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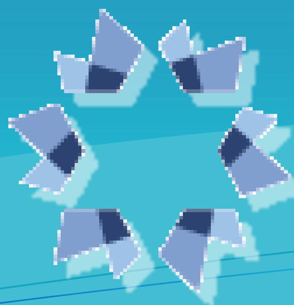


Comparison of Viability Performance of a New Flocked and Foam Swab Transported in E-Swab Liquid Amies Medium at Ambient Temperature.

B. GANDHI*[1], T. MAZZULLI[1,2]

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

Revised Abstract

Background: The objective of this study was to evaluate the performance of four swabs: a flocked swab (A) (Copan Diagnostics inc.), a new HYDRAflock swab[tm] (B), a multilength flocked swab (C), and a macrofoam swab (D) (Puritan Medical Products inc.) using the E- swab™ transport device containing modified liquid Amies medium.

Method: A viability study was performed in triplicate at room temperature (RT) using the following ATCC strains: *Neisseria gonorrhoeae* (NG), *Haemophilus influenzae* (HIN) and *Streptococcus pyogenes* (SP) *Pseudomonas aeruginosa* (PSA), *Streptococcus pneumoniae* (SPN) *Bacteriodes fragilis* (BF), *Fusobacterium nucleatum* FN), *Prevotella melaninogenica* (PM), *Peptostreptococcus anaerobius* (PA) and *propionibacterium acnes* (POA). Using a 0.5 McFarland suspension for each organism/swab, viable counts were performed at 0, 24 and 48 hrs according to the CLSI M40A roll plate method. From this working suspension, five 1:10 serial dilutions were prepared: 1:10, 1:100, 1:1000, 1:10,000 and 1:100,000 representing $1.5{\times}10^7$, $1.5{\times}10^6$, $1.5{\times}10^5$, $1.5{\times}10^4$ and $1.5{\times}10^3$ CFU/mL, respectively. Using an Eppendorf pipette, 100µl volumes of each organism suspension were transferred into wells of a round bottom microtiter plate. Each swab type was immersed into 100 µl of the organism suspension and allowed to absorb for 15 seconds with gentle twisting, and then inserted into the E-swab tube. Each organism/dilution was removed from the transport device after 15 minutes. The E-swab tube, including the swab, was vortexed prior to ringing the swab. The swabs were discarded and the tubes vortexed again for 5 seconds. 100µl of the suspension were pipetted onto dried agar surface, streaked and incubated at 37ºC in appropriate atmospheric conditions until countable colonies were observed. Colonies were counted and averaged for three swabs for each time point and dilution. Average colony counts at 24 and 48 hrs for each specific dilution and organism were compared to the 0 hr inoculated swabs, for the same dilution and organism.

Results: For HIN, all four types of swabs were acceptable at 24 hours, but none met the M40A acceptable criteria at 48 hours. For GC, only (A) and (C) were acceptable at 24 hours with (C) avg' counts just within the lower limits. For SP, (A) and (B) were acceptable at 48 hours with (B) having triple the colony counts compared with (A). (C) and (D) were acceptable at 24 hours only. For, SPN (B), (C), and (D) was acceptable at 48 hours while (A) was not acceptable. For PSA, growth greater than base line count at 0 hr was observed for (A), (B), (C) and (D).

Conclusions:
__Unacceptable recovery__ was observed for **HIN**, **PM, and PA** with all four swab types, for **GC** with swabs (B) and (D) for **SP** with swabs (C) and (D) and for **SPN** with swab (A).
__Acceptable recovery__ was observed for **PAS, FUS, POA,** and **BF.** The overall performance of the Puritan flocked and macro foam swabs were comparable to the flocked swab from Copan. Further studies are warranted using more isolates, including both clinical and reference strains and testing them at 4ºC incubation as well as the elution method at both room temperature and 4ºC. The general design of the shafts may be a factor in the performance of both Puritan flock swabs (B and C). Both are thinner and more flexible at the neck than Copan flock swab (A) and the Puritan macrofoam swab (D).

Introduction

Following the publication of the Clinical and Laboratory Standards Institute (CLSI) M40A document "Quality Control of Microbiological Transport Devices" in 2003, swabs made of new materials such as flocked (nylon) and modified foam have become available and are being addressed in the revised document. These novel swabs were designed to be an improvement over the rayon and Dacron fibers used in standard applicator swabs because of their greater absorption and release capability and in turn better recovery of potential causative microorganisms. As well, the M40A document does not address the use of liquid based modified Amies medium as part of a new platform for automation.

The objective of this study was two fold: (1) To assess the release and recovery of seeded organisms from these new swab types and (2) To evaluate the performance of these novel swab types in comparison to the E-swab transport system following the CLSI M40A recommendations with a slight modification by using 5% sheep blood agar for the anaerobes instead of brain heart infusion (BHI) agar.

Methods

The following organisms, media and incubation conditions were used during the study.

Species	ATCC No.	Plate Media	Incubation Temp °C	Incubation atmosphere	Testing Time (hours)
Neisseria gonorrhoeae	43069	Chocolate agar	35-37	5% CO_2	0, 24
Haemophilus influenzae	10211	Chocolate agar	35-37	5% CO_2	0, 24, 48
Streptococcus pyogenes	19615	5% sheep blood agar	35 -37	5% CO_2	0, 24, 48
Streptococcus pneumoniae	6305	5% sheep blood agar	35-37	5% CO_2	0, 24, 48
Pseudomonas aeruginosa	27853	5% sheep blood agar	35-37	O_2	0, 24, 48
Bacteroides fragilis	25285	5% sheep blood agar	35-37	Ano_2	0, 24, 48
Peptostreptococcus anaerobius	27337	5% sheep blood agar	35-37	Ano_2	0, 24 48
Fusobacterium nucleatum	25586	5% sheep blood agar	35-37	Ano_2	0, 24, 48
Propionibacterium acnes	6919	5% sheep blood agar	35-37	Ano_2	0, 24, 48
Prevotella melaninogenica	25845	5% sheep blood agar	35-37	Ano_2	0, 24, 48

Swabs

A. Copan ESwab flocked swab
B. Puritan HYDRAflocked swab
C. Puritan multilength flocked swab
D. Puritan macrofoam swab

Inoculum preparation:

1. Each test organism was reconstituted from a lyophilized ATCC culture and subcultured twice to chocolate agar or 5% sheep blood agar plates.
2. A fresh 18 -24 hour culture of each bacterial strain, (48 hour for anaerobes) was used to prepare inoculum suspensions that matched 0.5 McFarland turbidity standard (~ 1.5×10^8 cfu/ml) prepared in 0.85 % sterile physiologic saline (pH 6.8-7.2) using a DensiCHEK™ turbidity meter (bioMerieux).
3. This suspension was further diluted 1 log10 in 4.0ml of sterile saline to obtain a working suspension of ~ 1.5×10^7 cfu/ml.
4. The DensiCHEK™ turbidity meter was first validated before actual testing by preparing five log10 serial dilutions in 4.0 ml saline tubes from the working suspension and plating out 100 µl in duplicate.

Inoculum procedure:

1. 100µl aliquots of the working suspension were transferred into 27 wells of a microtitre plate using an Eppendorf pipette. Twenty seven swabs of each type were placed into the wells and allowed to absorb the inoculums for approximately 15 seconds with a gentle twist to aid in the absorption before inserting into their respective transport device.
2. Each microorganism/device combination was performed in triplicate for each time period (0, 24 and48 hr).

Viability colony counts:

1. After the designated incubation period, each transport device with the swab inside was vigorously vortexed for 15 seconds, expressing all the liquid by rotating the swab on the inside of the transport device.
2. Just prior to inoculation the transport device was vortexed for another 5 seconds and, 100µl aliquots of each dilution pipetted on to appropriate culture media and spread over the entire surface of the plate with a sterile bent rod.
3. Inoculated culture plates were incubated in the appropriate atmospheric condition for optimal growth. Results for the triplicate plate culture were counted and averaged.
4. The zero hour swabs were performed within 20 minutes after returning them to their transport devices.

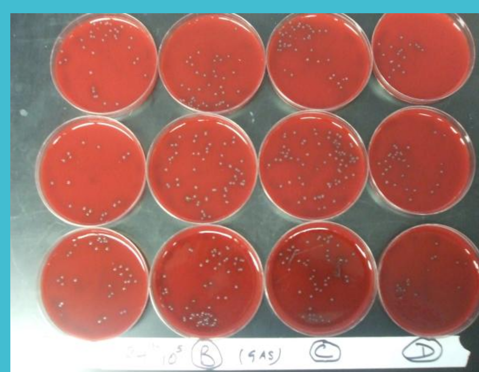
Results

Interpretation

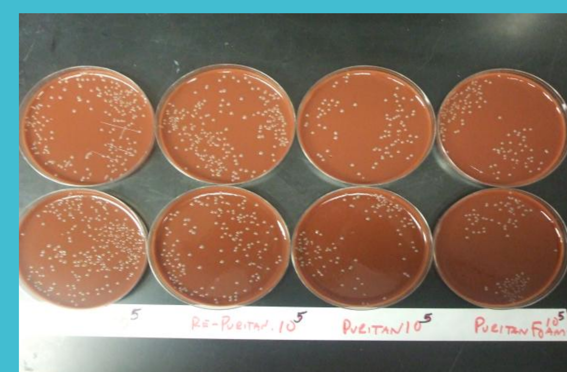
The M40-A document indicates acceptable recovery as ≥ 5 colonies recovered following a specific holding time from the specific dilution that yields a baseline count closest to 300 colonies.

Discussion

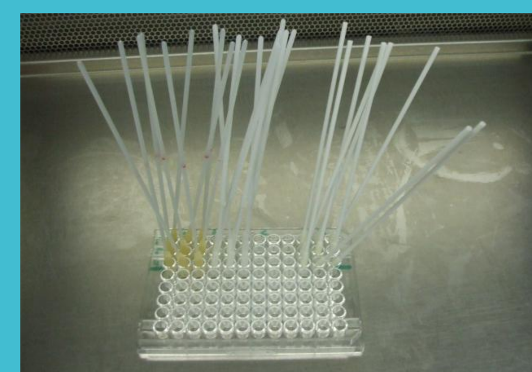
Photos 1 and 2 highlight how the absorption and release of the different swabs at zero hour appear. More in depth quantitative results are shown in Tables 1 & 2.



Streptococcus pyogenes zero hour count



Haemophilus influenzae zero hour counts



Swabs A, B, and D in wells

Copan swab inoculum with particulate before streaking

Recovery of aerobic and anaerobic microorganisms from 4 swabs types in Colony Forming Units/ mL (CFU/mL) at room temperature.

Table 1

Organism	Swab type	0 h	24 h	48 h
N. gonorrhoeae	A	381	27	0
	B	167	2	0
	C	274	5.3	0
	D	287	2.3	0
H. influenzae	A	248	49	1.66
	B	266	17	0.66
	C	101	10	1
	D	125	12	1.33
S. pyogenes	A	116	29	7.3
	B	159	57	24
	C	97	59	2.3
	D	82	21	2
S. pneumonia	A	281	5	1.3
	B	263	22.3	5.6
	C	196	29.3	11.3
	D	258	40.6	18.3
P. aeruginosa	A	Tntc	Tntc	Tntc
	B	Tntc	Tntc	Tntc
	C	Tntc	Tntc	Tntc
	D	Tntc	Tntc	Tntc

Table 2

Organism	Swab type	0 h	24 h	48 h
P. anaerobius	A	116	0.6	0
	B	86.3	1.3	0
	C	92.3	2	0
	D	73.6	2.3	0
B. fragilis	A	74	100	≥1000
	B	56.6	8	13.6
	C	42	6.5	101
	D	62.3	10	115
F. nucleotum	A	79	66.6	55.3
	B	110.6	39	12
	C	90.6	36.6	37.5
	D	86.3	43	16.6
P. acnes	A	164	103.3	106.6
	B	124	131.6	108
	C	93	92	87
	D	87.3	66	62.3
P.melaninogenica	A	161.6	8.3	0
	B	142	10.6	0
	C	96.6	6.3	0
	D	136.6	2.3	0

Tntc (Too numerous to count) Swabs: **A** - Copan. **B,C,D** – Puritan .

We tested the ten organisms recommended in the M40-A document only once and found each organism behaved differently. In the aerobic group, some organisms such as *P. aeruginosa* grew greater than the zero hour count. Others, such as *Neisseria gonorrhoeae* die off quickly and did not survive the 48 hour incubation at room temperature. In the literature, *H. influenzae* has been reported as having a "spike" or increase in colony counts after 24 hours incubation but to then start dying off by 48 hours, The results of our study did not show this pattern for *H. influenzae.*

In the anaerobic group, *B.fragilis* demonstrated erratic behavior with the Copan swab with a marked increase in growth at 48 hours. Van Horn et al. also observed this at 24 hours compared to zero hour colony counts.

In this study the zero hour results are similar visually. The Copan swabs are yellow brown in colour, as seen in photo 3, and appear to release some particulate matter in the Amies broth which is then transferred on to the culture media as seen in photo 4.
In seven out of ten organisms studied, the Copan swabs appear to have produced a greater colony count at zero hour possibly due to this coating. All three Puritan swab types are white in colour, as seen in photo 3, and do not transfer anything in the broth or onto the culture plates.

Conclusion

The absorption and release capabilities of all four swab types appears to be comparable. The M40-A requirements for the anaerobic organisms may be improved if the inoculum is adjusted to produce ~ 300 cfus instead of ~150 cfus at zero hour, if the inoculation preparation procedures were performed under anaerobic conditions, and if a larger volume of saline was used instead of the 4.0ml as currently recommended. Further studies are warranted using more isolates both clinical and reference .

References

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