Recovery of *Streptococcus pneumoniae* Using the New Puritan Liquid Amies Transport System and Copan ESwab System at Room Temperature

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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 uncoated flocked swab (**P**) (Puritan Medical Products Company LLC) and Copan's E swab (Elution Swab) with a coated flocked swab **(C**) (Copan Diagnostics Inc.) intended for processing using automated plating systems for the recovery of *Streptococcus pneumioniae* (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 test strains, **P** recovered 7 after 24 hours and an additional 13 after 48 hrs, whereas **E** recovered 9 strains at 0 hrs, an additional 7 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 appear 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 specimens during collection, maintain viability of bacterial pathogens involved in infectious diseases during transport and subsequent recovery of pathogens in the laboratory.

Recently, flocked swabs have become a subject of great interest. Flocked swabs differ from the traditional fibre wound swabs. They are made using nylon fibres and blends attached perpendicularly to the plastic applicator thus preventing entrapment of the clinical sample and resulting in greater release in the liquid medium and greater recovery of pathogens when cultured. Furthermore to improve the survival of fastidious bacteria during transport, swabs are often coated with proteins.

The present study is an assessment of two brands of flocked swabs placed in Liquid Amies broth using a fastidious aerobic organism, *Streptococcus pneumoniae* and comparing how well they comply with the CLSI M40A standards.

Method

Bacterial Strains:

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

Table 1.

Organism	Media	Incubation Temperature (°C)	Atmosphere	Testing Time (hours)
Streptococcus pneumoniae	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 MacFarland 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 $\log_{10}$ or 1:10 dilution to obtain a working suspension of ~ 1.5 ×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 10^6, 10^5, and 10^4.
6. The concentrations used to perform reference counts were 10^5, 10^4 and 10^3
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 CO_2 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.

Table 2. SPN isolate #900 colony counts (cfu)

	Dilution	0hrs	0hrs	0hrs	24hrs	24hrs	24hrs	48hrs	48hrs	48hrs
Puritan	10^6	500 -700	500 -700	500 -700	>1000	>1000	>1000	~1000	~1000	~1000
	10^5	285	308	326	194	316	340	~400	500 -700	500- 700
	10^4	45	46	50	8	25	39	**33**	**47**	**45**
Copan	10^6	300 -500	300 -500	300 - 500	300 -500	300-500	300 -500	34	20	33
	10^5	166	173	196	37	39	67	0	3	1
	10^4	**32**	**34**	**39**	1	2	5	0	0	0
Purity plate counts		10^5 (500-700)),(500-700)			**10^4 (230), (261)**			10^3 (40), (44)		

Table 3. SPN isolate # ATCC 49619 colony counts (cfu)

	Dilution	0hrs	0hrs	0hrs	24hrs	24hrs	24hrs	48hrs	48hrs	48hrs
Puritan	10^6	500 -700	500 -700	500 -700	240	462	~500	303	~400	~400
	10^5	141	142	266	89	165	186	72	58	90
	10^4	19	22	39	10	11	24	**5**	**7**	**10**
Copan	10^6	500 -700	500 -700	700-1000	74	109	116	6	22	11
	10^5	166	207	214	7	8	8	1	2	5
	10^4	**43**	**49**	**63**	0	1	2	0	0	1
Purity plate counts		10^5 (~1000), (~1000)			**10^4 (426), (540)**			10^3 (76),(76)		

Table 4. SPN isolate # 4 colony counts (cfu)

	Dilution	0hrs	0hrs	0hrs	24hrs	24hrs	248hrs	48rs	48hrs	48hrs
Puritan	10^6	700 -1000	700 -1000	700 -1000	~700	~700	~700	300 -500	300 -500	300 -500
	10^5	121	154	120	94	77	95	43	62	40
	10^4	18	16	18	**8**	**8**	**9**	0	5	3
Copan	10^6	500 -700	500 -700	500 -700	104	113	113	2	3	0
	10^5	87	80	113	7	7	8	0	0	2
	10^4	**17**	**8**	**12**	0	1	3	0	0	0
Purity plate counts		10^5 (500-1000),(500-1000)			**10^4 (189),(213)**			10^3 (18),(30)		

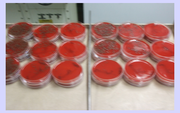

Figure 1: Puritan (left) Copan (right), three isolates in triplicate at 10^6, 10^5, 10^4 @ 48 hrs.

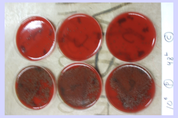

Figure 2: *S. pneumoniae* clinical isolate non-mucoid strain after 48 hrs.

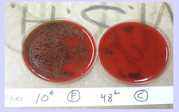

Figure 3: Close up of Figure 2.

Acknowledgement: This study was supported by Puritan Medical Products Inc.

Results

Table 5. Recovery times for several strains of *Streptococcus pneumoniae* using the Puritan and Copan Swabs/ Transport Systems.

	Puritan Transport System			Copan Transport System		
Strain	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 at at 4°C.

References

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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.