Julie C. Turner, M.S. 101 N. Chestnut St. Suite 101 Winston-Salem, NC 27101 336-397-1900 jturner@cleanearthtech.com Session ID: T89

THE CHARACTERIZATION OF THE ABSORPTION AND RELEASE PROPERTIES OF VARIOUS CLINICAL SWAB TYPES



Julie C. Turner, Kathryn Harry, Denene Lofland, and Kunapuli T. Madhusudhan CET, LLC., Winston-Salem, NC, USA

Abstract

An ideal swab must have the following properties: (1) the ability to absorb organisms from the infection site, (2) to maintain the viability of organisms during transport, prior to culturing, and (3) to allow the release of organisms from the swab into the appropriate media. The type and physical structure of fibers comprising the swab are important because they are in direct contact with the organism to be recovered. Diagnostic sensitivity varies with the number of organisms collected and released by the swab. The purpose of this study is to determine the absorption and release properties of Nylon Flocked, Rayon Flocked, Hydra Flock, and Macrofoam swabs of Puritan Medical Products and the Nylon Flocked swab of Copan Diagnostics.

Water and protein absorption capacities of these whole swabs ranged from 14% to 21% with the Hydra Flock swab exhibiting the highest absorption capacity. Next, the water absorption of the material of the swab was studied, by first removing it from the tip. Macrofoam tip material had the highest water absorption properties, followed by Hydra Flock. The water absorption capacity of a swab enables it to be moistened, which enhances the physical removal process of microorganisms from the swab.

A simulated qualitative study on the ability of swabs to absorb and release microorganisms was done. The swabs were dipped into a suspension of 1 μ m polystyrene beads, and then briefly washed. Pictures were taken using a scanning electron microscope before and after washing. The results of the study demonstrate the superior ability of Hydra Flock to absorb beads; however, the release was only comparable to or less than the other swabs. On the other hand, the Macrofoam swabs showed a high efficiency of both bead absorption and release.

The validity of the model study with polystyrene beads was verified experimentally by using suspensions of Hemophilus influenzae, Neisseria gonorrhoeae, Staphylococcus aureus, Streptococcus pneuomoniae, S. pyogenes, Bacteriodes fragilis and Peptostreptococcus anaerobius for the absorption and release of bacteria by the swabs. Statistical analysis (ANOVA) of the data was performed. The experimental results revealed that the Hydra Flock swab demonstrates a significantly superior recovery (69%) of all bacteria, while the Macrofoam swab had 62% recovery and the Nylon Flocked swabs of Puritan and Copan only recovered ~ 54%. Interestingly, the recovery of Gram-negative bacteria was significantly higher (68%-83%) than Gram-positive bacteria (37%-57%), across all swab types. However, no significant difference in the recovery of aerobic and anaerobic bacteria was found.

In summary, the results of the study demonstrate the overall superiority of Puritan Medical Products' Hydra Flock swab over other swabs in this study and its potential to increase the diagnostic sensitivity of clinical tests by collecting and releasing significantly larger number of bacteria. Additionally, the commonly-used Macrofoam swab also demonstrated its value to absorb and release bacteria by coming in a close second.

Materials and Methods

Puritan Medical Products

Copan Diagnostics Nylon Flocked

Nylon Flocked Rayon Flocked

Macrofoam

Hydra Flock

Absorbance studies

Water and protein absorption of entire swab

• Swab (N=3) was immersed in 1 ml of distilled water or 22% bovine serum albumin for 15 sec and percent water or protein absorption was determined after weighing the wet swab.

Water absorption of swab head material

- Using a scalpel blade, swab head material was removed from the swab shaft (N=8), transferred into a pre-weighed 1.7 mL tube and 1 mL of distilled water was added. To facilitate water absorption, tubes were shaken for 1 min at
- To remove the unbound water, tubes were centrifuged and excess water was removed to determine percent water

Scanning electron microscopy (SEM) measurements

- To simulate the collection of bacteria, swab tips were placed in a 1% polystyrene bead suspension (Microbead, μm), then removed and air-dried.
- To measure the release of bacteria in the same model system, swab tips adhering polystyrene beads (as before were placed in 2 ml of distilled water, vortexed at high speed for 10 sec, and air-dried. SEM images of untreated swabs were also done to record the surface morphology.
- A Hitachi S-3200 Variable Pressure SEM was used to obtain electron micrographs after each sample was subjected to critical point drying and gold-palladium sputtering using a DC plasma sputtering machine (Pathan et al., 2008).

Materials and Methods (Continued)

Table 1. Summary of organisms, media, and culture conditions used in the study.

Organism	Culture Medium	Culture Conditions
Staphylococcus aureus (ATCC 25904) Streptococcus pneuomoniae (ATCC 6305) Streptococcus pyogenes (ATCC 19615)	5% Sheep blood agar	37°± 1° C for 18-24 h, 5% CO2
Hemophilus influenza (ATCC 49247) Neisseria gonorrhoeae (ATCC 43069)	Chocolate agar	37°± 1° C for 18-24 h, 5% CO2
Bacteriodes fragilis (ATCC 25285)	5% Sheep blood agar	37°± 1° C for 18-24 h, anaerobic
Peptostreptococcus anaerobius (ATCC 27337)	5% Sheep blood agar	37°± 1° C for 48 h, anaerobic

- The CLSI document M40-A (CLSI, 2003) served as a guide for culture studies.
- Bacterial cell suspension equivalent to 0.5 McFarland in 0.85% sterile saline was prepared and diluted to $\sim 10^7$
- The test swabs (N=10) were immersed in the diluted bacterial suspension, and cultured on appropriate media after necessary dilutions to obtain recovery.

Dilution Factor x B x C x D

A = Number of bacteria recovered (CFU/mL) (adjusted for serial dilutions) Dilution Factor x A

B = Number of viable organisms in the starting culture (CFU/mL) (adjusted for serial dilutions) C = Average water absorption capacity of the swab (mL)

Data Analysis

absorption capacity.

swab (Figure 1).

- Statistical analysis of data collected was done by using JMP-7 (SAS Institute, Cary, NC).
- One-way Analysis of Variance (ANOVA) was used to test the equality of several means and to establish the test of

D = Average dry weight of swab (g)

- significance. The p-values were computed and then the test of significance was applied ($\alpha = 0.05$).
- Tukey-Kramer Honestly Significance Different Test (p ≤ 0.05) was used to determine which means are significantly different from one another.

Figure 1. One-way ANOVA of water The average water absorption capacity of all swabs ranged from 17.1% to 21.5%. Hydra Flock swab exhibited the maximum water absorption and its ability to retain water was significantly greater than the Rayon Flocked Nylon Rayon Macrofoam Hydra Al Pairs Tukey-Kramer

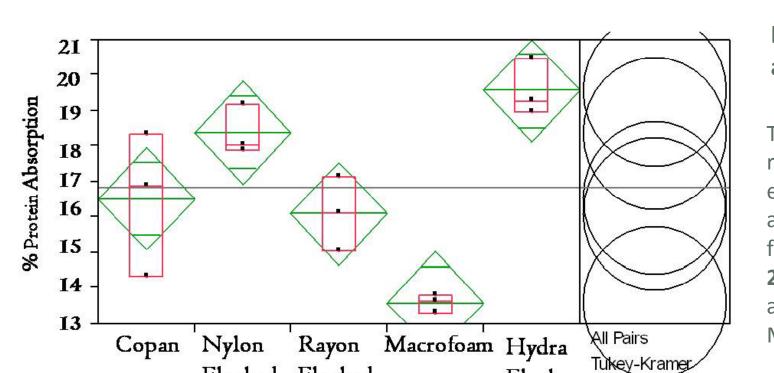


Figure 2. One-way ANOVA of protein absorption capacity.

The protein absorption capacity of all swabs ranged from 13.6% to 19.6%. Hydra Flock swab exhibited the maximum protein absorption and its ability to retain protein was significantly different from Rayon Flocked and Macrofoam swabs (Figure 2). The data showed that the Hydra Flock swab is about 46% more protein absorptive than the Macrofoam swab.

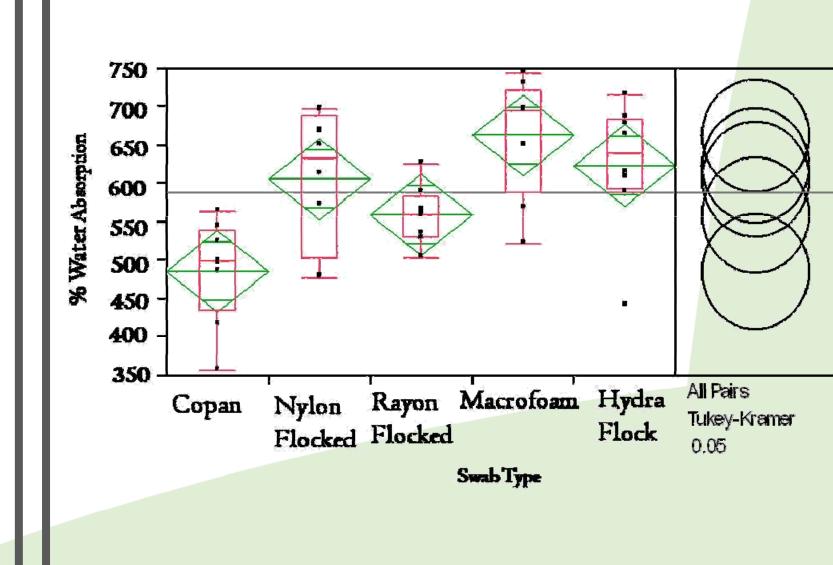
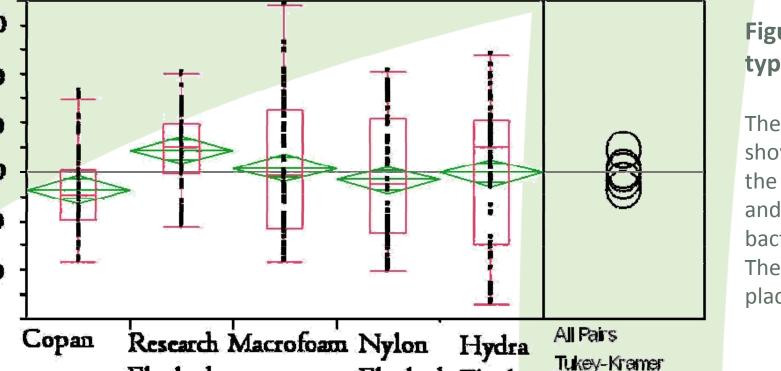


Figure 3. One-way ANOVA of water absorption capacity of fibers or foam of

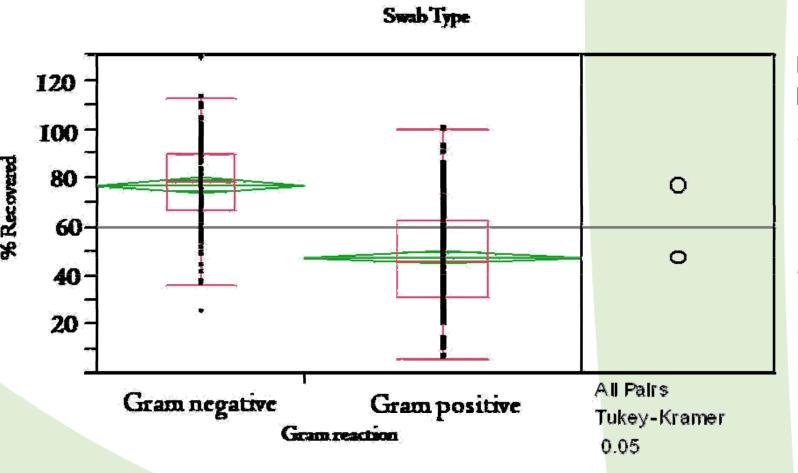
fibers or foam were used for measurements (Figure 3). Conversely, when whole swabs were used, Hydra Flock and Rayon Flocked ranked the highest and lowest, respectively with Copan's Nylon Flocked in the middle.



Flocked Flock

Figure 4. One-way ANOVA (by swab type) of recovery of all bacteria.

The recovery of all bacteria by swab type is shown in **Figure 4**. One-way ANOVA showed the highest recovery with the Hydra Flock and the lowest recovery of all viable bacteria by Copan's Nylon Flocked swab. The Macrofoam swab ranked in second



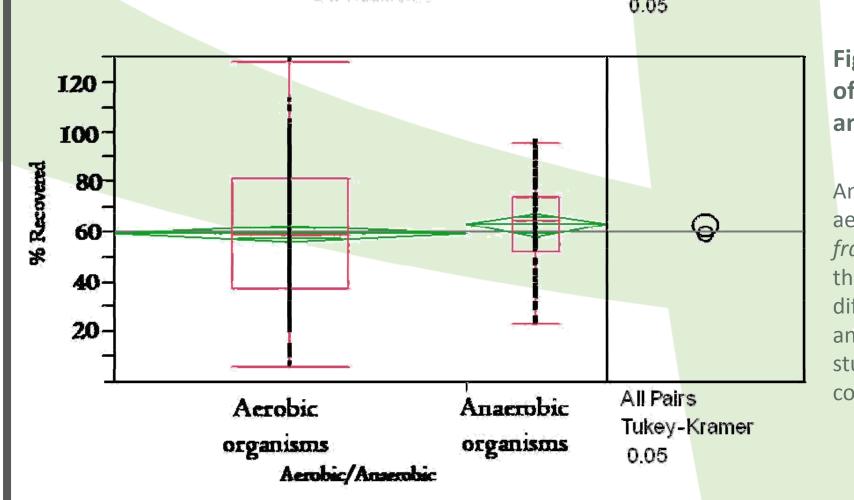


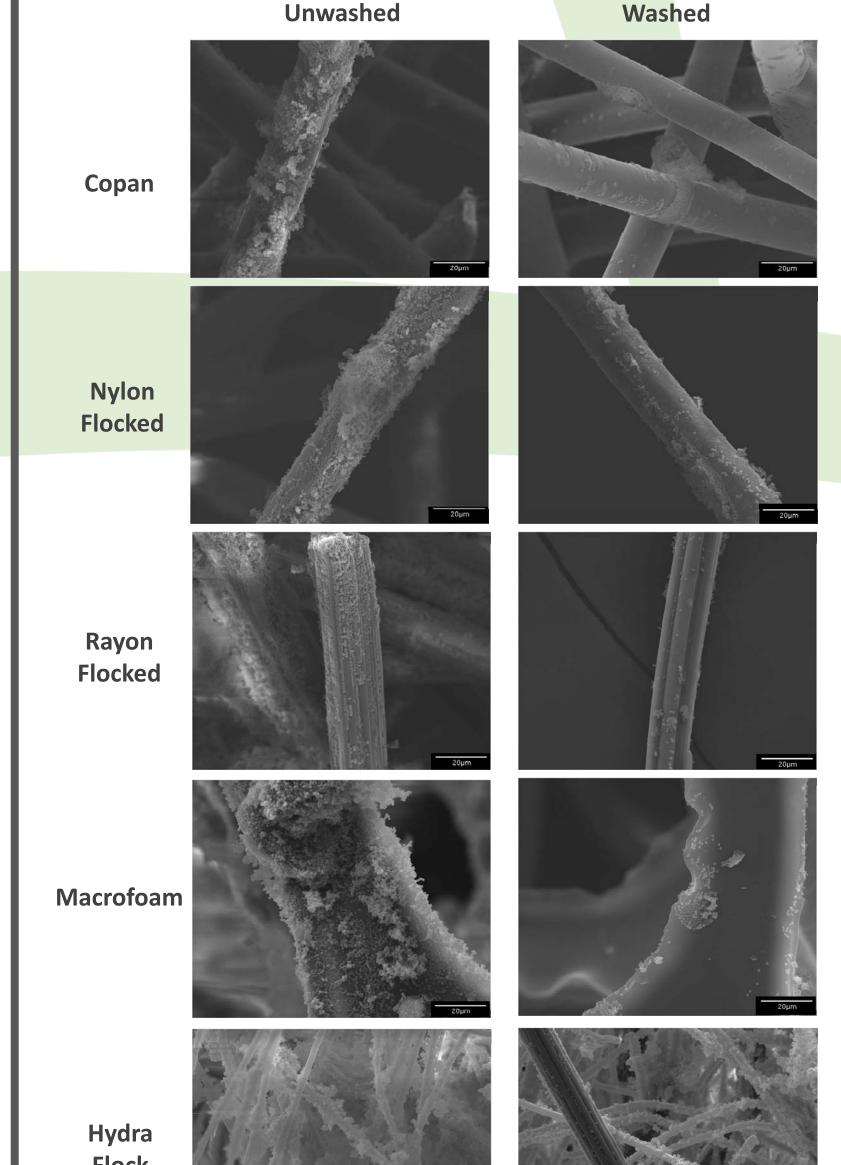
Figure 5. One-way ANOVA of recovery of bacteria analyzed by Gram-reaction.

The study consisted of three Gram-negative (N.gonorrhoeae, H.influenzae, and B. fragilis) bacteria and four Gram-positive (anaerobius, S. aureus, S. pneumonia, and S. pyogenes) bacteria. The results showed that Gram-negative bacteria had significantly higher recovery than Gram-positive bacteria (Figure 5).

Figure 6. One-way ANOVA of recovery of bacteria- analyzed by aerobiosis/ anaerobiosis.

Among the test bacteria there were 5 aerobes and 2 anaerobes. Except for B fragilis and P. anaerobius, all organisms in the study were aerobes. No significant difference in the recovery of aerobes and anaerobes was evidenced in the current study (Figure 6) when all swabs were compared together.





SEM measurements

Swabs were dipped in a polystyrene bead suspension and SEM photomicrographs of swab heads were recorded before and after washing (Figure 7) to modal capture and release of bacteria. The Hydra Flock demonstrated superior ability to collect beads, although the fractional release of beads by the Hydra Flock was comparable to or less than other swabs. On the other hand, the efficiency of bead collection for the Macrofoam swabs was about equal to the release of beads.

- 1. A key finding of this study is the overall superior performance of Hydra Flock Swab based on
- high water and protein absorption capacities highest recovery efficiency of various test bacteria
- Coupling these two properties will likely allow the Hydra Flocked design to have a greater effect on diagnostic
- Further, the ability of the Macrofoam swab to recover the second largest amount of test bacteria deserves
- Finally, it should be noted that the overall performance of Copan's Nylon Flocked swab to recover viable bacteria did not surpass any one of the swabs under the test conditions.

CLSI (2003) Quality Control of Microbiological Transport Systems; Approved Standard, CLSI document M40-A (ISBN 1-56238-520-8), pp 1-33, CLSI, Pathan, A.K. et al (2008) Micron 39: 1049-1061.

Sall, J. et al (2007) JMP Statistics: A guide to statistics and data analysis using JMP, 4th Edn., SAS Publishing, Cary, NC

© 2010 CET, LLC. All rights reserved.