

Comparative Evaluation of Puritan and Copan Liquid Amies Transport System for the Recovery of *Streptococcus pneumoniae* against the CLSI M40A Standard

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Revised Abstract

Objective: To assess the performance of two modified liquid Amies transport systems, Puritan Liquid Amies Transport System with a standard flocked swab (**P**) (Puritan Medical Products Company LLC) and Copan's E swab (Elution Swab) (**C**) (Copan Diagnostics Inc.) intended for processing using automated plating systems for the recovery of *Streptococcus pneumoniae* (SPN).

Method: The CLSI M40A Roll-Plate method at room temperature was followed to test the recovery rate and viability for up to 48 hours of 18 SPN strains acquired from the Toronto Invasive Bacterial Disease Network (TIBDN) stock and other medical laboratories in Ontario. As well, two ATCC reference strains (ATCC 6303 and ATCC 49619) were also tested. At time 0, 24, and 48 hours, the average colony counts were calculated based on triplicate swabs for each organism/dilution/time combination. Culture plates were read manually. Results were compared to control plates at time zero. For viability studies to be considered acceptable, there had to be an average of ≥ 5 colony forming units (CFU) following the specific holding time from the specific dilution that yielded zero-time plate counts closest to 300 CFU.

Results: Of the 21 SPN strains, **P** recovered 7 after 24 hours and an additional 14 after 48 hrs, whereas **E** recovered 9 strains at 0 hrs, an additional 8 strains after 24 hours and 4 strains (all mucoid strains including one ATCC strain which was repeated and 2 clinical isolates) after 48 hrs.

Conclusion: Our study suggests that based on the CLSI M40A standard roll plate protocol the Puritan system appears to outperform the E system. The mucoid strains appears not to be a problem with the E system. Further study is required to understand the inhibitory nature of the Copan transport system on SPN.

Introduction

Transport system devices continue to undergo improvements in their ability to maximise the absorption of clinical specimen during collection, maintain viability of bacterial pathogens involved in infectious diseases during transport and their subsequent recovery in the laboratory.

In recent times flocked swabs have become a subject of great interest. Flocked swabs differ from the traditional fibre wound swabs by nylon fibres and blends attached perpendicularly to the plastic applicator that prevents entrapment of clinical sample and thus results in greater release and recovery of pathogens when cultured.

This present study is an assessment of two brands of flocked swabs in Liquid Amies broth system using one type of an aerobic fastidious organism *Streptococcus pneumoniae* and how well they comply to the CLSI M40A standards.

Secondly some obvious physical appearance of these two brands were also noted (Figure 2-4).

Method

Bacterial Strains:

- Streptococcus pneumoniae* ATCC 6303 (mucoid strain)
- Streptococcus pneumoniae* ATCC 49619 (non mucoid strain)
- Streptococcus pneumoniae* (18 wild strains)

Organism	Media	Incubation Temperature (°C)	Atmosphere	Testing Time (hours)
<i>Streptococcus pneumoniae</i>	BA 5%sheep	35-37	5% CO ₂	0,24,48

Method Continued

M40A Roll Plate Method:

1. All organisms were harvested from frozen stock cultures and subcultured three times prior to testing.
2. Working suspensions were made from growth of 18-24 hr culture plates and adjusted to match 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL) in 10.0 ml saline using a Vitek nephelometer.
3. Each suspension was further diluted in 1 log₁₀ or 1:10 dilution to obtain a working suspension of $\sim 1.5 \times 10^7$ CFU/ml
4. From this suspension, four 1:10 dilutions were prepared: 1.5×10^6 to 1.5×10^3 CFU/mL
5. The final working concentrations used to inoculate swabs were 10⁶, 10⁵, and 10⁴.
6. The concentrations used to perform reference counts were 10⁵, 10⁴ and 10³
7. 100µL of the working suspension was transferred into 18 wells of a microtitre plate using an Eppendorf pipette.
8. Nine swabs of each brand were placed into the wells and allowed to absorb the inoculum, performing a turning motion for greater absorption. After inserting into their respective labelled transport device, the applicator sticks were snapped off at the score mark and the caps screwed on.
9. Each micro organism/device combination was performed in triplicate for each time period (0, 24 and 48 hr).
10. The 0 hr swabs were removed from the transport devices containing 1.0ml Amies broth after ~15 minutes incubation and discarded.
11. Using an Eppendorf pipette, 100µl of the inoculum was transferred onto the centre of a blood agar plate containing 5% sheep blood, streaked for isolation and incubated at 35°C in CO₂ environment.
12. The remaining swabs were incubated at room temperature for 24 and 48 hrs.
13. Results were calculated by taking an average of the colony counts from triplicate tests.
14. Counts at 24 and 48 hrs were compared to 0 hrs counts.

Results

Strain	Puritan Transport System			Copan Transport System		
	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
6303 ATCC			***			***
6303 ATCC (rep)			***			***
49619 ATCC			***	***		
Spn#1			***	***		
Spn#2			***	***		
Spn#3		***		***		
Spn#4		***		***		
Spn#5			***	***		
Spn#6		***			***	
Spn #900			***	***		
Spn #901		***		***		
Spn #902		***			***	
Spn #903		***			***	
Spn #904		***		***		
Spn #866			***		***	
Spn #909			***			***
Spn #115			***			***
Spn #881			***		***	
Spn #884			***		***	
Spn #885			***		***	
Spn #886			***		***	

Conclusion

1. Based on this study, Puritan flocked swabs demonstrated superior absorption and release abilities as evidence by the higher counts. Copan E Swab also demonstrated superior absorption but it is difficult to assess if the E swab released all the inoculum based on its viability performance.
2. Possibilities for this could be the flocked swabs has impurities in the glue/adhesives or substandard/impurities in the raw material which may have some antibacterial effect on SPN or the Copan modified Amies broth formula is not optimal for the growth of this organism to permit it to survive up to 48hrs in most cases.
3. There are numerous other factors, not presently examined which can affect swab performance.
4. Strains that survived up to 48 hrs were mucoid strains which were not affected by any antibacterial effect due to their polysaccharide capsule.
5. Other researchers have also documented the poor recovery of *Streptococcus pneumoniae* with the Copan E swab system (2).
6. Further studies are warranted using more strains both mucoid and non-mucoid.

References

1. Clinical and Laboratory Standards Institute (2003) Quality Control of Microbiological Transport Systems. Approved Standard M40-A. Wayne, PA: CLSI; 2003
2. Gandhi, B., Mazzulli, T. (2010) Comparison of viability performance of a new flocked and foam swab transported in E-Swab liquid Amies medium at ambient temperature. Presented at the 110th General Meeting of the American Society for Microbiology, San Diego, California.
3. Humans, R. P., Jones, G.A. (2006) A New concept for transporting clinical material on flocked swabs in liquid Amies medium. Presented at the 106th General Meeting of the American Society for Microbiology, Orlando, Florida
4. Birrel, K. (2007) Investigation of the ability of transport swabs to release collected microorganisms- using the roll plate method. Presented at the 107th General Meeting of the American Society for Microbiology, Toronto, Ontario.



Figure 1: Serial Dilutions 1:10



Figure 2: Puritan Flocked Swab (left) Copan Flocked Swab (right)

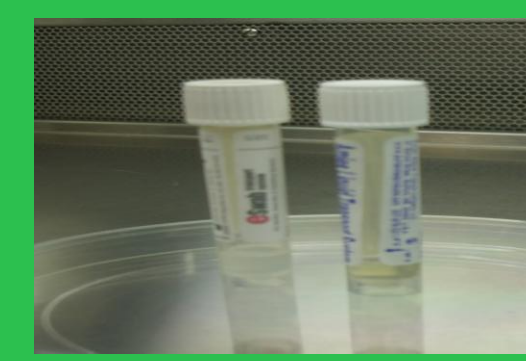


Figure 3: Copan Amies Broth (left) Puritan Amies Broth (right)

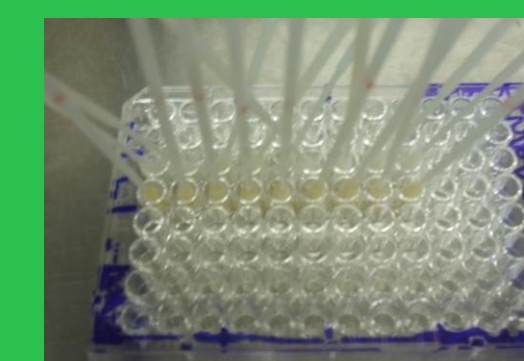


Figure 4: Puritan (top) Copan (bottom)



Figure 5: Three isolates at 10⁶, 10⁵, 10⁴

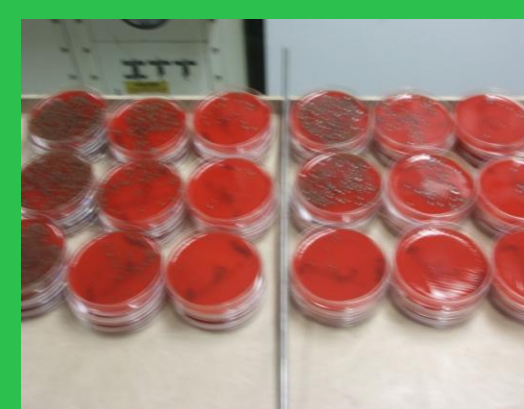


Figure 6: Puritan (left) Copan (right), three isolates in triplicate at 10⁶, 10⁵, 10⁴ @ 48 hrs

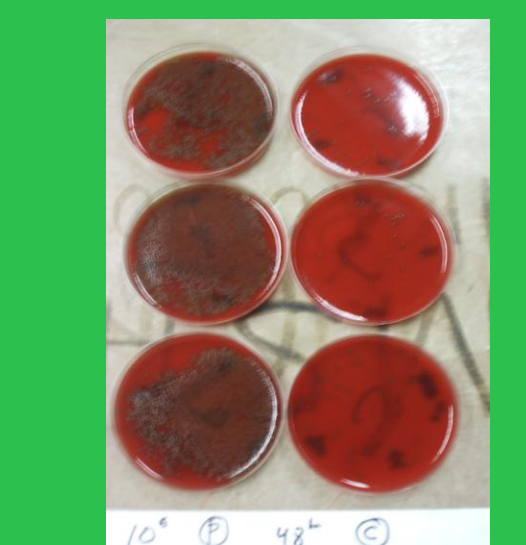


Figure 7: S. Pneumoniae clinical isolate non-mucoid strain after 48 hrs

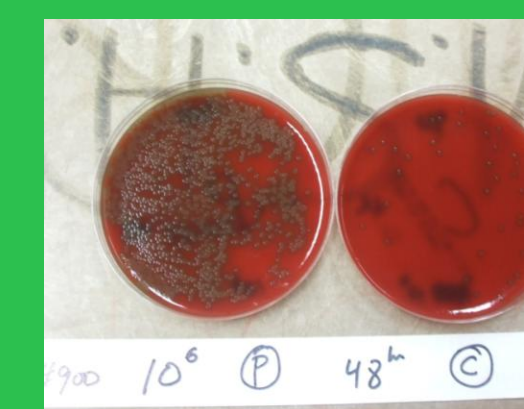


Figure 8: Close up of Figure 7

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