Qualification of High-Recovery, Flocked Swabs as Compared to Traditional Rayon Swabs for Microbiological Environmental Monitoring of Surfaces

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ABSTRACT: In microbiological environmental monitoring programs, swabs are widely used for hygiene monitoring of surfaces and operators. Traditional rayon swabs are generally used and considered the gold standard in swab collection. Two experimental studies were conducted to validate the performance of a new collection device for environmental monitoring of surfaces, called *flocked swabs,* manufactured by microRheologics (Brescia, Italy). The first experimental study consisted of comparing flocked swabs' recovery and release capacity to traditional rayon swabs from known microorganism inocula (spiked samples); the second experimental study compared the recovery capacity from samples obtained in routine environmental surfaces sampling of pharmaceutical areas. microRheologics flocked swabs compared to traditional rayon swabs showed an improvement in the percentage of recovery of contamination from surfaces from 20% up to 60%, and the findings were confirmed from a preliminary evaluation of routine environmental surface sampling of pharmaceutical areas. microRheologics flocked swabs also displayed an instant and nearly complete release of absorbed samples of more than 80%.

KEYWORDS: Microbiological environmental monitoring of surfaces, microorganisms recovery, bacteria release, flocked swab, rayon swab, microbial contamination, swab collection.

1. Introduction

While processing in an aseptic environment, one of the most important controls is the environmental monitoring program. Environmental monitoring should promptly identify potential sources of contamination, allowing for implementation of corrective actions before product contamination occurs. The monitoring program should include air, gases, water, operators, floors, walls, and equipment surfaces, including the critical surfaces that come in contact with the product, container, and closures (1). Samples should be taken throughout the classified areas of the aseptic processing facility using hygienic sampling procedures. In particular, acceptable surface monitoring methods include touch plates, swabs, and contact plates (2). Environmental monitoring methods are not always able to recover microorganisms present in the sampled area. In particular, low-level contamination can be particularly difficult to detect. Rayon swabs are traditionally used for microbiological environmental sampling, despite their limited recovery capacity of contamination from surfaces, which is estimated around 20%. A new swab with a nylon fiber coating, called a *flocked swab,* is supposed to improve the recovery and release capacity. We define recovery as the ability of the device, a swab, to collect and retrieve viable microorganisms from a surface. The release capacity is an expression of the device elution and discharging properties. In other words, the swab's ability to release any collected sample into a solution or media.

Overview of microRheologics Flocked Swabs Technology

Flocking is the process of applying a fiber directly onto a surface. The new flocked swab is a pre-shaped plastic applicator onto which a thin layer of nylon fiber is sprayed by a flocking process (Figure 1).

The nylon-flocked swab is designed to improve sample absorption by strong capillary action and to release more than 80% of the collected sample. Traditionally, wound fiber swabs (rayon or polyester swabs) trap a large percentage of the sample in the fiber matrix, retaining the sample. (Figure 2).

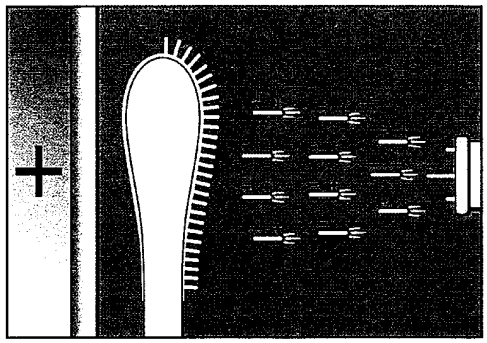

Figure 1

Flocking process: fiber is propelled perpendicularly onto a plastic pre-shaped device.

2. Materials

The following materials were used to conduct the qualification study: unidirectional laminar flow hood, stainless steel plates (electro-polished 316-L with finishing RA - surface roughness- 0.04) in which a "Target Area" of 36 cm^2 is delimited, aerosol spray devices, sterile flocked swabs manufactured by microRheologics (Brescia, Italy), sterile rayon swabs manufactured by microRheologics, 90-mm petri dish of Tryptic Soy Agar (TSA), sterile gloves, automatic pipettor.

Panel of Tested Microorganisms

The microorganisms tested both with rayon and nylon-flocked swabs were *Staphylococcus aureus* American Type Culture Collection (ATCC) 6538, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633 (spore).

Additional microorganisms were only tested with nylon-flocked swabs: *Aspergillus niger* ATCC 16404

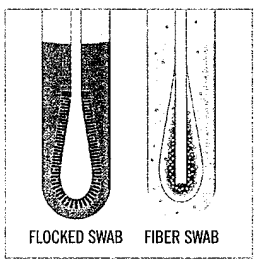

Figure 2

Releasing effect from a flocked swab compared to a fiber swab.

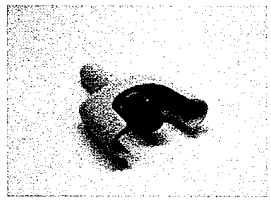

Figure 3

Spraying device used to distribute the inoculum onto the sampled surface

(spore), *Salmonella abony* National Collection of Type Cultures (NCTC) 6017, *Micrococcus luteus* (environmentally isolated), *Bacillus parabrevis* (environmentally isolated).

3. Methods

3.1. Experimental Study: Comparison between Nylon-Flocked Swab and Traditional Rayon Swab Recovery and Release Capacity from Known Microorganism Inocula (Spiked Samples)

The first experimental study comprised of four experiments: the first (3.1.1) aimed at substantiating the improved recovery capacity of flocked swabs versus standard rayon swabs obtained with traditional sampling technique methodology for environmental monitoring of surfaces and operators, the second (3.1.2) and third (3.1.3) experiments were performed in order to evaluate the release efficiency of flocked and rayon swabs, and the fourth (3.1.4) experiment was aimed at determining the maximum recovery rate achievable by combining swab and contact plate collections.

The biggest challenge in the design of our testing protocol was to clearly define the main variables that would directly influence the test results, and in particular how to distribute the bacterial suspension (inoculum) onto the sampling surface, the characteristics of the sampled surface, and the maximum time allowed for the test execution, in order to reproduce as realistically as possible a contaminated environmental surface to be tested in a standardized and quantitative fashion.

In order to evenly distribute a thin layer of bacterial suspension onto a *target sampling zone* (36 cm^2) (Figure 5), a *spraying device* (Figure 3) was charged with an aliquot of the inoculum. The spraying device was positioned at a distance of 16 cm from the target

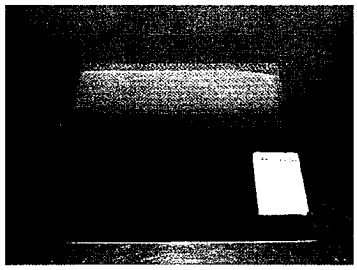

Figure 4

Stainless steel surface sampled.

sampling zone. The inoculum distribution analyses showed that 30% of the initially sprayed inoculum would fall outside the target sampling area; therefore a 30% reduction from the initial bacterial suspension count was applied in order to compare the data. The second variable defined was the potential variability of microorganisms recovery depending on the surface characteristics of the sampling area. An *electro-polished stainless steel AISI 316L surface* (Figure 4) was selected, as commonly found in the industry. The third variable was the maximum time allowed for the execution of each single experiment, including inoculum spraying, drying, and collection by swabbing that would not compromise microorganisms viability and therefore recovery. Bacterial viability losses between various time limits (after 3, 4 and 5 minutes) were compared and the lowest one (3 minutes) was selected. The maximum time of 3 min for each collection was therefore applied to the experiments run in the first experimental study.

3.1.1. The first experiment consisted in preparing 50–150 cfu per 100 µL fresh bacterial suspensions for all the strains listed in the panel of tested microorganisms. One hundred microliters of each suspension was directly transferred onto a TSA plate, incubated at 32 ± 2°C for 5 days, and colonies were counted to determine the suspension bacterial initial charge in terms of cfu (colony forming units). An additional 100 µL of each suspension was transferred into the spraying device. The spraying devices must be sterilized prior to the transfer of the inoculum, and the stainless steel plate must be cleaned with sterile water at 70–80 °C for 5 min prior to being placed under unidirectional laminar flow hood and let cool down for at least 20 min. Tested microorganisms were sprayed onto the plate surface at a nebulisation distance of 16 cm. The target area of 36 cm^2 was sampled with the swab, previously moistened with a non-nutrient solution, by rubbing the swab horizontally and then vertically. The maximum time allowed for spraying and swabbing did not exceed 3 min.

After collection, swabs were directly plated on a 0.90-mm Petri dish of TSA and incubated at 32 ± 2°C for 5 days. Colonies were counted. All testing was performed in duplicate and on three different swab lots. The same above procedure was also followed for all the next experiments. The assessment on the swab recovery capacity was indicated by the ratio between recovery after swab plating and recovery from direct inoculation with a 30% reduction to standardize the recovery data due to the spray loss.

3.1.2. The second experiment examined flocked swab release capacity by measuring the swab sample retention. After swab plating as executed in experiment one, the swab tip was cut into a test tube containing 10 mL of sterile physiological solution and vortexed for 30 s. The solution extracted was filtered using a 0.2-µm filter, the membrane placed onto a TSA plate and incubated for 5 days at 30–35 °C. At the end of the incubation period, colonies were counted to evaluate the extra recovery from the swab.

3.1.3. The third experiment verified that flocked swabs claimed release capacity of discharging more than 80% of a given collected sample. The test was performed by direct inoculation with no surface swabbing step. One hundred microliters of the initial inoculum were transferred onto the flocked swab and was immediately followed by direct plating onto a TSA plate. TSA plates were incubated for 5 days at 30–35 °C. At the end of incubation period, colonies were counted and compared to the initial inoculum.

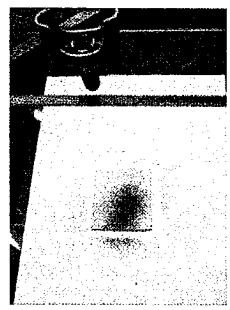

Figure 5

Target sampling zone swabbed (36cm^2)

3.1.4. The last experiment was aimed at assessing whether the use of a combination of surface sampling methods, such as swab and contact plate, might significantly increase the recovery rate. This test was conducted by making two collections in succession: first by swabbing the surface with a flocked swab (immediately afterwards plated onto TSA plates) and then, on the same target area, by means of a contact plate of TSA. Both plates were incubated for 5 days at 32 ± 2 °C and colonies were counted.

3.2. Experimental Study: Comparison between Nylon-Flocked Swab, Traditional Rayon Swab, and Contact Plate Recovery Capacity from Routine Environmental Surface Sampling of Pharmaceutical Areas

Two experiments were run to test the recovery capacity of the flocked swabs in comparison to traditional rayon swabs from routine environmental surface sampling of pharmaceutical production areas, such as non-classified areas (high-medium contamination) and unidirectional laminar flow hood (low–no contamination); and comparing flocked swabs to contact plate recoveries from grade C or ISO 8 pharmaceutical production areas.

3.2.1. The purpose of the first experiment was to test the recovery capacity of flocked swabs versus traditional rayon swabs applying the routine methodology for environmental monitoring of surfaces in pharmaceutical production areas. Various areas within the microbiology laboratory were tested, as well as the top of unidirectional laminar flow hoods. In each testing area, two adjacent "target zones" of 36 cm^2 were identified and swabbed: the first with the flocked swabs and the second with the traditional rayon swab. After collection, swabs were directly plated on a TSA plate and incubated at 32 ± 2 °C for 5 days. Colonies were counted. Swabbing of both zones was executed at close range and almost simultaneously, but the possible variability of microbial contamination between the two areas, even if contiguous, should be considered.

3.2.2. The recovery capacity obtained from nylon-flocked swabs was compared to the recovery obtained from contact plates, from samples collected in grade C or ISO 8 pharmaceutical production areas. Surfaces in injectable product preparation areas were monitored. Adjacent "target zones" of 36 cm^2 were sampled, the first by means of flocked swabs afterwards plated onto a TSA plate, and the second by means of a contact plate. Both plates were incubated for 5 days at 32 ± 2 °C and colonies counted. Also in this case, variability in the contamination of the two adjacent areas should be considered.

4. Results and Discussion

Recovery Capacity from Known Microorganism Inocula of Nylon-Flocked Swabs Compared to Traditional Rayon Swabs

Recovery of microorganisms obtained from flocked swabs was on average approximately 60% (Table I), while recovery from rayon swabs was approximately 20% (Table II).

Release Capacity from Known Microorganism Inocula of Nylon-flocked Swabs Compared to Traditional Rayon Swabs

Experiment 3.1.2 showed an average extra recovery from flocked swabs after vortexing of less than 6%, thus not significantly affecting flocked swab initial recovery capacity of about 60% (Table III). Standard rayon swabs showed rather poor recovery of about 20%, which was not improved by swab vortex processing to allow better sample release (Table IV). Flocked swabs used for environmental monitoring of surfaces and properly plated onto TSA plates release initial contamination in a very efficient and instantaneous way and are preferable to standard rayon swabs. Experiment 3.1.3 confirmed flocked swabs' instant and nearly complete sample release in solution through capillary action (Tables V and VI).

Maximum Recovery

The test highlighted that part of the bacteria still remains on the surface after the collection and that a combination of multiple tests could further improve the recovery to more than 70% (Table 7). The use of a swab plus contact plate, due to physical or procedural constraints, may be of limited application, but an investigation is ongoing on the impact of multiple flocked swabs collection in terms of increased recovery.

Comparison between Nylon-Flocked Swab and Traditional Rayon Swab Recovery Capacity from Routine Environmental Surface Sampling of Pharmaceutical Areas

Twenty specimens were collected with both traditional rayon swabs and the new flocked swabs from the

TABLE I
Flocked Swab Recovery Capacity

Recovery Capacity—Flocked Swabs					
Microorganism	Strain	Initial inoculum cfu/inoculum (100 µL)	Minus 30% Spray Loss (100 µL)	Average cfu/plate	% Recovery
S. aureus	ATCC 6538	109	76.3	32	41.94
Candida albicans	ATCC 10231	65	45.5	29	63.74
Escherichia coli	ATCC 8739	139	97.3	52	53.44
P. aeruginosa	ATCC 9027	51	35.7	19.5	54.62
Bacillus subtilis	ATCC 6633	53	37.1	23.5	63.34
				AVERAGE %	55.42
Additional Microorganisms Tested on Flocked Swabs					
Aspergillus niger	ATCC 16404	59	41.3	27	65.38
Salmonella abony	NCTC 6017	145	101.5	58.5	57.64
Micrococcus luteus	Environmentally isolated	73	51.1	31	60.67
Bacillus parabrevis	Environmentally isolated	81	56.7	27	47.62

bacteriology section of the microbiology laboratory (high contamination zone—see Figure 6), 20 specimens were collected with both traditional rayon swabs and the flocked swabs from the biology section of the microbiology laboratory (medium contamination—see Figure 7), and 10 specimens were collected with both traditional rayon swabs and the flocked swabs from the unidirectional laminar flow hoods in the microbiology laboratory (low–no contamination). All the swabs were plated on 0.90-mm Petri dish TSA plates and incubated at 32 ± 2 °C for 5 days. Colonies were counted during the 5-day incubation time; colonies were counted daily to monitor growth.

Comparison between Nylon-Flocked Swab and Contact Plate Recovery Capacity from Routine Environmental Surface Sampling of Pharmaceutical Areas

Twelve specimens were collected with contact plates and 12 specimens with flocked swabs within injectable product preparation areas (freeze drying). The swabs

TABLE II
Rayon Swab Recovery Capacity

Recovery Capacity—Traditional Rayon Swabs					
Microorganism	Strain	Initial Inoculum cfu/inoculum (100 µL)	Minus 30% Spray Loss (100 µL)	Average cfu/plate	% Recovery
S. aureus	ATCC 6538	140	98	18.5	18.88
Candida albicans	ATCC 10231	56	39.2	10	25.51
Escherichia coli	ATCC 8739	113	79.1	17.5	22.12
P. aeruginosa	ATCC 9027	98	68.6	10	14.58
Bacillus subtilis	ATCC 6633	78	54.6	9.5	17.40
				AVERAGE %	19.70

TABLE III
Flocked Swab Sample Retention

Swab Sample Retention—Flocked Swabs								
Microorganism	Strain	Average cfu/ inoculum (100 µL)	Minus 30% Spray Loss (100 µL)	Average Result cfu/plate	% Recovery	Average Swab Retention cfu/filtered	% Extra Recovery	% Total Recovery
S. aureus	ATCC 6538	81	56.7	27.5	48.50	2.5	4.41	52.91
Candida albicans	ATCC 10231	101	70.7	39.5	55.87	0.5	0.71	56.58
Escherichia coli	ATCC 8739	59	41.3	21	50.85	5	12.11	62.95
P. aeruginosa	ATCC 9027	60	42	23	54.76	3	7.14	61.90
Bacillus subtilis	ATCC 6633	78	54.6	30.5	55.86	0.5	0.92	56.78
				AVERAGE %	53.17		5.06	58.22
Additional Microorganisms Tested on Flocked Swabs								
Aspergillus niger	ATCC 16404	52	36.4	22.5	61.81	0.5	1.37	63.19
Salmonella abony	NCTC 6017	71	49.7	21.5	43.26	9	18.11	61.37
Micrococcus luteus	Environmentally isolated	82	57.4	28	48.78	1.5	2.61	51.39
Bacillus parabrevis	Environmentally isolated	93	65.1	34.5	53.00	1.5	2.30	55.30

TABLE IV
Rayon Swab Sample Retention

Swab Sample Retention—Traditional Rayon Swabs								
Microorganism	Strain	Average cfu/ inoculum (100 µL)	Minus 30% Spray Loss (100 µL)	Average Result cfu/plate	% Recovery	Average Swab Retention cfu/filtered	% Extra Recovery	% Total Recovery
S. aureus	ATCC 6538	93	65.1	14	21.51	0.5	0.77	22.27
Candida albicans	ATCC 10231	83	58.1	11.5	19.79	0	0.00	19.79
Escherichia coli	ATCC 8739	85	59.5	10	16.81	0	0.00	16.81
P. aeruginosa	ATCC 9027	53	37.1	8	21.56	0	0.00	21.56
Bacillus subtilis	ATCC 6633	85	59.5	10	16.81	0.5	0.84	17.65
				AVERAGE %	19.30		0.32	19.62

TABLE V
Flocked Swab Release Capacity

Release Capacity—Flocked Swabs				
Microorganism	Strain	Average cfu/inoculum (100 µL)	Average Result cfu/plate	% Release
S. aureus	ATCC 6538	145	134	92.64
Candida albicans	ATCC 10231	83	78	93.98
Aspergillus niger	ATCC 16404	92	85	92.39
Escherichia coli	ATCC 8739	129	120	93.28
P. aeruginosa	ATCC 9027	59	53	89.27
Bacillus subtilis	ATCC 6633	98	89	91.16
Salmonella abony	NCTC 6017	118	105	88.70
Micrococcus luteus	Environmentally isolated	90	85	94.81
Bacillus parabrevis	Environmentally isolated	105	94	89.84
			AVERAGE %	91.79

were plated on 0.90-mm Petri dish TSA plates. TSA Petri dish and contact plates were incubated at 32 ± 2 °C for 5 days. Colonies were counted during the 5-day incubation time; colonies were counted daily to monitor growth (Figure 8).

5. Summary and Conclusion

Multiple tests were performed to validate nylon-flocked swab performance in terms of recovery capacity, release capacity, and maximum recovery rate as compared to traditional rayon swabs. The recovery test showed that the recovery percentage for all microorganisms was approximately 60% for flocked swabs, compared to 20% for rayon swabs. Release tests results showed that swab vortexing does not significantly improve the recovery capacity and that flocked swabs through capillary action instantly and nearly completely, up to more than 90%, release the sample. Flocked swabs demonstrate extremely favourable recovery capacity and low sample retention rate as compared to traditional swabs.

TABLE VI
Rayon Swab Release Capacity

Release Capacity—Traditional Rayon Swabs				
Microorganism	Strain	Average cfu/inoculum (100 µL)	Average Result cfu/plate	% Release
S. aureus	ATCC 6538	111	23	20.72
Candida albicans	ATCC 10231	90	21	23.33
Aspergillus niger	ATCC 16404	67	15	22.38
Escherichia coli	ATCC 8739	101	20	19.80
P. aeruginosa	ATCC 9027	69	13	18.84
Bacillus subtilis	ATCC 6633	84	18	21.42
Salmonella abony	NCTC 6017	118	24	20.33
Micrococcus luteus	Environmentally isolated	138	29	21.01
Bacillus parabrevis	Environmentally isolated	93	21	22.58
			AVERAGE %	21.15

TABLE VII
Maximum Recovery

Microorganism	Strain	Average Recovery Contact cfu/plate	% Recovery Contact Plate	% Total Recovery Flocked Swab	% Result Contact + Swab
S. aureus	ATCC 6538	12	16.64	58.95	75.59
Candida albicans	ATCC 10231	14.5	21.14	52.48	73.62
Aspergillus niger	ATCC 16404	9	22.96	57.40	80.36
Escherichia coli	ATCC 8739	11	14.29	64.94	79.22
P. aeruginosa	ATCC 9027	7	19.61	57.42	77.03
Bacillus subtilis	ATCC 6633	10.5	18.75	57.14	75.89
Salmonella abony	NCTC 6017	15	23.29	52.80	76.09
Micrococcus luteus	Environmentally isolated	9	11.80	53.08	64.88
Bacillus parabrevis	Environmentally isolated	11.5	11.57	53.82	65.39
		AVERAGE %	17.78	56.45	74.23

The study also shows that part of bacteria remaining on the sampled surface could be recovered by applying multiple collections, such as, for instance, multiple flocked swabs. Further evaluation of this application is in progress.

The second experimental study consisted of routine environmental sampling of adjacent areas of the bacteriology, biology, and unidirectional laminar flow hood sections within the microbiology laboratory, which are considered to be high, medium, and low–no contamination, respectively. All sections were swabbed by means of the new flocked swabs and the traditional rayon swab. Both swabs performed satisfactorily, taking into account the possible variability in the microbial load of the adjacent sampled zones in uncontrolled airflow areas. In any case, the recovery rate and the overall microbial contamination detected from specimens collected by flocked swabs is considerably higher than by rayon swabs, as shown in Figure 6 and 7. This result could be explained by the improved recovery and release capacity of the new device into the culture media. The absence of surface contamination inside a unidirectional laminar flow hood was confirmed with both sampling devices. This area is classified at low or no contamination and it is representative of sterile production areas.

A comparison of the recovery obtained from flocked swabs and contact plates inside grade C or ISO 8 pharmaceutical areas (preparation areas/freeze-drying)

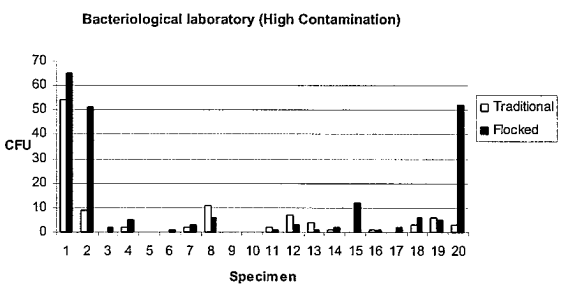

Figure 6

Comparative recovery data between flocked and traditional swabs in high contamination zone.

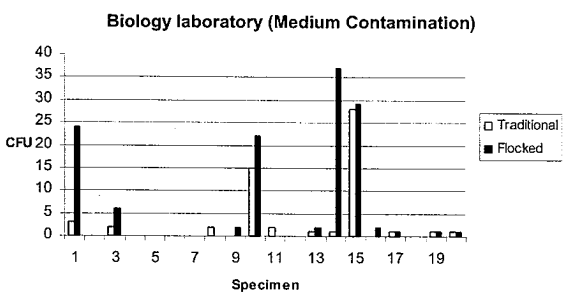

Figure 7

Comparative recovery data between flocked and traditional swabs in medium contamination zone.

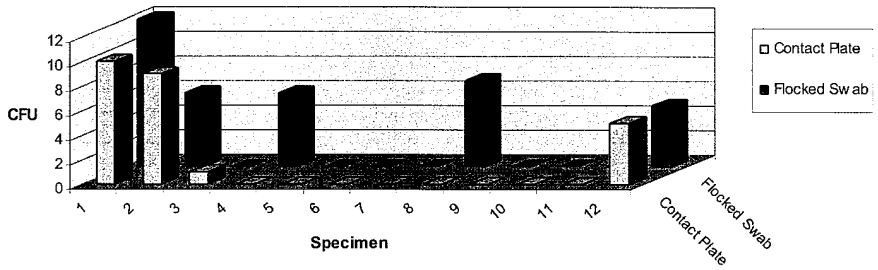

Figure 8

Comparative recovery data between flocked swab and contact plate in freeze-drying area.

was performed. As shown in Figure 8, the new flocked swab showed excellent recovery capacity.

Additional testing will be performed throughout a 1-year period in order to collect statistically significant data and confirm the actual percentage increase in recovery using a flocked swab as compared to a traditional rayon swab. Further investigation is also ongoing to examine the distribution of microorganism populations in the different areas.

Microbiological environmental monitoring of surfaces with the new flocked swab is a validated method, and the flocked swab is highly recommended to improve the recovery of microorganisms, especially in low-level contamination areas (classified production areas).

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