Comparative Performance of Two Liquid AmiesTransport Systems for the Recovery of Fastidious Bacteria Following the CLSI M40A Swab Elution protocol at Room Temperature Incubation.

BHARAT. GANDHI*[1], TONY. MAZZULLI[1,2]

[1]Department of Microbiology., Mt. Sinai Hospital and University Health Network, Toronto, Canada,
and [2]University of Toronto, Toronto, Canada

Introduction

Organism viability, survival rate and subsequent release from the transport device are crucial components in the isolation of clinically significant organisms from commercial swab systems. Many factors play a role in the recovery of bacteria from clinical specimens. These factors range from the type of swabs and transport media used, to the length of time and temperature of transportation.

The new Flocked swab with liquid Amies is designed to improve the sensitivity obtained with the traditional transport swab systems. In this new design organisms are theoretically completely released into the 1 mL of liquid Amies broth from which up to ten samples of 100µL can be derived for culture plates and Gram stain. This potential advantage of this liquid based platform is that it can be used for either manual inoculation or with automated equipment.

We therefore decided to evaluate a novel patent pending flocked swab from an exclusive swab manufacturer with their liquid Amies formulation and compare the results with two different flocked swabs from another manufacturer that are already on the market.

Abstract

Objective: To compare the performance of Puritan's liquid Amies transport medium with HydraFlock® swab, **(P**) (Puritan Medical Products Company LLC), with Copan's ESwab™ transport system containing a treated flocked swab and ESwab™ transport medium with COPAN's standard FLOQSwab (**C**) (Copan Diagnostics Inc.). **Method:** Viability tests were performed using a modified Swab-Elution Method (CLSI M40-A) on swabs incubated at room temperature for 0, 24 and 48h prior to processing. All test plates were inoculated in triplicate for more accurate averaged colony count results. Six ATCC strains were tested including *N.gonorrhoeae* (ATCC 43069 ,& ATCC 19424), (NG), *N. menengitidis* (ATCC 13077), (NM), *H.influenzae* (ATCC 49247,& ATCC 10211), (HIN), and *S.pneumoniae* (ATCC 49619) (SPN). Nine swabs of each brand were inoculated by absorbing 100µl of ~1.5×10^7 CFU/ml organism suspension and then placing them in their respective devices and kept for 0, 24 and 48h at room temperature. Subsequently, 100µL from each of three swab/devices from each of the three swab types was serially diluted 10-fold in 0.9ml sterile saline and four dilutions prepared. 100µL of each dilution was pipetted onto chocolate agar or blood agar plates and incubated under optimal conditions for subsequent colony counts. After 48hrs incubation, countable colonies for each swab cultured at each time point was recorded. An average CFU count was determined from each triplicate set of swabs and dilution for each incubation period. Product performance was compared for each swab transport with the zero time counts at the dilution that produced 30 to 300 - 500 colonies. **Results:** All three swab types produced comparable CFUs at 0hr. **Three out of six** strains were recovered from the **Puritan system** after **48h** incubation **NM (1/1), HIN (1/2), and SPN (1)** and **5/6** strains including **NG (2/2),** NM (1/1), HIN ((1/2) and SPN (1/1) after **24 h**. **three out of six** strains were recovered from **COPAN's ESwab system** with treated flocked swab after **48h** incubation **NM(1/1), HIN(1/2),** and **SPN(1),** and **3/6** strains NM (1/1), HIN ((1/2)) and SPN ((1/1) after **24h**. **Two out of six** strains were recovered from **COPAN's standard FLOQSwab** after **48h** incubation **NM ((1/1),** and **SPN (1/1**), and **2/6** strains NM ((1/1) and SPN (1/1) after **24h**. **Conclusions:** In general, based on this limited study, all three flocked swabs placed in their respective liquid Amies transport systems appeared to perform comparably. Treating the flocked swabs does not appear to influence the recovery of these fastidious organisms. Further study with more isolates and different strains may be required to detect a significant difference in performance between these three systems. Only the Puritan system was able to recover both strains of NG after 24h, while both COPAN swabs failed to pass the CLSI M40A requirement.

Method

Protocol Tested (CLSI Swab Elution Quantitative Method):
The protocol used for viability studies was modified based upon the Swab Elution Method as described in the CLSI standard Quality Control of Microbiology Transport System. M40-A Vol 23 No. 34, 2003.

Method Continued

Fastidious Bacteria tested:
Neisseria gonorrhoeae ATCC 43069 (NG)
Neisseria gonorrhoeae ATCC 19424 (NG)
Neisseria meningitides ATCC 13077 (NM)
Haemophilus influenzae ATCC 49247 (HIN)
Haemophilus influenzae ATCC 10211 (HIN)
Streptococcus pneumoniae ATCC49619 (SPN)

Transport swab system:
HydraFlock® Puritan Medical Products Company LLC **(P)**
Modified Liquid Amies Medium, Puritan Medical Products Company LLC
ESwab™ Transport System (Copan Diagnostics Inc.) **(CT)**
FLOQSwab™ (Copan Diagnostics Inc.) **(CS**)

Specimen preparation:
Inoculum used for each investigation was prepared by making a direct suspension in sterile saline of isolated colonies selected from an 18 to 24h culture. The initial bacterial suspension was prepared to a concentration of approximately 1.5 ×10^8 CFU/ml using a DensiCHECk™. Nephelometer (BioMerieux Vitek Inc).

Preparation of growth control:
Six 10-fold dilutions were made using .333uL test suspension added to 3.0 ml saline. This dilution protocol provides suspensions with concentrations of approximately 1.5×10^7 CFU/ml to 1.5×10^2 CFU/ml.
To verify these concentrations, duplicate samples of 100µl each of the three tubes were plated out and colonies counted after 24 to 48 h incubation.
Experimental design (in triplicate):
For each organism, an aliquot of 100µl (~1.5×10^6 CFU/ml) of the ~1.5×10^7 CFU/ml suspension (second tube) was transferred into 9 wells of a round bottom microtitre plate (3 @time zero, 3 @24h and 3 @ 48h) for each transport system under test.
One swab was placed into each of the wells and allowed to absorb the suspension for ten seconds.
The inoculated swabs were then placed in their respective devices containing 1.0 ml of liquid Amies broth.
At each of the time points, the swabs were removed from their devices and vortexed in 0.9 ml sterile saline for 30 seconds from which four 1:10 serial dilution were made.
100 µl aliquot of each dilution was pipetted onto chocolates agar plates and or blood agar plate, streaked, and incubated in CO_2 at 37°C for 24 -48h.
Bacterial recovery was determined by counting the colonies recovered in each of the dilutions.
The number of the organisms recovered is expressed as an average from triplicate samples and from triplicate plates (9 plates/dilution /organism).
CLSI M40A recommends that the dilution to be used is that which has a zero time reading of ~30 to~ 300 CFU on a plate.
In this experiment the 10^{-3} dilution (~1.5×10^4 CFU/ml) was the one that most closely met these criteria.

Figure 1: HyrdaSwab (left), ESwab (centre), FLOQSwaba(right)

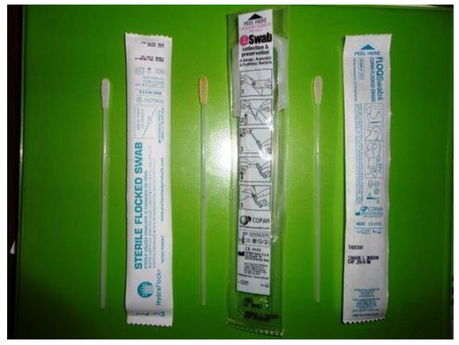

Figure 2: HyrdaFlock (left) ESwab (centre), FLOCQSwab (right))

Results

Average colony counts (CFU)

		O hr (RT)				24 hr (RT)				48 hr (RT)		
		10^5	10^4	10^3	10^2	10^5	10^4	10^3	10^2	10^5	10^4	10^3
NG ATCC 43069	P	>500	300-500	40	1	52	3.3	0	0	0	0	0
	CT	>500	300-500	69	4.5	1.3	0	0	0	0	0	0
	CS	>500	300-500	51	3	2	0	0	0	0	0	0
NG ATCC 19424	P	>500	300-500	59	6.3	71	7.6	0	0	0	0	0
	CT	>500	300-500	63	7	3.6	0	0	0	0	0	0
	CS	>500	300-500	61	6.6	0	0	0	0	0	0	0
NM ATCC 13077	P	>500	345	68	6.3	42	3	0	0	6	3	0
	CT	>500	352	81	6	77	4.6	.3	0	19	1.3	0
	CS	>500	326	59	5.3	83	9.3	1.3	.3	3	1	0
HIN ATCC 10211	P	>500	300-500	298	34	G500	322	73	8.3	7	2	0
	CT	>500	300-500	303	25	G500	330	95	17	9	3	0
	CS	>500	300-500	288	35	310	61	10	1	0	0	0
HIN ATCC 49247	P	>500	255	29.6	3.3	18	0	0	0	0	0	0
	CT	>500	242	31	2.6	22	0	0	0	4	0	0
	CS	>500	129	23	1.3	16	0	0	0	0	0	0
SPN ATCC 49619	P	>500	211	32.6	3	166	28.6	3	0	37.6	6.6	0
	CT	>500	198	30.6	2	248	45.6	5	0	35	4.6	0
	CS	>500	150	13	2.5	315	67.6	6.6	0	73	18.6	1.3

Conclusion

1. In general based on this limited study, Puritan's HydraFlock and the Copan's ESwab systems were comparable in their ability to recovery fastidious bacteria after 24 and 48 hours at room temperature incubation.
2. Only the Puritan's HydaFlock swabs completely absorbed the 100 µl inoculums (fig1).
3. The release /recovery function of all three systems appears to be comparable at zero hour incubation.
4. The zero hour colony counts were lower for one of the two HIN (HIN atcc 49247), as a result all three systems failed to recover at 48h incubation.
5. These results point to the fact that treating the swabs may not be beneficial for the recovery of all organisms.
6. Further studies will be performed and data presented using more fastidious organisms.