Comparison of Sample Size and Methods of Cell Removal for the Enumeration of *Bacillus cereus* in Artificially Preserved Wipes

Irene Tchagou[1], Roma Adu-Osei[1], Travis Canida[2], Thomas Hammack[1], Mei-Chiung Jo Huang[3], and Rachel Binet[1]

[1]FDA, Office of Regulatory Science, [2]FDA, Office of Analytics and Outreach, [3]FDA, Office of Cosmetics and Colors; 5001 Campus Dr. College Park, MD 20740.

Abstract

Microbial contamination in cosmetic wipes can cause health issues as well as economic loss due to product recalls. [1] The microbiological analysis of cosmetic wipes using the procedures in FDA's BAM[2] Chapter 23 for solid products consists of diluting a 1g-sample size 1:10 with 1 ml Tween® 80 and 8 ml Modified Letheen Broth (MLB), using a vortex mixer to recover microbes for enumeration. This sample size may not be optimal for the analysis of wipes. In this study, we tested an entire wipe using a vortex mixer (EV) or a stomacher lab blender (ES) for recovery of *B. cereus* and compared the recovered counts with those obtained using 1g sample size with a vortex mixer (1gV) or 1g sample with a stomacher lab blender (1gS).

Two types of dry wipes were humidified with 0.45% Sodium Benzoate (SB) or 0.002 % Benzalkonium chloride (BAK) solutions inoculated with *Bacillus cereus* 3A spore[3] suspensions at three different levels of contamination, low (~4.7 log CFU/ml), medium (~5.7 log CFU/ml), and high (~6.7 log CFU/ml), and at 5 replicates per level. After 14 days at room temperature, the wipes were either cut to a 1g-sample size in the center or left untouched. The samples were diluted 1:10 with the same proportion of Tween® 80 and MLB as above, similar to the BAM protocol. All the samples were enumerated (CFU/g) on nonselective MLA (Modified Letheen Agar) plates, and on selective BACARA™ agar plates[4], after cell removal with a vortex mixer for 30 seconds, or with a stomacher for a minute. Uninoculated wipes were used as negative controls.

No significant difference in *B. cereus* counts was observed between the vortex mixer samples and the stomacher samples, regardless of sample size ($p=0.70$). However, differences were observed between agar plates ($p<0.001$), the type of wipes ($p<0.001$), and the sample size ($p<0.001$). Higher microbial counts were seen on non-selective MLA compared to counts on BACARA™ agar. Microbial recovery was also higher from 1g-sample size than from the entire wipe, which might be due to possible death of cells in dried edge areas of a sheet and will be further investigated.

Introduction

The U.S. cosmetic wipes market is steadily growing with a projected compound annual growth rate of 5% reaching $ 950 million from 2022-2032.[5] Wipes are utilized for baby care, hand washing, feminine and other personal cleansing, removing makeup, and applying products such as deodorants and sunless tanners, among other uses. [6] They are made of materials such as polyester, polypropylene, cotton, wood pulp, or rayon fibers formed into sheets and may be packaged individually, or in small or bulk packaging. Additionally, they are moistened with water, cleansing and moisturizing agents, and may contain preservatives to prevent the growth of bacteria and molds. [6] However, even with preservatives, wipes are susceptible to microbial contamination. FDA BAM Chap 23 suggests analyzing a 1g-sample size as recommended for Solids and Powders in Section G-2. However, this size may not be practical for wipes. Therefore, we compared *B. cereus* 3A recoveries between contaminated and preserved lab-made wipes from a 1g-sample as well as the entire wipe. Cells were removed from the wipe using the vortex mixer for 1g and stomacher for the entire wipes.

Materials and Methods

Two types of dry wipes, one made of cotton and the other of non-woven material, were moistened to 99.9% with a preserved solution of 0.002% of BAK or 0.45 % SB contaminated with *B. cereus* 3A at the High (6.7 log CFU/ml), Medium (5.7 log) and Low (4.7 log) levels. After 14 days, the wipes were analyzed. Bacterial counts were compared on MLA non selective agar plates and on *B. cereus* BACARA selective agar plates.

Sample Preparation

- Read OD~ 0.5 BC, ~ 6.7 log CFU/ml
- Prepare serial dilution and plate the 4th and 5th dilution on Trypticase soy agar (TSA)
- Spike 300µl of d0, d1, and d2 each in 3 bottles of 70 ml of 0.002% BAK or 0.45% SB
- Pour 5 or 10 ml of the spiked level in quintuplet on individual sheet of wipe and analyzed after 14 days

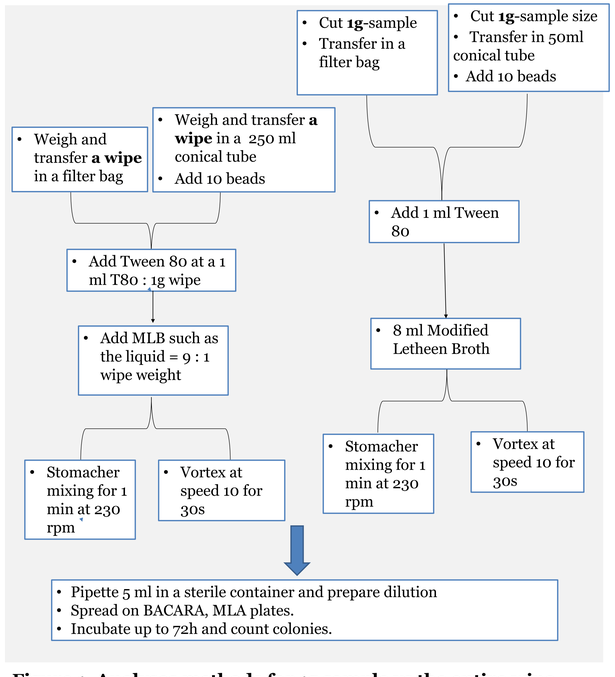

Figure 1. Analyses methods for 1g sample vs the entire wipe

Results

Statistical results revealed no differences between the stomacher and vortex mixer cell removal methods (Fig. 2). However, significant differences ($p<0.001$) were noted between MLA and BACARA agar plates, non-woven material and 100% cotton types of wipes, and between 1g and entire wipe sample sizes (Table 1). For example, cells recovered from non-woven materials with vortex mixer from 1g-sample size yielded 4.38 and 2.29 log CFU/g compared to 4.29 and 2.20 log CFU/g cells removed with stomacher from the entire wipe, at the high and low levels of inoculation, respectively. Likewise, cells recovered from 1g-sample size made of 100% cotton, yielded 4.30 and 3.28 log CFU/g compared to 4.21 and 3.19 log CFU/g recovered from the entire wipe removed with the stomacher.

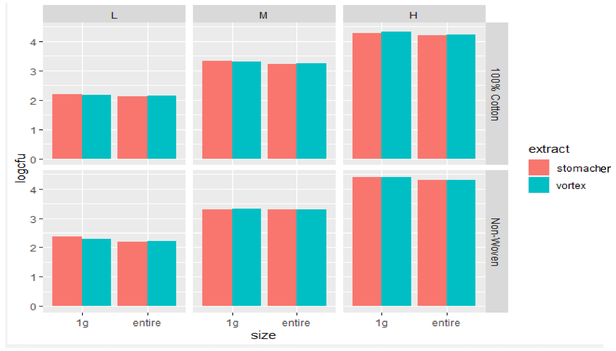

Figure 2. *B. cereus* 3A (log CFU/g) recovery from 1g and entire wipe samples using two cell removals, stomacher and vortex-mixer

- Wipes: Non-woven – Cotton = 0.080 ($p<0.001$)
- Size of Sample: 1g – entire = 0.083 ($p<0.001$)
- Method: Stomacher – vortexing = -0.007 ($p=0.70$)

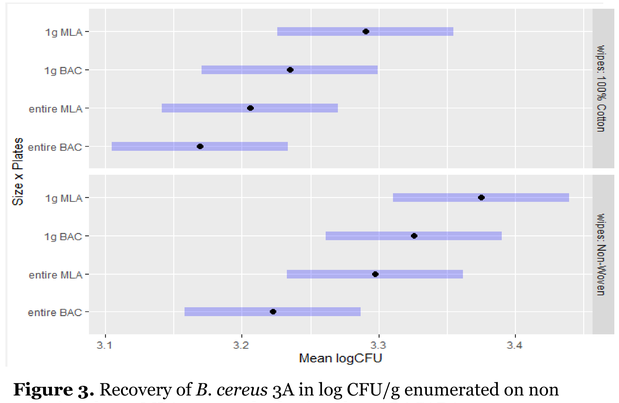

Figure 3. Recovery of *B. cereus* 3A in log CFU/g enumerated on non selective plates, MLA agar, and selective plates, BACARA.

- Overlapping bars means non statistically significant difference

Table 1. Recovery of *B. cereus* cells (log CFU/g) from 1g and entire wipe samples

		1gV[a]	1gS[a]	EV[a]	ES[a]
Wipes[b]	Level	Mean[c] (95% CI)[d]	Mean[c] (95% CI)[d]	Mean[c] (95% CI)[d]	Mean[c] (95% CI)[d]
Non woven + BAK	L	2.29 (2.23-2.35)	2.30 (2.25-2.36)	2.21 (2.16-2.27)	2.20 (2.14-2.26)
	M	3.36 (3.31-3.42)	3.38 (3.32-3.43)	3.28 (3.23-3.34)	3.27 (3.22-3.33)
	H	4.38 (4.32-4.44)	4.39 (4.34-4.45)	4.30 (4.24-4.36)	4.29 (4.23-4.35)
100% Cotton + SB	L	2.21 (2.15-2.27)	2.21 (2.15-2.26)	2.15 (2.09-2.20)	2.12 (2.06-2.18)
	M	3.28 (3.23-3.34)	3.28 (3.22-3.34)	3.22 (3.16-3.28)	3.19 (3.14-3.25)
	H	4.30 (4.24-4.36)	4.30 (4.24-4.35)	4.24 (4.18-4.29)	4.21 (4.15-4.27)

[a] 1gV: 1g-sample size + vortex mixer; 1gS: 1g-sample size + stomacher
EV: entire wipe + vortex mixer; ES: entire wipe + stomacher
[b] Lab-made spiked wipes at low, medium, and high contamination levels (6.7, 5.7 and 4.7 log CFU/ml) and preserved with 0002% BAK or 0.45% SB.
[c] Mean of five replicate portions per batch after logarithmic transformation: $\log_{10}$[CFU/g].
[d] Standard deviation for the test portions within the level.
95% CI = Lower and upper confidence limit for difference of means.

Discussion and Conclusion

1. Results for lab-made wipes spiked with *B. cereus* 3A revealed higher cell recoveries from non-woven materials preserved with BAK than those recovered from 100% cotton preserved with SB. These results could be due to the types of materials used in the wipes or the types of preservatives.
2. Cell recoveries from 1g-sample size were higher than recoveries from the entire sheet regardless of the cell removal methods, vortex mixer or stomacher. These results could be due to loss of moisture around the edge of the wipe during 14-day aging. The spiked cells of 1g-sample taken from the center of the wipe might be better supported.
3. We are continuing our investigations to address whether a gradient in moisture creates differences in the survival of microbial cells on the entire wipes, and overall lowers the microbial count per gram of sample.

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