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Examination of Swab Capture-and-Release of Pathogenic Bacteria on Representative Commercial Food Contact Surfaces

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1. Identification of experimental objectives

The objectives of this investigation were to: 1. identify swab performance as a quantifiable measure of the capture-and-release of viable pathogenic bacteria, 2. To follow up on the original study performed in January 2012 using only two testing surfaces and three microorganisms 3. provide Puritan Medical Products LLC with data from which they may draw conclusions on performance.

The purpose of this follow up study will be to show, through results, the performance of comparable swabs from Puritan and 3M on High Density Polyethylene (HDPE) plastic cutting boards and on stainless steel surfaces. The surfaces chosen for this further study reflect parameters found commonly in commercial food service kitchens as well as residential kitchens. The microbes chosen reflect pathogens of concern in the food industry.

Katahdin's role in this study has been two-fold: first to work with Puritan to develop a meaningful testing protocol using more selective criteria and second, to perform the actual surface swabbings and subsequent microbiological assays, and to collect and report findings of those assays.

2. Materials and methods

2.1 Swabs

The swabs used in this study consisted of Puritan Medical Products Company LLC (hereafter referred to as "Puritan") and 3M brand swabs. Puritan swabs included: EnviroMax© and EnviroMax Plus©; 3M swabs included: Enviro Swab© (dry), Enviro Swab© (pre-moistened), and Sponge-Stick Swab© (dry).

2.1.1 Swab handling and labeling

The tamper-evident envelopes encasing the Puritan swabs were removed and discarded. All 3M swab tamper seals remained intact until the beginning of the appropriate trial sessions. A coding system was employed for efficient labeling and easy identification of swabs (see Figure 1.1). The labels were printed on adhesive 1" x 3" labels and adhered to the swab containers. The swabs were then segregated according to the parameters identified below and collected in sealable, transparent plastic bags. All swabs were handled using aseptic technique.

Figure 1.1 Swab label coding system

(1) Brand
P = Puritan
3M = 3M

(2) Surface material
SS = Stainless steel
HDPE = HDPE plastic

(3) Trial group
E1
F1
G1

(4) Bacterium
BC = *Bacillus cereus*
LM = *L monocytogenes*
EC = *Escherichia coli*

(5) Number
1, 2, 3, … 20

Figure 1.2 Trial group identification

	Puritan		3M	
E1	EnviroMax Plus	+ 50% Neutralizing buffer & 50% (0.1%) Peptone Water	Enviro Swab	+ Water, polysorbate 80, & sodium chloride (ingredient list obtained from attached MSDS)
F1	EnviroMax	*dry*	Sponge-Stick	*dry*
G1			Enviro Swab	*dry*

2.2 Preparation of surface materials

Two surface material types (provided by Puritan) were used in this study: stainless steel (30cm x 30cm; qty. = 5) and high-density polyethylene (HDPE; 30cm x 45.5cm; qty. = 4). The protective plastic laminate layer covering the testing side of each stainless steel tile was removed and discarded. Each HDPE tile was scuffed with sandpaper (90 Grit) to produce a textured surface mimicking those found on heavily-used commercial food preparation and processing areas.

All surface tiles were placed in the laboratory dishwasher and exposed to a "heavy-duty" wash cycle the evening prior to the start of each trial. Laboratory-grade powder detergent (Labconco, FisherSci product # 04-334) was used as the cleaning agent. Each surface was prepared for inoculation (immediately prior to the start of each test trial) with the application of a 70% methanol solution. The surfaces remained exposed to the solution for no less than 30 seconds before each surface was wiped dry with paper towels. (Note: testing areas were not inoculated until uniformly dry).

Testing area boundaries (10cm x 10cm) were drawn on each surface tile using a cardboard template and permanent black marker. Four testing areas were drawn on each of the five stainless steel tiles. Six testing areas were drawn on three HDPE tiles; two testing areas were drawn on the remaining tile. The testing areas were labeled "1" through "20" to correspond with the numbered swabs in each trial group. All surface materials were handled using aseptic technique.

2.3 Preparation of inoculum

Preparation of each inoculum was begun by adding one lyophilized pellet to 1mL of sterile phosphate buffer solution (PBS; 3M, product # FTPHB9966) and incubated at 35±0.5°C for 30 minutes. Ninety-nine milliliters of PBS were added to the 1mL suspension and incubated for an additional 30 minutes. Finally, 10mL of the secondary suspension were added to 90mL of PBS to create the inoculum. The same method was used to create the inoculum for each bacterium: *Listeria monocytogenes* ATCC 19115, *Escherichia coli* ATCC 11229, *Bacillus cereus* ATCC 10876 (Microbiologics, product # 0687V, 0681V, 0998V, respectively).

2.4 Preparation of workbench surface

The workbench was cleaned using a surface bactericidal solution (Cavicide, FisherSci product # 22-998-800). All workbench areas intended for use during each testing trial were sprayed with the decontaminant solution using a pump-trigger spray bottle. The solution remained on the workbench for no less than 30 seconds before the surface was wiped dry with paper towels.

2.5 Inoculation

At the start of each trial, one milliliter of inoculum was deposited in the center of each testing area. A stainless steel plate spreader was used to evenly distribute the inoculum across the testing area. A one-hour exposure period followed inoculation of the testing areas. Proper aseptic technique and good laboratory practice were employed throughout the duration of the experiment.

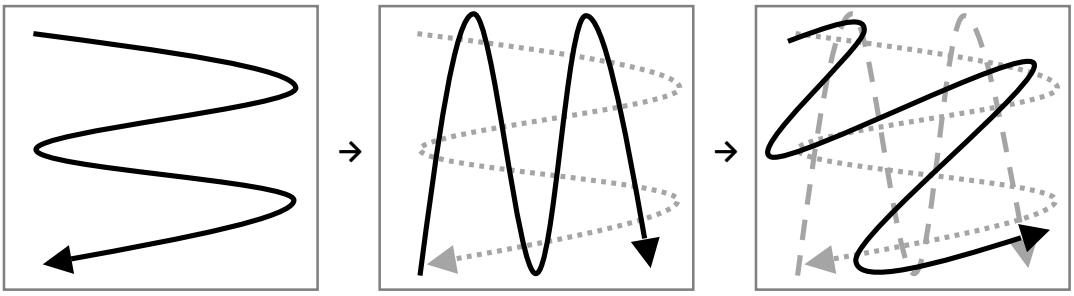
2.5.1. Total surface exposure time and cross-contamination prevention

The inoculated surfaces were left exposed to the laboratory environment with special care taken to ensure no sample preparation, analysis, etc. occurred within several feet of the surface materials. Extra measures to prevent contamination by foreign bacteria were taken during the *Bacillus cereus* testing trial; access to the laboratory was limited to include only the microbiology staff and project manager. Any incoming containers or samples were handled by the laboratory staff and were processed at an appropriate distance away from the testing surfaces using aseptic technique.

2.6 Swabbing

Swabbing began immediately following the one-hour exposure period. Each swab's identification label was matched to the numbered testing area before swabbing. The technique used to swab each testing area has been illustrated below (Figure 1.3). Once the testing area was properly sampled, the swab was returned to its original container and sealed.

Figure 1.3 Illustration of swabbing technique



This figure demonstrates the three-directional swabbing pattern employed to capture bacteria existing within a defined area. The perimeter lines in this illustration represent the boundary lines drawn for each testing area. The faded, dashed lines indicate the previous swab path over which the next path has been superimposed (solid line).

2.7 Sample dilution, plating, incubation, and analysis

2.7.1 Sample dilution

Ten milliliters of sterile PBS was added to each contaminated swab. The swab container was grasped securely and repeatedly squeezed (Sponge-Sticks) or inverted (all other swabs) for 15-20 seconds to promote the release of captured bacteria and to create a homogeneous sample.

2.7.2 Sample plating

Two plating technologies (and three plating techniques) were used in this study. *Listeria monocytogenes* was plated using purchased CHROMagar *Listeria* plated media (Gibson Laboratories, product # 11820). One milliliter of homogenized swab sample solution was drawn

from the swab container, deposited onto the media surface, and spread using a stainless steel spreader.

Escherichia coli was plated using the pour-plate method. One milliliter of homogenized swab sample solution was drawn from the swab container and deposited into a sterile Petri dish. Violet Red Bile Agar with MUG (Neogen, product # 7359A) was poured over the aliquot to cover the bottom of the dish. The dish cover was reapplied and a gentle, multi-directional swirling movement was used to homogenize the aliquot/medium mixture and ensure uniform distribution of the bacteria. (Note: the dish remained in contact with the workbench while swirling.) The plates were left to acclimate to room temperature, solidifying the medium and fixing the uniformly distributed aliquot in the gel matrix.

Bacillus cereus was plated using aerobic plate count (APC) Petrifilms (3M, product # 6400). APC Petrifilms are comprised of two layers: 1. a semi-transparent top film layer, the underside of which is covered with dehydrated media and 2. a slightly stiff card-stock backing covered with a yellow 1cm x 1cm grid. The layers are adhered along the top edge, and the film layer (7.5cm x 10.2cm) is slightly longer than the backing (7.5cm x 9.5cm). To inoculate, the film layer was lifted up and 1mL was deposited onto the center of the backing layer. The film was then rolled down, and a plastic spreader was centered over the aliquot and gently, but firmly, pressed onto the film to distribute the aliquot. (Note: the spreader is provided by the manufacturer (3M) with each group of APC Petrifilms purchased and is designed to uniformly distribute the aliquot across a $20cm^2$ circular area.)

2.7.3. Sample incubation and data analysis

L. monocytogenes, E. coli, and B. cereus samples were incubated at 35±2°C for 2 days, 1 day, and 2 days, respectively. Sample analysis consisted of counting colony-forming units (CFUs) observed macroscopically (backlit by a 60-watt fluorescent light-box). Analyst counts were documented in the appropriate bench-logs and a second analyst confirmed the number of CFUs counted to within 10% of the primary analysts documented count. All counts were then approved by the project manager. Aseptic technique was used and proper personal protective equipment (PPE) was worn by all counting analysts. Data analysis was comprised of average viable CFUs with regard to swab specificity and segregated by bacterium. Conclusions based on statistical analyses have not been drawn in this report.

3. Results

Table 3.1 Trial group E1 – Puritan EnviroMax Plus© and 3M Enviro Swab© (pre-moistened)

	Listeria monocytogenes (CFUs/mL)				*Escherichia coli* (CFUs/mL)				*Bacillus cereus* (CFUs/mL)			
	Puritan		3M		Puritan		3M		Puritan		3M	
Stainless steel	191	528	364	168	4320	4640	6528	11200	567	433	101	320
	462	736	176	216	3168	5120	6368	0	587	313	467	387
	167	400	400	440	9824	4640	4320	0	137	433	307	347
	253	560	336	600	6592	0	3328	0	307	480	280	347
	440	600	512	264	11200	3328	3360	0	627	220	353	407
	214	824	344	344	7232	0	2080	2784	553	178	240	160
	515	704	472	456	9312	7584	1	1632	460	267	287	98
	126	832	496	480	0	1456	0	0	347	260	155	114
	640	384	352	560	5792	3136	10496	3296	413	500	333	172
	728	576	472	352	9088	10240	6400	560	427	200	220	247
High-density polyethylene	0	0	0	0	0	2	67	22	160	63	18	43
	0	1	0	0	0	0	6	3	93	69	19	44
	0	0	0	0	0	12	332	7	50	42	8	7
	0	0	0	1	0	0	19	1	66	92	11	12
	1	0	0	1	1	0	0	6	115	120	14	27
	0	0	0	0	0	3	10	0	91	89	55	20
	0	0	0	0	0	0	5	2	60	49	15	29
	0	0	10	0	0	0	8	2	190	102	15	18
	1	0	0	0	1	5	10	14	53	102	23	30
	0	0	3	0	10	4	12	0	213	57	22	12

Table 3.2 Trial group F1 – Puritan EnviroMax© and 3M Sponge-Stick© (dry)

	Listeria monocytogenes (CFUs/mL)				*Escherichia coli* (CFUs/mL)				*Bacillus cereus* (CFUs/mL)			
	Puritan		3M		Puritan		3M		Puritan		3M	
Stainless steel	528	744	203	137	15520	16274	11744	15874	105	249	8	18
	456	592	40	80	14624	16250	25600	13925	206	236	23	54
	126	432	2	163	7552	15875	14592	30000	152	229	26	69
	0	528	7	100	8832	14640	15552	12374	227	81	11	16
	512	408	265	70	15150	15000	21952	25195	203	245	37	6
	592	640	84	72	14880	17000	8384	25275	291	133	10	11
	656	592	58	58	15270	17775	25000	16012	198	138	65	36
	624	824	22	43	15780	20000	25000	12525	286	61	68	3
	432	752	95	43	16275	25760	25000	10500	114	356	35	54
	728	648	113	93	15552	22780	12572	15661	387	201	6	5
High-density polyethylene	0	0	0	0	1	1	0	442	23	3	6	0
	0	0	0	0	0	2	1	1	8	10	3	4
	0	0	0	0	4	2	0	1	4	10	3	5
	0	0	0	0	1	2	25000	1	2	16	3	4
	0	1	0	0	1	3936	0	12500	5	13	4	6
	0	0	0	0	1	0	0	12500	12	12	0	3
	1	0	0	0	0	0	0	12500	6	14	2	2
	0	0	0	0	4	0	1	1	11	43	7	1
	0	0	0	0	1	0	15572	13592	6	8	2	3
	0	0	0	0	14	0	8	12568	2	19	7	5

Table 3.3 Trial group G1 – 3M Enviro Swab© (dry) (Puritan trial group F1 data duplicated for comparison)

	Listeria monocytogenes (CFUs/mL)				*Escherichia coli* (CFUs/mL)				*Bacillus cereus* (CFUs/mL)			
	Puritan		3M		Puritan		3M		Puritan		3M	
Stainless steel	528	744	137	186	15520	16274	4352	8608	105	249	10	187
	456	592	165	262	14624	16250	25000	4320	206	236	45	154
	126	432	80	255	7552	15875	12000	4064	152	229	2	172
	0	528	27	365	8832	14640	11785	15000	227	81	91	131
	512	408	583	281	15150	15000	3312	1248	203	245	145	135
	592	640	437	392	14880	17000	15090	5000	291	133	181	146
	656	592	306	272	15270	17775	19423	15000	198	138	72	56
	624	824	245	464	15780	20000	20198	5000	286	61	103	5
	432	752	542	272	16275	25760	26372	1250	114	356	145	151
	728	648	422	472	15552	22780	26000	344	387	201	232	160
High-density polyethylene	0	0	0	0	1	1	0	0	23	3	14	16
	0	0	0	0	0	2	0	0	8	10	17	31
	0	0	0	0	4	2	203	1336	4	10	27	18
	0	0	0	0	1	2	912	592	2	16	17	9
	0	1	0	0	1	3936	0	472	5	13	19	32
	0	0	0	0	1	0	0	520	12	12	18	37
	1	0	0	0	0	0	0	350	6	14	26	31
	0	0	0	0	4	0	68	572	11	43	12	28
	0	0	0	0	1	0	136	0	6	8	11	12
	0	0	0	0	14	0	3392	0	2	19	26	12

(Table 3.1) *The data in Table 3.1 represent data collected from Puritan EnviroMax© and 3M Enviro Swab© (pre-moistened) as CFUs per mL (10^1). A noticeable difference in total viable CFUs exists between the stainless steel and HDPE groups for both Puritan and 3M during the trials involving L. monocytogenes and E. coli. Further investigation is needed to determine the cause of such a significant lack of growth. (Note: similar data can be found in Tables 3.2 and 3.3 in the same trial groups.)*

(Table 3.2) *The data found in Table 3.2 are similar to those shown in Tables 3.1 and 3.3.*

(Table 3.3) *The data shown in Table 3.3 demonstrate trends similar to those found Tables 3.1 and 3.2.*

Figure 3.1 Average viable CFUs captured and released by Puritan and 3M swabs (segregated by trial group and surface type).

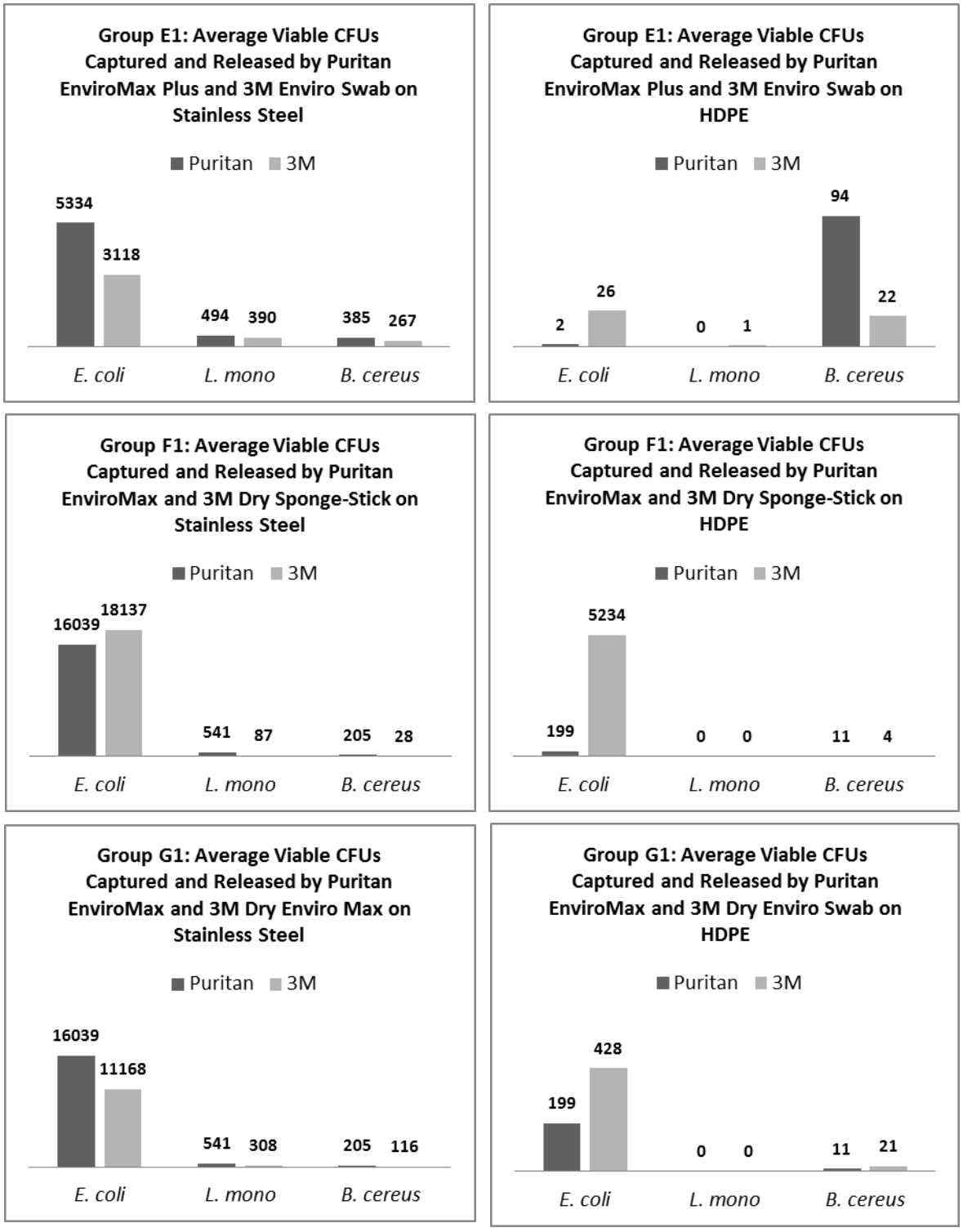
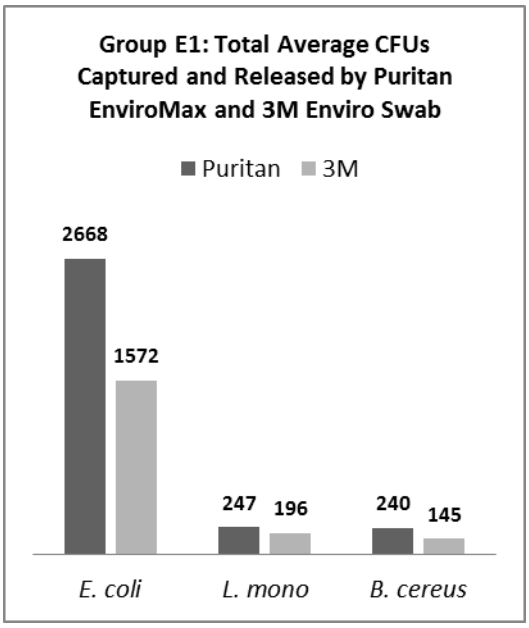
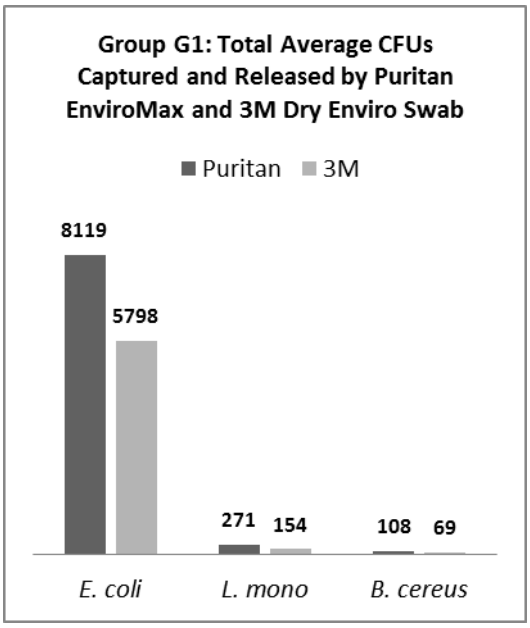
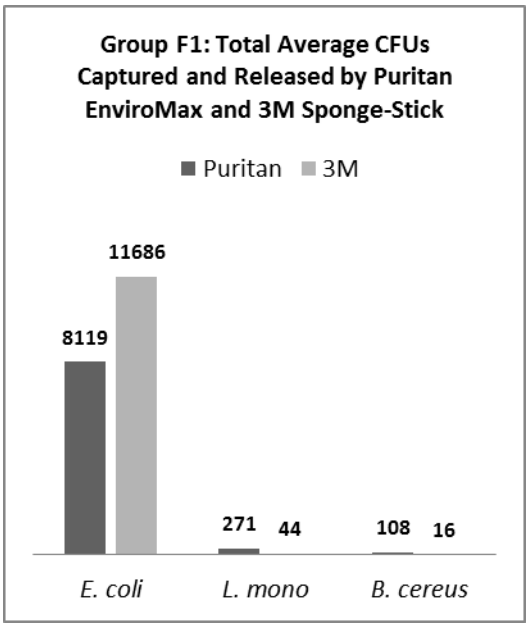


Figure 3.2 Total average viable CFUs captured and released per trial group. Data per surface type (as shown in Fig. 3.1) averaged together for visualization of total performance with regard to bacterium-specific categories.







The charts in Figures 3.1 and 3.2 demonstrate a superficial comparison of average CFUs captured and released by Puritan and 3M swabs. The averages shown in Fig. 3.1 were calculated by determining the mean of each trial group by bacterium. Side-by-side orientation of the charts was employed to show data collected from stainless steel test areas (right) and HDPE (left).

The charts in Fig 3.2 demonstrate the means of stainless steel and HDPE per trial group. The resulting data show overall capture-and-release per trial group.

4. Discussion of findings

The data for this study shows a clear difference between the two study surfaces – with bacterial growth being much lower on the HDPE surfaces than on the stainless steel surfaces. As far as the microorganisms, there does not appear to be a definitive difference in growth of the three pathogens between the two brands of swabs.