer.