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Comparison of physical characteristics and collection and elution performance of clinical swabs

Kathryn H Harry[1], Julie C Turner[1] and Kunapuli T Madhusudhan[2]*

[1]Wake Forest University Health Sciences, Winston-Salem, NC 27157, USA.
[2]559 Stafford Estates Drive, Salisbury, NC 28146, USA.

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Diagnostic sensitivity of a clinical test is a function of the number of organisms collected and eluted from the swab head that is attributed to the type of fibers and their physical characteristics. To relate these properties, water and protein absorption of nylon flocked, rayon flocked, and HydraFlock® and macrofoam swabs manufactured by Puritan Medical Products, and nylon flocked swab made by Copan Diagnostics were determined. The water and protein absorption capacities of whole swabs ranged from 17 to 21.5% and 13.6 to 19.6%, respectively. The HydraFlock® swab exhibited the highest water and protein absorption capacities in the group. Among the swab tip materials tested, macrofoam exhibited the highest water absorption capacity. Zeta potential of flocked swab fibers as a function of pH showed differences in charged groups depending on fiber types. The collection and release of bacteria with swabs using suspensions of clinically important pathogenic bacteria demonstrated the highest (69%) overall recovery by HydraFlock® swab and macrofoam swab ranked second (62%). The recovery of Gram-negative bacteria was significantly higher compared to Gram-positive bacteria across the swab types. The results of the present study indicate potential superiority of HydraFlock® swab over other swabs in a clinical setting and by collecting and releasing significantly larger numbers of bacteria.

Key words: Flocked swabs, water absorption, zeta potential, and pathogenic bacteria.

INTRODUCTION

The detection efficiency of proteins, nucleic acids, viruses, cells, and cellular parts depends on the collection efficiency of various pre-analytical devices. Due to the ease of use, swabs are commonly employed for the collection of cellular samples; although swabs are inefficient sampling devices compared to aspirates and biopsies for a successful primary culture (Nelson et al., 2006; Walsh et al., 2008). As the diagnostic sensitivity for a given clinical test varies with the number of cells collected and released by the sampling devices such as swabs, there is a constant need for proper specimen collection for reliable and accurate diagnosis. Swabs made of spun fibers such as rayon, Dacron or cotton shaped to form the swab tip, are popular for clinical and environmental applications. It is estimated that a small fraction of the organisms collected on traditional spun fiber swabs can be recovered. For example, Rose et al. (2004) demonstrated that the macrofoam swab, a non spun fiber swab that has cellular structure, recovered ≥ 30% more of *Bacillus anthracis* Sterne spores than rayon and polyester swabs, which is attributed to the entrapment of organisms between fibers. In addition to the physical structure, the chemical composition of swab material also influences recovery. Dalmaso et al. (2008) also reported significant differences between the nylon flocked swab and spun rayon swab in their recovery capacity of various bacteria and fungus. Additionally, toxicity of cotton swabs (Ellner and Ellner, 1966) to some of the delicate organisms and inactivation of organisms on the swabs was attributed to the chemical components

*Corresponding author. E-mail: ktmadhusudhan2000@yahoo.com. Tel: 515-710-5695.

present in the swab material or the handle (Pollock, 1947; Perry et al., 1997). Furthermore, the ability of different types of swabs to hold and maintain moisture to prevent the death of organisms on the swab has also been considered a very critical factor, because there is considerable delay from the point of collection to sample processing in the laboratory, which may lead to desiccation of cells on the swab dramatically decreasing their survival (Roelofsen et al., 1999). The fact that *S. aureus* can withstand desiccation (Chaibenjawong and Foster, 2011) whereas *N. gonorrhoeae* is very sensitive to dehydration (Harvey et al., 2007) reveals differences in the ability of various bacteria to survive on dry surfaces. To prevent cellular death on swabs and also to minimize the overgrowth of commensals that coexist with the test organism, transport media are used (Miller and Holmes, 1999). An ideal swab would collect many cells and allow for their release into media, while demonstrating conformity to the directive requirements for the intended use as specified by the manufacturer.

As the fibers used in the swab head are in direct contact with the organism to be recovered from the sampling site, the construction of the swab also plays an important role. An efficient swab design enhances the amount of diagnostic specimen recovered. This is of paramount importance in view of the myriad of different pathological agents that mediate disease conditions; a need exists to efficiently collect a clinical sample for accurate diagnosis. Additionally, other important parameters often considered for swabs include the ability to preserve the target without affecting diagnostic method sensitivity, while maintaining the viability of organisms. The purpose of this investigation was to determine the physicochemical characteristics, evaluate the performance of nylon flocked, rayon flocked and HydraFlock® swabs, plus the macrofoam swab manufactured by Puritan Medical Products (PMP), and compare them to the nylon flocked swab of Copan Diagnostics, Inc (Copan). To accomplish this objective, collection and release performance of test bacteria by swabs was compared and correlated to physical characteristics of swab head material, namely water/protein absorption and zeta potential.

MATERIALS AND METHODS

Bacteria, swabs and media

Swabs from freshly manufactured lot numbers were obtained for the study. HydraFlock®, macrofoam, nylon flocked, and rayon flocked swabs were provided by Puritan Medical Products, LLC., Guilford, ME. Nylon flocked swabs of Copan Diagnostics, Inc. was obtained through VWR International. Pre-poured Tryptic Soy Agar II containing 5% sheep blood (SBA) and Chocolate II Agar were from BBL™. Pre-reduced Anaerobic Sterilized (PRAS) Dilution Blanks were from Anaerobic Systems, Morgan Hill, CA. Anaerobic Indicator (BR0055B) and AnaeroGen were from Oxoid, UK. Bovine serum albumin (B4287) was obtained from Sigma-Aldrich. *Streptococcus pyogenes* ATCC 19615, *S. pneuomoniae* ATCC 6305, *Staphylococcus aureus* sub sp. *aureus* ATCC 25904, *Hemophilus influenza* ATCC 49247, *Neisseria gonorrhoeae* ATCC 43069, *Bacteriodes fragilis* ATCC 25285 and *Peptostreptococcus anaerobius* ATCC 27337 were obtained from ATCC, Manasas, VA. The source for the 0.5 McFarland standard and 1% (w/v) Microbeads (1 µm) suspension were PML Microbiologicals, Wilsonville, OR and Polysciences, Warrington, PA, respectively. All chemicals used in the study were of reagent grade.

The composition of the GC broth without supplements was 15 g of Bacto protease peptone (# 3), 4 g of K_2HPO_4, 1 g of KH_2PO_4, and 5 g of NaCl in 1 liter of distilled water (pH 7.2). GC broth was sterilized by autoclaving.

Absorbance studies

To measure the fluid absorption, each pre-weighed swab was immersed in 1 ml of distilled water for 15 s. Following the water absorption, the swab was removed, and absorption values were determined by weighing the wet swab to compute percent water absorption (N=3). To determine the protein absorption, a 22% bovine serum albumin solution was used instead of water and the test repeated with fresh swabs. To determine the water absorption of the swab head material, the swab tip material (~0.5 g) was removed from the swab shafts using a scalpel blade. A pre-weighed quantity of the swab tip material was transferred into a 1.7 ml micro-centrifuge tube. Following the addition of 1 ml of distilled water, the tube was shaken at ambient temperature for 1 min to facilitate water absorption. The tube containing the wet swab tip material was centrifuged for 5 min at 10,000 rpm, followed by the removal of unbound water using a bibulous paper, before the mass of the wet fibers was determined (Nugent et al., 2007). This process was repeated twice and the percent water or protein absorption was computed.

Scanning electron microscopy (SEM) measurements

To study the ability of swabs to collect bacteria in a model system, swab tips were immersed in 1% polystyrene beads (1 µm) suspension (Microbead) for 15 s, then removed, air-dried for 45 s and prepared for SEM. To measure the release of bacteria in the same model system, swab tips adhering the polystyrene beads (as described above) were washed by placing them in 2 ml of distilled water, vortexed at high speed for 10 s, and dried before preparing for SEM examination (Nugent et al., 2007). To prepare for SEM, swabs harboring polystyrene beads were subjected to critical point drying under reduced helium pressure at 230 Pa for 20 min followed by sputtering of a gold-palladium layer on it, using a DC plasma sputtering machine (Pathan et al., 2010). A Hitachi S-3200 Variable Pressure high resolution thermionic SEM, which allows control of the specimen chamber vacuum level and the environment, was used for this purpose. Revolution SEM, an active digital imaging system, was incorporated into the SEM to accurately control the beam and image acquisition for obtaining sharp digital images. Electron photomicrographs were obtained at various magnifications (100X to 3,000X). SEM photomicrographs of swab tips were recorded to examine the physical structure of swab fibers.

Zeta potential (ξ)

For measuring the ξ of swab head fibers, swabs were carefully shaved with sterile razor blades and size reduced. The fibers were suspended in 0.001M KCl solution and ξ of fibers in the pH range of 3.0 to 9.0. The ξ was measured using cylindrical cells with SurPASS Electrokinetic Analyzer (Anton Paar) which has integrated titration unit for automatic pH adjustment and automated ξ

Table 1. Culture media and conditions used in the study

Organism	Dilution medium	Culture medium	Culture condition
S. aureus *S. pneumoniae* *S. pyogenes*	0.85% saline	Sheep blood agar	37° ± 1° C for 18-24 h, 5% CO_2
H. influenzae *N. gonorrheae*	GC broth without supplements	Chocolate agar	37° ± 1° C for 18-24 h, 5% CO_2
B. fragilis	PRAS Dilution Blanks	Sheep blood agar	37°± 1° C for 18-24 h, anaerobic
P. anaerobius	PRAS Dilution Blanks	Sheep blood agar	37°± 1° C for 48 h, anaerobic

measuring ability. About 200 mg of flocked fiber was packed into the glass cylindrical cell, rinsed with KCl at a maximum pressure of 400 mbar for 3 min before measurement of streaming potential at 400 mbar for 20 s. Each ξ measurement was performed in two directions and two replicate measurements were done in each direction to generate one data point at a given pH. The instrument was calibrated according to the manufacturer's recommendations.

Culture studies

Well-isolated colonies from each of the bacterial strains were streaked for isolation on SBA with the exception of *H. influenzae* and *N. gonorrhea*, which were streaked on chocolate agar. After the incubation of cultures at 37°± 1° C for 18 to 24 or 48 h (for *P. anaerobius*), several well-isolated colonies were selected and transferred using a nylon flocked swab pre-wet with 0.85% NaCl to a tube containing 5 ml of 0.85% sterile saline (pH 6.8) in a glass test tube and vortexed to prepare a cell suspension. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard (1.5×10^8 CFU/ml) either by adding sterile saline or by adding more bacterial growth. The first $\log_{10}$ dilution was made in saline to obtain a bacterial suspension containing ~10^7 CFU/ml.

A fresh inoculum of each test organisms was prepared prior to the performance of each set of experiments. For culture studies, the test swab was placed in 5 ml of bacterial suspension (~10^7 CFU/ml) in a glass test tube for 10 s to allow liquid absorption and the adherence of bacteria to the swab head. Due to the porous structure of the macrofoam, the swab head was pressed against the tube wall to expel air bubbles after placing it into the bacterial suspension. Bacterial adhesion to the macrofoam swab was achieved by placing it in the tube for 10 s after air expulsion. The swabs were removed from the bacterial suspension, held in air for 45 s, transferred to the dilution medium, and vortexed for 15 s to release the bacteria. This was followed by serially diluting the viable organisms in the dilution medium (Table 1). Quantitation of viable organisms was done by spreading samples in duplicate for each of the dilutions on the culture media (Table 1) followed by enumeration.

All the operations described above were performed at ambient temperature unless otherwise specified. Due to the obligate anaerobic nature of *P. anaerobius,* all dilutions were performed at ~ 4°C to minimize the metabolic activity of bacteria. The whole procedure was completed within 30 min, to prevent the loss of the organism's viability in the inoculum prior to culturing. All bacteria except *B. fragilis* and *P. anaerobius* were cultured under 5% CO_2 as shown in Table 1. For culturing *B. fragilis* and *P. anaerobius,* AnaeroGen sachets were placed in an anaerobic jar according to the manufacturer instructions to create an anaerobic environment, reducing oxygen level in the jar to < 1%, and maintaining CO_2 levels from 9 to 13%. The anaerobic condition was checked with an anaerobic indicator strip prior to placing the cultures in the anaerobic jar according to the manufacturer recommendations. The anaerobiosis is indicated by a visual change of the indicator from pink to white.

The number of organisms (CFU/ml) recovered was expressed as an average for the duplicates at a given dilution (A). The number of viable organisms (CFU/ml) present in the bacterial suspension (~10^7 CFU/ml) was also determined after performing serial dilutions and enumeration of viable organisms at each dilution (B). Average water absorption capacity of the swab in milliliters (C) as well as the average dry weight of the swab (D) was used also in the computation of percent recovery. Appropriate negative controls were run in parallel. Ten swabs of each swab type were used to determine the recovery of each organism. A summary of culture media and conditions used in the study is given in Table 1.

$$\text{Recovery (\%)} = \frac{\text{Dilution Factor x A}}{\text{Dilution Factor x B x C x D}} \times 100$$

Quality control

All microbiological tests are quality controlled to guarantee that the result is true and comparable to other studies conducted in a similar manner. Lyophilized cultures of the test organisms were rehydrated according to the manufacturer's instructions, streaked on appropriate media to obtain isolated colonies, and sub-cultured at least twice. The identity of the organisms was also confirmed by Gram staining and observation of the bacterial morphology under the microscope at 1,000X magnification. Necessary in-house QC measures were instituted throughout the study to maintain the study quality (CLSI, 2003; CLSI, 2004).

Data analysis

Statistical analysis of data was done by using JMP-7 (SAS Institute, Cary, NC). The *p*-values were computed by Analysis of Variance (ANOVA) and then the test of significance was applied ($\alpha = 0.05$).

Table 2. Comparison of the mean water and protein absorption capacities of swabs and swab head material*.

Swab type	Water absorption, % by:		Protein absorption, % by
	Whole swab	Swab head material	Whole swab
HydraFlock®	21.5 (A)	624 (A)	19.6 (A)
Macrofoam	19.8 (AB)	662 (A)	13.6 (C)
Rayon flocked	17.1 (B)	558 (AB)	16.1 (BC)
Nylon flocked (PMP)	19.0 (AB)	606 (A)	18.4 (AB)
Nylon flocked (Copan)	19.7 (AB)	486 (B)	16.5 (ABC)

*Letters in parentheses denote significance. Levels not connected by the same letter are significantly (p<0.05) different.

In this approach, the independent variable, Y = % recovery, X = swab, Gram reaction, or aerobe/anaerobe. Statistical significance of the difference between means were compared by Tukey-Kramer Honestly Significant Difference test, an exact α-level test (as sample sizes are the same), and the traditional letter-coded report is reported, where means not sharing the same letter are significantly different (Sall et al., 2007).

RESULTS

Absorbance studies

The water absorption capacity of all swabs ranged from 17.1 to 21.5%. Among the group, the HydraFlock® swab exhibited the highest water absorption and its ability to retain water was significantly greater than the rayon flocked swab (Table 2). Differences in water absorption among macrofoam, and nylon flocked (Copan and PMP) swabs were minimal. In addition to measuring the water absorption capacity of the whole swab, swab head material (fibers or foam) were separated to determine their water absorption, as described under the Materials and Methods. Determination of the water absorption of the swab head material is necessary, because the weight of the swab head material is a fraction of the weight of the swab (<1%); therefore, water absorption of the whole swab is not a true reflection of the physical property. This is corroborated by significant differences in water absorption capacities between whole swabs and swab head material (Table 2). For example, HydraFlock® swabs and rayon flocked swabs had the highest and lowest water absorption capacities, respectively with nylon flocked (Copan) swab in the middle, when whole swab was used. On the other hand, macrofoam and nylon flocked (Copan) ranked the highest and lowest, respectively with nylon flocked (PMP) in the middle, when the swab head materials (fibers or foam) were used for the water absorption measurements (Table 2).

The protein absorption capacity of all swabs ranged from 13.6 to 19.6%. Among the group, the HydraFlock® swab exhibited the maximum protein absorption. Its ability to retain protein was significantly different from rayon flocked and macrofoam swabs (Table 2). Differences in protein absorption among HydraFlock® and rayon flocked or macrofoam swabs were significant (Table 2). The data demonstrates that the HydraFlock® swab is about 46% more protein absorptive than the macrofoam swab.

SEM measurements

Capture and release of polystyrene beads was studied and qualitatively evaluated using a model system to simulate bacteria. Swabs were dipped in a polystyrene bead suspension and SEM photomicrographs of swab heads were recorded before and after washing. A close examination of several SEM photomicrographs revealed the superior ability of the HydraFlock® swab to collect beads; although, the fractional release of beads was comparable to or less than the other swabs studied (Figure 1 C and D). On the other hand, the efficiency of bead collection for the macrofoam swab was about equal to the release of beads (Figure 1 E and F). SEM images of the swab tips without dipping in polystyrene suspension were also recorded to examine the physical structure of the swab head material at higher magnification. At 1,000X magnification, we were able to visualize several stalks of bundles of fibers in the HydraFlock®, each consisting of approximately 25 fibrils that open as a tuft at the end (Figure 1A and B), resembling a Hydra (simple fresh-water animal). The length of each fibril of the tuft was variable and ranged from ~ 15 to 40 µm. SEM photmicrographs of rayon flocked swab showed ridges along the fiber length (Figure 1 G and H) in contrast to the smooth surface of the nylon flocked swab fibers. Furthermore, the SEM photomicrographs obtained at 20 µm and 5 µm magnifications revealed that the diameter of fibers of the nylon flocked swab of Copan was slightly higher than the diameter of fibers of both rayon flocked swab and nylon flocked swab manufactured by PMP (figure not shown). Thus, SEM experiments provided some important structural details of the swabs.

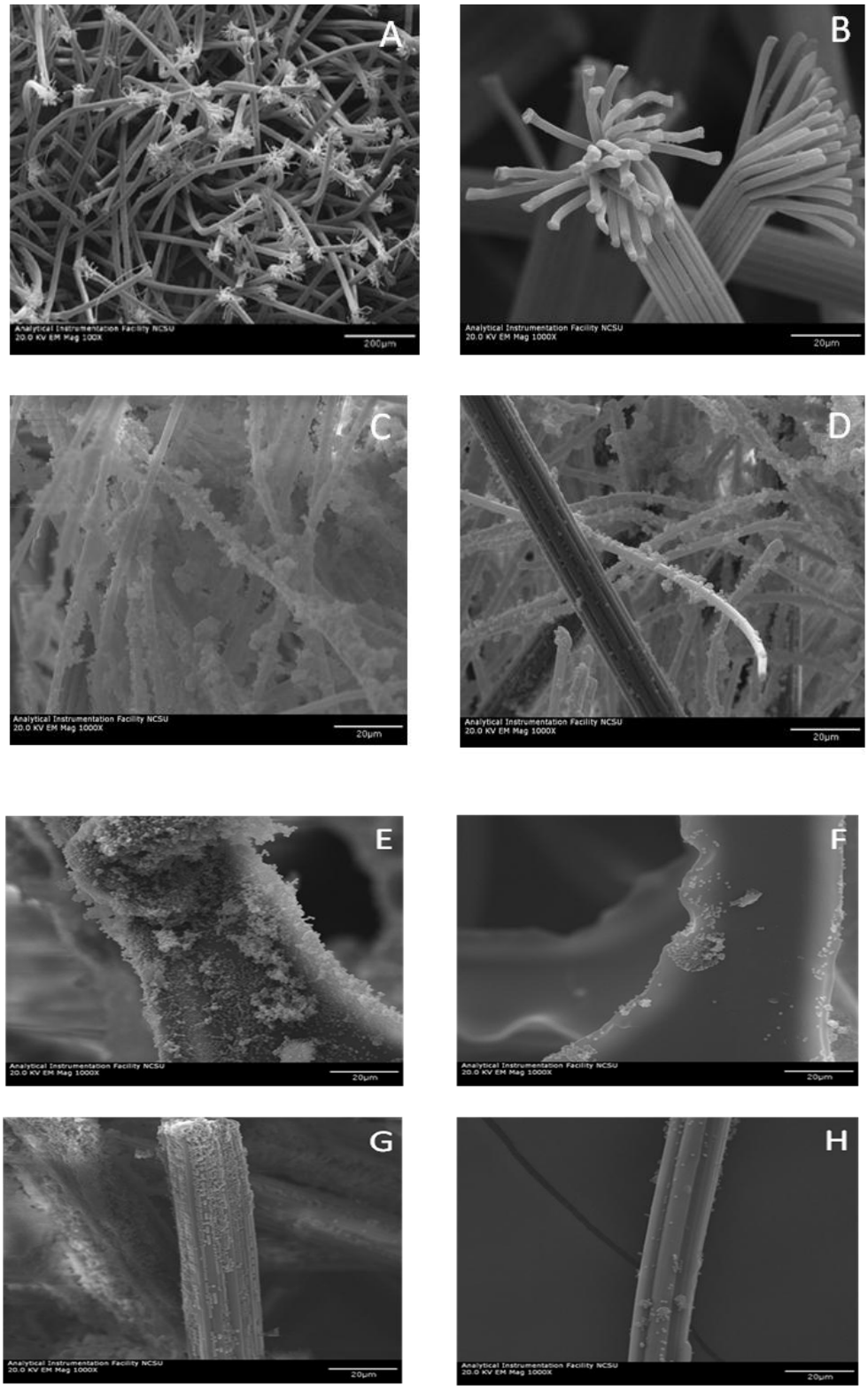

Figure 1. SEM photomicrographs of unwashed and washed swabs after dipping in a polystyrene beads suspension depicting the collection and release of bacteria in a model system. The magnification of photomicrographs of all swabs is shown. The photomicrographs of HydraFlock® swab at 100X (A) and 1,000X (B) magnifications 3 and note HydraFlock® swab tip has flower like appearance. C, Unwashed HydraFlock® swab; D, Washed HydraFlock® swab; E, Unwashed macrofoam swab; F, Washed macrofoam swab; G, Unwashed rayon flocked swab; H, Washed rayon flocked swab.

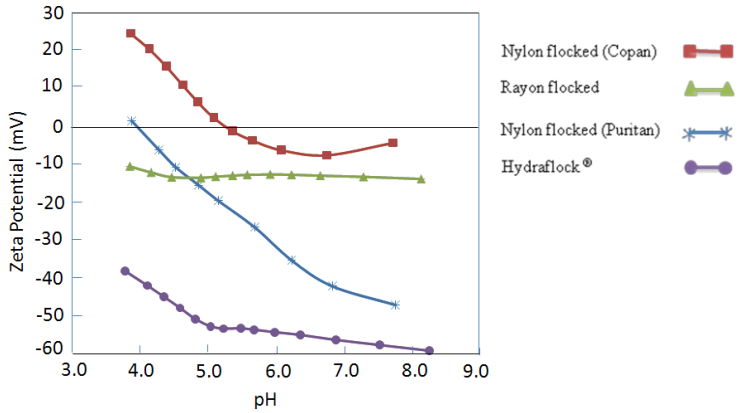

Figure 2. Zeta potential (streaming potential) of swab head fibers as a function of pH in 0.001M KCl solution.

Table 3. Comparison of the mean percent recovery of test organisms by various swabs*

Analysis criteria	Percent recovery by swab				
	HydraFlock®	Macrofoam	Rayon flock	Nylon flocked (PMP)	Nylon flocked (Copan)
Pooled data-by swab type	69 (A)	62 (AB)	60 (AB)	57 (B)	53 (B)
Gram negative bacteria-by swab type	74 (B)	86 (A)	84 (A)	84 (AB)	56 (C)
Gram positive bacteria-by swab type	65 (A)	43 (BC)	41 (BC)	37 (C)	50 (B)

*Letters in parentheses denote significance. Levels not connected by the same letter are significantly (p<0.05) different.

Zeta potential

The ξ of various flocked swab fibers at a single conductivity value (0.001M KCl) as a function of pH is shown in Figure 2. The ξ of fibers from nylon flocked, rayon flocked and HydraFlock® swabs of PMP exhibited negative values where as nylon flocked fibers of Copan demonstrated positive ξ in the pH range of 3.8 to 5.1. The ξ of rayon flocked fibers was invariant with pH change; however, fibers of nylon flock and HydraFlock® swab of PMP showed increasing negative ξ with increasing pH in the range of 5 to 8 although their magnitude was different. The ξ of nylon flocked fibers of PMP increased from -18 to -48 with increase in pH from 5 to 8 although nylon flocked fibers of Copan demonstrated no dramatic increase in ξ in the same pH range. The change in ξ of HydraFlock® swab fibers was also marginal with increase in pH from 5 to 8.

Culture studies

To examine differences in swab performance, the recovery of bacteria from all swab types was pooled and an ANOVA was performed using the approach, Y = % recovery, X = swab, Gram reaction, or aerobe/anaerobe. The null hypothesis (H_0) is that all swabs perform equally; all organisms are recovered at the same rate irrespective of their Gram reaction or aerobic/anaerobic nature. The recovery of all organisms from swabs was pooled prior to analysis by swab type to rank overall swab performance to recover organisms. In the present study, the HydraFlock® swab (69%) and nylon flocked (Copan) swabs (53%) had the highest and the lowest recovery of all viable bacteria, respectively with significant differences (Table 3). The macrofoam swab with 62% recovery ranked the second place. Analysis of recovery from the pooled data, by organism, demonstrated the highest and lowest recovery values for *N. gonorrhoae* (83%) and *S. pyogenes* (37%), respectively showing a dramatic difference. The study consisted of three Gram-negative (*N. gonorrhoeae*, *H. influenzae*, and *B. fragilis*) bacteria and four Gram-positive (*P. anaerobius, S. aureus, S. pneumonia*, and *S. pyogenes*) bacteria. The combined recovery of Gram-negative and Gram-positive bacteria, each by all swabs, was 77 and 47%, respectively and the differences among both groups were also significant. Among the Gram-negative bacteria, recovery of *B. fragilis* was significantly lower and different from the recovery of *N. gonorrhoeae* and *H. influenza.* However, there were no significant differences in recovery of *N. gonorrhoeae and H. influenzae.* Among Gram-positive bacteria,

Table 4. Comparison of mean percent recovery of each test organism by various swabs*.

Analytical criteria	*N. gonorrhoeae*	*H. influenzae*	*B. fragilis*	*P. anaerobius*	*S. aureus*	*S. pneumoniae*	*S. pyogenes*
Percent recovery	83 (A)	79 (A)	68 (B)	57 (C)	55 (C)	40 (D)	37 (D)

*Letters in parentheses denote significance. Levels not connected by the same letter are significantly (p<0.05) different.

recovery of *P. anaerobius* and *S. aureues* was signify-cantly higher (57%) than the recovery of *S. pneumoniae* (40%) and *S. pyogenes* (37%) combining recovery from all the swabs.

Except for *B. fragilis* and *P. anaerobius*, five test organisms in the study are aerobes. Among the aerobic organisms, the recovery of *N. gonorrhoae* (83%) and *S. pyogenes* (37%) was the highest and lowest, respectively (Table 4). The overall recovery of aerobic and anaerobic bacteria by all swabs showed no significant difference and it was 59 and 62%, respectively. Among anaerobes, recovery of *B. fragilis* (68%) was higher than *P. anaerobius* (57%), and the differences were significant (Table 4).

Examination of the recovery of Gram-negative bacteria by various swabs (Table 3) revealed the highest (86%) and the lowest (56%) recovery by macrofoam and nylon flocked (Copan) swabs, respectively; the differences were statistically significant. Examination of the recovery of Gram-positive bacteria by various swabs (Table 3) revealed the highest (65%) and the lowest (37%) recovery by the HydraFlock® swab and nylon flocked swabs, respectively. It is noteworthy, that recovery by the HydraFlock® swab is significantly different from the rest. The recovery of various Gram-positive bacteria by nylon flocked (Copan) was not significantly higher than recoveries obtained with macrofoam and rayon flocked swabs. Finally, the recovery was analyzed by organism and by swab type, so that swabs exhibiting higher recovery of viable organisms can be recommended for specific applications (data not shown). From this data, it can be concluded that the swabs exhibiting the highest and the lowest recovery of *H. influenzae*, *N. gonorrrhoeae*, and *P. anaerobius* were macrofoam and nylon flocked (Copan), respectively. Additionally, nylon flocked (PMP), HydraFlock®, and rayon flocked were superior to nylon flocked (Copan) in recovering *B. fragilis.* The HydraFlock® swab recovered the largest number of viable *S. aureus* and *S. pyogenes.* The recovery of *S. pneumoniae* was the highest with nylon flocked (Copan).

DISCUSSION

Specimen collection and transportation has been often overlooked or undervalued; even though they have been critical components of a quality health care system. Careful evaluation of swab systems is important by understanding product limitations, as well as, challenges to obtain a good clinical specimen that yields best diagnostic result during laboratory analysis. For specimen collection, aspirates of fluids and exudates or infected tissues from suspected/infected sites are superior to samples collected on swabs (Brook, 1987; Perry et al., 1997). However, because of the ease of using swabs, swab specimens represent a significant percentage of diagnostic samples processed in clinical microbiology laboratories (Perry et al., 1997). Careful evaluation of swab transport devices for their ability to maintain viability of bacteria during transit to the laboratory is important to ensure delivery of quality specimens.

In the initial phases of the study, physical characteristics of the swabs studied that could potentially influence the collection and release of bacteria were evaluated. In this part, we compared the ability of each swab to collect and release a group of 7 different types of bacteria. The water absorption measured in this experiment includes total (free and bound) water which is dependent on the chemical nature of fibers and surface coatings. It is known that two-dimensional structures made of synthetic fibers, for example polyamide or polyester fibers and filaments, have a poor absorptivity; therefore, storing low amounts of water. On the other hand, fiber materials can be rendered hydrophilic by applying binders carrying hydrophilic groups (-OH or -COOH groups) onto them or adding water-insoluble cellulose ethers (> 50% by weight). Addition of such functional groups results in higher water absorption and retention of large amounts of bound water (U.S. Patent No. 3,965,091; U.S. Patent No. 4136218). Water absorption capacity of a swab plays an important role to extract microorganisms from the collection site by the capillary action and prevents dehydration of organisms. From the absorption data, it is expected that the HydraFlock® swab will have better extraction performance than the other swabs, especially the rayon flocked swab. Water absorption by swab fibers is determined by the chemical composition, microstructure, and surface polarity. These properties may influence other physical properties, such as ξ by replacing electrolyte ions (Bismarck et al., 2002; Bellman et al., 2004). Furthermore, water absorption of swab fibers might play an important role in protecting bacteria because desiccation is a leading cause of death of bacteria on swabs (Roelofsen et al., 1999).

Protein absorption by swabs is likely to contribute to

increased bacterial adsorption when they are used to collect specimens from wounds, mucous membranes, blood, etc., because body fluids contain water and variable amount of protein. Furthermore, bacteria contain appendages, such as pili which are comprised of proteins and used for adhesion, attachment to receptors, conjugation, etc (Forbes et al., 2007) and these proteins may be easily adsorbed to the swab fibers. Protein and water absorption capacity of swabs can be improved by imparting hydrophilic functional groups (such as –OH, -COOH, $-NH_3$, etc). Charged functional groups on the surface of microbial sampling devices are important for attraction and adhesion of bacterial cells based on the observations that electrostatic interaction is an important factor in bacterial adhesion to surfaces (Terada et al., 2005; Terada et al., 2006). We measured streaming potential of swab fibers as a function of pH to understand the charge and adsorption properties under fixed conductivity conditions to derive meaningful conclusions because some researchers demonstrated potential changes in ξ at high electrolyte concentrations (Horvath et al., 2006; Cadena et al., 2009). Zeta potential of fibers was obtained based on streaming potential or streaming current detected by electrodes when a dilute electrolyte is circulated. Observation of significant ξ differences of nylon flocked swab fibers as a function of pH between PMP and Copan products is a hall mark of the study. The ξ of nylon flocked swab fibers of Copan transitioned from positive to negative (-5) when pH was increased from 5 to 7.8; however, nylon flocked swab fibers of PMP showed a constant increase of negative ξ (-18 to -48) in the same pH range. Such differences might reflect differences in chemical nature of fibers as well as surface treatments. The ξ describes the nature of the electrostatic potential near the surface of a particle and it is important in establishing whether the repulsive electrostatic potential barrier between neighboring particles is high enough to attract each other due to short-range attractive Van der Waals forces. Higher ξ of swab fibers will result in repulsion, possibly resulting in less entrapment of bacteria in between. No information on either ξ of swab fibers or linking ξ of swab fibers to viability or recovery of bacteria were reported in the literature although correlation between electrokinetic measurements of jute and banana fibers to swelling properties was established (Buschle-Diller et al., 2005). However, some studies proposed that ξ can also be used to predict microbial adhesion to swab fibers and water absorption, as well as, surface hydrophobicity to some extent (Iyer and Jayaram, 1971; Espinosa-Jimenez et al., 1997; Bismarck et al., 2002).

SEM photomicrographs revealed morphological and structural differences between various swabs. For example, differences in fiber diameter between Copan nylon flocked and PMP rayon flocked were evident. Additionally, we found interesting fiber organization for the HydraFlock® swab in the present study. The stalk portion of the HydraFlock® swab has bundles of fibrils and offers higher mechanical strength during the swabbing operation, while the tuft of fibrils at the end is likely to facilitate efficient collection of microorganisms.

The test organisms utilized in this study were those specifically prescribed in M40-A for establishing performance claims and quality control of swab transport systems. They include a representative panel of aerobes, anaerobes, fragile/non-fragile organisms and fastidious bacteria. We preferred to use the swab elution method, instead of the roll-plate method although the latter is widely used in clinical laboratorties. A limitation of the roll-plate method for bacterial viability performance testing is that it is not a quantitative method; it is, at best, a semi-quantitative method. Although the swab elution method does not reflect the standard protocol used in most clinical laboratories, it allows a quantitative measurement of the ability of a transport system to maintain viable organisms (Abiagom and VanHorn, 2002). Recovery of viable bacteria with various swabs under the test conditions were compared to each other. As the current study does not utilize a transport medium, our results cannot be compared to peer-reviewed published studies in which the researchers compared the performance of a bacterial transport system (containing swab plus transport medium) and bacterial recovery (or viability) studied as a function of time and temperature of incubation.

The results of the study revealed the highest recovery of all organisms by the HydraFlock® swab. Macrofoam ranked second place in recovery, while nylon flocked (Copan) ranked the lowest. The difference in recovery of all Gram-negative bacteria between any swab of PMP and the nylon flocked (Copan) swab was between 18 and 30%, under the test conditions suggesting the potential to use PMP's swabs for selective applications. The difference in recovery between the highest and the lowest recovery of PMP swabs was ~ 12%. It is interesting to note that Gram-negative bacterial infections represent one third of the microbiologically documented infections in febrile series of neutropenic patients (Glauser et al., 1997). The HydraFlock® swab recovered the largest number of viable *S. aureus* and *S. pyogenes*. The recovery of *S. pneumoniae* was the highest with nylon flocked (Copan). Excellent recovery of gonococci by all swabs (70 to 100%) observed in the study in comparison to the poor stability and viability of dilute suspensions of *N. gonorrhoeae* WP (T4) in various solutions and buffers reported by Norrod and Williams (1979) could be attributed to the use of GC broth for dilutions by us. It is interesting to note consistently lower recovery (<50%) of *S. pneumoniae* and *S. pyogenes* with all swabs used in the study (Table 4). Roelofsen et al. (1999) compared the recovery of various bacteria with viscose swabs (Copan) to polyurethane swabs (Becton Dickinson). The results revealed significantly higher recovery of various bacteria with polyurethane swabs than viscose swabs without

transport medium at zero hours.

It can be argued that some of the observed differences in the recovery of organisms from the test swab are attributable to a direct toxic effect of one of the components of the swab. Aero-tolerance by *B. fragilis* (Takeuchi et al., 1999; Baughn and Malamy, 2004) might have contributed to higher recovery of viable cells. The aero-intolerance of *P. anaerobius* (Carlsson et al., 1978; Rolfe et al., 1978) might have contributed to its lower recovery although utmost precautions were taken to maintain an anaerobic environment. In contrast to *B. fragilis*, the sensitivity of *Peptostreptococcus* sp to brief exposure of air was demonstrated (Bowler et al., 2001).

Under most conditions of routine clinical practice, a swab carrying pathogenic microorganisms is used to provide sufficient information to direct antimicrobial therapy. A comparative study of this nature, as described here, cannot be performed with actual clinical specimens without introducing uncontrollable variables. Clinical specimens exhibit variations in consistency and contain cellular and chemical constituents. Further, these constituents may act as nutrients or toxins derived from the body and poly-microbial flora has the potential to affect the organism's viability. The survival of test organisms reported in this study does not represent the results with actual clinical material or recovery rates; however, it allows comparisons of recovery rates to be made and most certainly permits insight into a swab's ability to sustain organism viability. An ideal swab is designed to collect many cells and allow for their release into media that needs to be verified by using scientifically competent and valid methods. Quantitative results obtained with swabs are similar to biopsy results, especially for wounds (Wheat et al., 1986). For example, a 75% concordance between the swab and biopsy specimens of peptic ulcers was observed (Sapico et al., 1986). Our method of collecting bacteria on the swab is one of the scientifically valid methods and offers a simple comparison of swabs' efficiencies. The results of the study reveal the number of viable bacteria released by each swab rather than the actual number of CFU present in the swab tip. It is important to note that the viability of all CFU in the swab cannot be guaranteed, because some organisms may have become nonviable, preventing colony formation on the agar media even after release. The test procedures employed for determining bacterial viability were based upon the quality control methods described in Clinical Laboratory Standards Institute M40-A (CLSI, 2003; CLSI, 2004) guidelines and can be compared to the performance characteristics of similar devices but not used in the present study. The performance of various flocked and macrofoam swabs of PMP were compared to the nylon flocked swab of Copan using scientifically competent and valid methods in a laboratory setting. A key finding of this study is the overall superior performance of HydraFlock® swab based on the high water and protein absorption capacities, and the high recovery of bacteria. Furthermore, the ability of the macrofoam swab to recover the largest number of *H. influenzae*, *N. gonorrrhoeae*, and *P. anaerobius* deserves attention. The overall performance of the nylon flocked (Copan) swab to recover viable bacteria did not surpass any one of the swabs manufactured by PMP used in this study. The overall highest recovery efficiency of various bacteria coupled with its high water absorption capacity with the HydraFlock® swab design is likely to have a greater effect on diagnostic sensitivity.

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