Cleaning to Achieve Sterility: An Evaluation of State-of-the-art Cleaning Techniques to Remove Particles of Biological Origin

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NASA planetary protection regulations stipulate that a surface may be considered "sterile" when a microbial burden of less than 300 aerobic bacterial spores per square meter can be treated to achieve a 10^4-fold reduction in viable endospores. In the cleaning process, single spores or small surface particles are immobilized by electrostatic charges, but can be removed only by extreme methods. If inexpensive cleaning processes exist that will produce a "sterile" surface without detrimentally affecting hardware, the process of readying spacecraft for final assembly and launch could be achieved simply and economically.

The NASA White Sands Test Facility (WSTF) was one of three participating facilities in a study to evaluate and refine existing cleaning techniques and identify cleaning methodologies that can achieve 4-log reduction levels on viable spore counts. Researchers evaluated the standard techniques used in the three facilities, and also explored variations in cleaning procedures and the chemistry of the cleaning solutions used.

Three cleaning procedures were evaluated at WSTF: (1) aqueous precision cleaning and verification without benefit of pre-cleaning; (2) pre-cleaning with a standard detergent called Simple Green® followed by aqueous precision cleaning and verification; and (3) pre-cleaning two groups of coupons in Simple Green® – the first with ultrasonics and flushing, and the second with ultrasonics, manual brushing, and flushing. Test results demonstrate that pre-cleaning with Simple Green® significantly improves cleaning efficiency, and the brushing method improves the efficiency of cleaning especially challenging surfaces. The WSTF cleaning procedure that included the brushing method was even more promising, averaging a 4.1-log reduction. However, due to limited sample size and variation between samples, statistical analysis indicates this method can reduce the bioburden by a 3.1 log with a 95% confidence level. Further studies with more replicates would determine whether this method will achieve a 4-log reduction level.

WSTF divides the cleaning and verification process into three distinct phases: pre-cleaning, precision cleaning, and verification. In pre-cleaning, we use chemicals, ultrasonic cleaning, steam cleaning, bead blasting, and other methods to remove grease, paint, and other contaminants, leaving the part visibly clean. For precision cleaning, we use solvents, ultrasonic cleaning, vapor degreasing, and other methods to clean parts to a specified level of particle and/or nonvolatile residue contamination. Finally, we use solvents to sample a part to verify the cleaning process, after which qualified technicians analyze the sample to accurately determine the cleanliness level of the part.

Pre-cleaning operations employ ultrasonic cleaning tanks, rinse tanks, and a nitrogen-purged drying oven. HEPA [high-efficiency particulate air] filtration alleviates the risk of contaminating already pre-cleaned components in the staging area. After inspection and staging work, these components go to the Class 100 clean room for precision cleaning and verification. The aqueous precision cleaning and verification equipment used in this study consist of a table-top ultrasonic tank, an ultrasonic probe and generator, clean sampling equipment, a deionized water source, and a low-pressure gaseous nitrogen source for blow-drying parts.

During early coupon testing, we were asked to use the aqueous precision cleaning and verification method without benefit of pre-cleaning the coupons. This method produced a 3-log reduction in viable spores, but did not meet the project objective of a 4-log reduction. Accordingly, we requested the opportunity to change the process to include pre-cleaning with Simple Green® followed by aqueous precision cleaning and verification. A new cleaning trial was arranged involving pre-cleaning two groups of coupons in Simple Green®: the first with ultrasonics and flushing, and the second with ultrasonics, manual brushing, and flushing. Both methods reduced the bioburden by more than 4-log. However, the coupons provided insufficient challenge to differentiate between the two pre-cleaning methods.

A simulated parts challenge used both cleaning methods (with brushing, without brushing) to pre-clean a mixture of 1/4- and 3/8-in. AN caps, elbows, and tees. WSTF received six batches of parts, all individually bagged to provide triplicate results for the two cleaning methods. Each batch consisted of five large (3/8-in.) AN caps, five small (1/4-in.) AN caps, five 1/4-in. AN tees, five 3/8-in. AN elbows, three contaminated stainless-steel coupons, and one clean, small (1/4-in.) AN cap, or 24 parts per batch.

Parts for each batch were debagged and placed in a basket before they were cleaned following the two prescribed methods. (AN refers to a manufacture's part identification).

The cleaned parts were bagged and returned to the NASA Jet Propulsion Laboratory (JPL) for biological analysis. Culturing at JPL determined spore counts and colony-forming units (CFUs), the results of which were transmitted to WSTF. We then tabulated detailed spore count and CFU/part data. Statistical analysis of these data sets suggests no batch-to-batch variation, so the data for each batch were pooled together. The CFUs before and after cleaning and the log reductions for each material are shown in Table 1 and figure 1.

Data indicate that fittings such as elbows and tees are more difficult to clean than caps or coupons. This is reflected in the higher spore counts, and is consistent with WSTF experience in previous cleaning studies. Based on earlier coupon cleaning studies, we anticipated that coupons would be cleaner than caps; this was not the case. Coupons were no cleaner than caps, and the coupons cleaned with brushing were not as clean as the brushed caps. We have been unable to explain this result based on the cleaning process. Analysis of this set of data indicates that these part-to-part variations are not statistically significant.

Early cleaning studies using coupons, together with coupon data from the multiple parts study, indicate that coupons do not provide a sufficient challenge to differentiate effective cleaning methods. Coupon studies appear to be limited to separating ineffective cleaning methods from those requiring additional evaluation. The data from this study suggest that, consistent with WSTF experience, a mixture of AN tube fittings provides a good cleaning challenge that will allow differentiation of different cleaning methods.

Based on data from this study, we conclude that normal pre-cleaning and precision cleaning using Simple Green® (with brushing) and deionized water, as conducted by our scientists, provides an average 4-log reduction in viable spore levels on parts of various materials and configurations. The data also suggest that manual brushing during pre-cleaning significantly improves the cleanliness level of a surface.

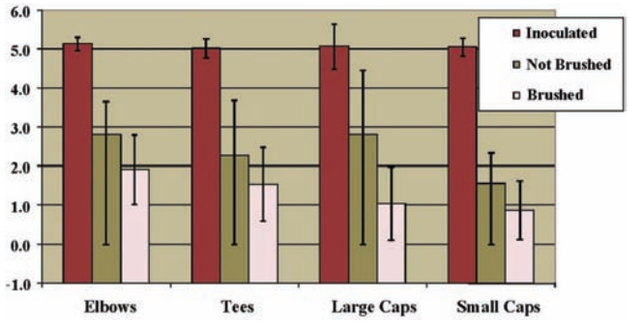

Fig. 1. *WSTF brushed and not brushed cleaning results.*

	Parts	No. of Replicates	Inoculation		After Cleaning		Log Reduction	
			CFUs	St. Dev.	CFUs	St. Dev.	Value	St. Dev.
No Brushing	Elbows	15	138,000	23,900	644.13	545.49	2.33	0.86
	Tees	15	104,000	26,100	189.50	266.58	2.74	1.43
	Large Caps	15	117,000	68,200	623.25	1030.24	2.27	1.75
	Small Caps	15	114,000	25,700	36.17	27.99	3.50	0.81
With Brushing	Elbows	15	138,000	23,900	82.25	73.17	3.22	0.91
	Tees	15	104,000	26,100	34.50	32.32	3.48	0.97
	Large Caps	15	117,000	68,200	11.00	10.29	4.03	1.10
	Small Caps	15	114,000	25,700	7.42	5.63	4.19	0.79

Table 1. *Comparison of cleaning efficiency for WSTF brushed and not brushed methods.*

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