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INTERNAL NOTE

EFFECT OF CURRENT CLEANING PROCEDURES ON STERILIZATION OF SPACECRAFT COMPONENTS

By

Manufacturing Research and Technology Division
Manufacturing Engineering Laboratory

GEORGE C. MARSHALL SPACE FLIGHT CENTER
Huntsville, Alabama

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ABSTRACT

This report covers the effect of current cleaning procedures on sterilization of spacecraft components. Spacecraft components consisting of aluminum panels and stainless steel tubes were cleaned using these current Marshall Space Flight Center procedures. The cleaning process significantly reduced the biological load on the test specimens. Current Marshall Space Flight Center cleaning procedures do not, however, sterilize the spacecraft components.

ACKNOWLEDGMENT

This report is based on the work accomplished under Engineering Project No. 2232-1 as reported by D. L. Stewart and J. C. Hurgeton, Methods Development Group, Hayes International Corporation, in the Manufacturing Engineering Laboratory Technical Report MD-159-67.

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INTRODUCTION

A program has been initiated to develop sterilization technology in the Manufacturing Engineering Laboratory under Engineering Project No. 2232-1. This technology will be applied to the manufacturing, assembly, monitoring, testing, handling and storage of typical spacecraft components and assemblies.

This report covers the investigation of the effect of current MEL non-viable cleaning procedures on certain elementary spacecraft components contaminated with known quantities of viable organisms. These spacecraft components consisted of aluminum panels and stainless steel tubing.

DISCUSSION

Applicable Documents

1. Engineering Plan EP-2232-1
2. M-ME-M PROC . 005.5B
3. MSFC-SPEC 164
4. Standard Procedures for the Microbiological Examination of Space Hardware
5. MIL Handbook 105

Materials

1. Twenty aluminum panels 4 inches by 9 inches
2. Thirty 6-inch by 0.125-inch diameter stainless steel tubes

3. Tryptic Soy Agar

4. One percent peptone water

5. Six-inch cotton tip applicators

6. Freon

7. Sumco - 30

8. Trichloroethylene

9. Turco 2014

10. Sulfuric acid

11. Potassium dichromate

Equipment

1. Incubator

2. Autoclave

3. Colony counter

4. Chemical cleaning console

5. Ultrasonic bath

6. Surface treatment tanks

7. Calculator

Procedures

1. Initial Decontamination. - Twenty aluminum panels and 30 pieces of stainless steel tubing were degreased with acetone, packaged in appropriate containers and sterilized by wet heat at 121° C for 18 minutes.

2. Re-Contamination. - An aqueous solution of *Bacillus subtilis var. niger* was prepared by washing a slant with 5 ml of saline solution and placing this suspension of cells in a sterile test tube with an additional 5 ml of saline. Serial dilutions of this suspension were made, and each dilution was plated with Tryptic Soy Agar to determine the number of bacterial cells per milliliter. This procedure was performed in order to select a dilution of cells to fulfill the requirement of 10^3 cells per square foot of surface.

Each side of the aluminum panels received 0.25 ml of the suspension containing 3.38×10^3 cells per ml and was allowed to air dry overnight. Extreme care was taken not to touch the panels with human hands during this procedure.

The interiors of the stainless steel tubing received 0.2 ml of the suspension of cells containing 3.9×10^2 cells per milliliter. Immediately following this step, the tubes were slanted at a 15 degree angle and allowed to air dry overnight. Forceps were utilized to hold the tubing during the contamination process.

One aluminum panel and one piece of stainless steel tubing received no viable contamination. These items were used as sterility controls for the assay procedure.

3. Microbiological Assessment: Aluminum panels. - A 4-inch square area on each side of the panel was sampled by the cotton swab method. Ten of the panels were sampled prior to cleaning, and the other ten were sampled after cleaning. The sterility control panel was included in the group sampled prior to cleaning. These samples were assayed according to the procedures illustrated in Figure 1. Although counts were recorded at 24 hours and 48 hours of incubation, only the 72 hour counts are presented in this report because these counts exceeded the 24 hour and 48 hour counts.

Microbiological Assessment: Stainless steel tubing. - Each piece of the stainless steel tubing was sampled by rinsing the interior of the tube with 5 ml of one percent peptone water. Ten pieces of tubing were sampled before the cleaning, and 20 pieces of tubing were sampled after the cleaning. Each rinse fluid sample was assayed in the same manner as the cotton swab analyses (Fig. 1) with one exception; the rinse fluid samples were not mechanically shaken.

4. Cleaning Methods. - Ten of the aluminum panels were cleaned by R-ME-D to M-ME-M PROC - 0005.5B.

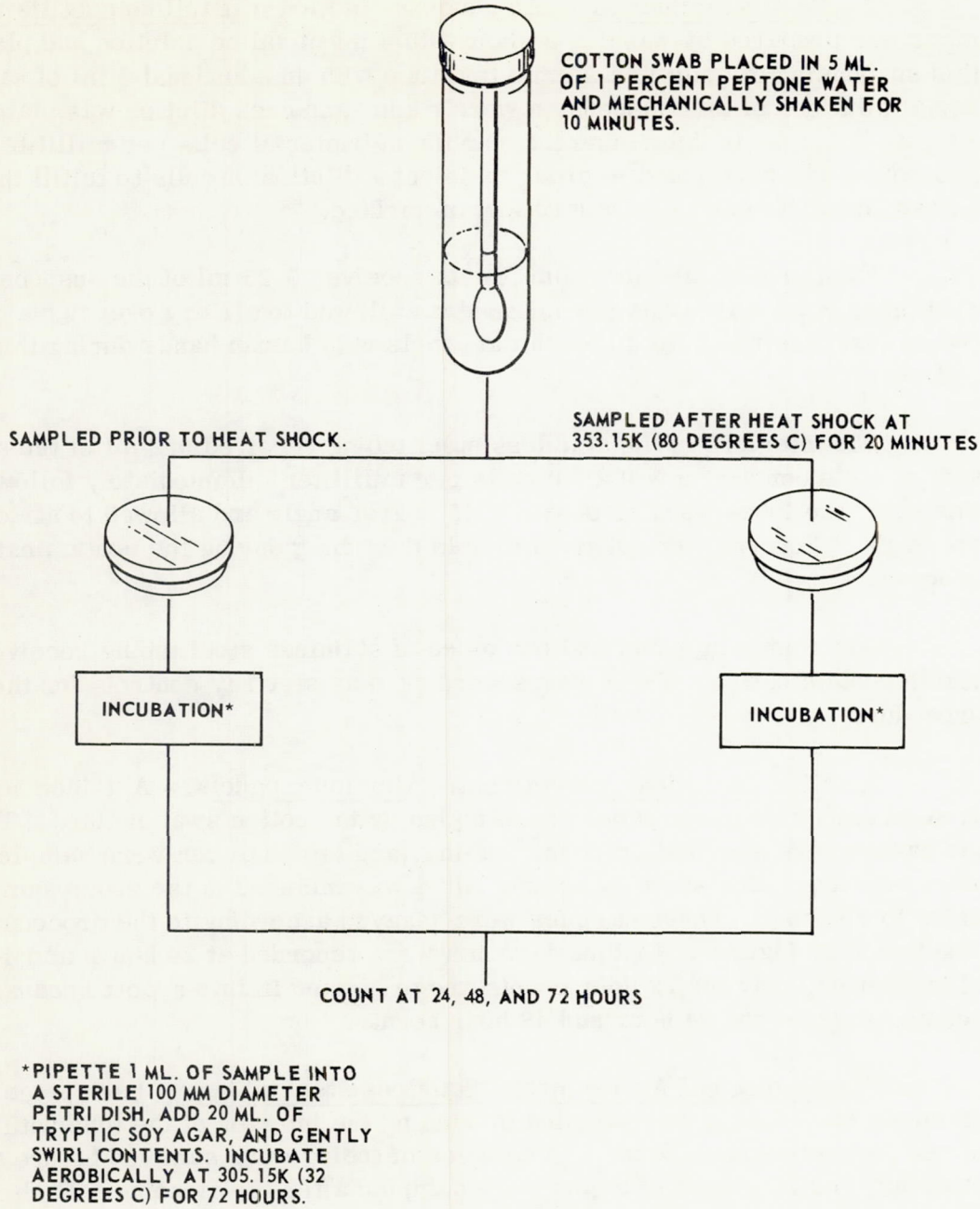

FIGURE 1. SCHEMATIC OUTLINE OF COTTON SWAB ANALYSIS

All 20 pieces of the stainless steel tubing were cleaned by R-ME-D according to MSFC SPEC 164. In the initial plan, 10 pieces of tubing were to be cleaned in an ultrasonic bath, and the other 10 pieces of tubing were to be cleaned by Freon flushing. However, a combination of these two cleaning methods was used for all 20 pieces of tubing.

Results

The individual colony counts - before (contaminated) and after cleaning - are listed in Tables I and II.

TABLE I. INDIVIDUAL BIOLOGICAL DATA - PANELS

Number of Colonies per Square Foot - Panels (72-hour Incubation)

Sample Number	Contaminated Non-heat Shock	Contaminated Heat Shock	Sample Number	After Cleaning Nonheat Shock	Heat Shock
Panels 1a	5220	2700	Panels 11a	180	0
Panels 1b	3060	1440	Panels 11b	180	0
Panels 2a	3240	1980	Panels 12a	360	0
Panels 2b	4680	4140	Panels 12b	0	0
Panels 3a	1080	1980	Panels 13a	0	0
Panels 3b	8280	6860	Panels 13b	0	0
Panels 4a	6300	3600	Panels 14a	0	0
Panels 4b	3960	1800	Panels 14b	0	0
Panels 5a	1980	2160	Panels 15a	180	0
Panels 5b	3060	1260	Panels 15b	0	0
Panels 6a	2880	2160	Panels 16a	0	0
Panels 6b	4860	3960	Panels 16b	2880	0
Panels 7a	3060	2340	Panels 17a	0	0
Panels 7b	5940	3120	Panels 17b	0	0
Panels 8a	6120	3600	Panels 18a	0	0
Panels 8b	1980	1260	Panels 18b	0	0
Panels 9a	1800	3240	Panels 19a	0	0
Panels 9b	1440	2160	Panels 19b	0	0
Panels 10a CONTROL	0	0	Panels 20a	0	0
Panels 10b	0	0	Panels 20b	0	0

TABLE II. INDIVIDUAL BIOLOGICAL DATA - STAINLESS STEEL TUBING

Number of Colonies per Square Foot - Stainless Steel Tubing
(72-hour Incubation)

Sample Number	Contaminated Nonheat Shock	Contaminated Heat Shock	Sample Number	Cleaned Nonheat Shock	Heat Shock
1	1000	2500	11	500	0
2	2500	500	12	0	0
3	2000	3500	13	0	0
4	4500	2000	14	0	0
5	2500	3000	15	0	0
6	5500	1500	16	0	0
7	4500	3500	17	0	0
8	3500	1000	18	0	0
9	3000	4500	19	0	0
10 CONTROL	0 CONTROL	0	20	0	0
			21	0	0
			22	0	0
			23	500	0
			24	0	0
			25	0	0
			26	0	0
			27	0	0
			28	0	0
			29	0	0
			30	0	0

Each major group is further reported as both heat shocked and nonheat shocked. All heat-shock counts made after cleaning were zero.

A sample size of 10 was chosen for these experiments because it appeared to balance the work load, material requirements and statistical analyses. The panels were contaminated on each side and separetely sampled for biological load. The sides are designated a and b in Table I.

Panel 3b, Table I, heat shocked and nonheat shocked, had colony counts significantly higher than the rest of the samples. The odds of this happening by

chance indicate that there probably was an assignable cause for these high values. The fact that these data points were out of control was not known at the time the experiment was run; therefore, no assignable reason was determined in these cases. Out of control data in future experiments will be carefully examined in an effort to assign causes now that significant limits have been established.

The number of colonies counted on the aluminum panels after 72 hours of incubation was in some instances higher than the calculated amount of contamination added. There are several possible explanations for this. The possibility of uneven distribution of bacteria on the panel because of the method of contaminant application should not be overlooked, as well as the fact that the panels were air dried and subjected to possible further air contamination during drying. Because this contamination discrepancy was not found in the stainless steel tube counts, it is quite probable that one of the above possibilities caused the abnormally high contamination value.

There are some colony counts for both panels and tubes where the heat shock colony counts are greater than the non-heat shock colony counts. Due to the fact that colonies may arise from one cell or several cells, this observation can be explained. The data were included because the counts were not out of limits when compared to other counts made within each group.

Table III summarizes the individual colony counts for both the panels and the tubes according to the major classifications listed in Tables I and II.

Using statistical tests it is possible to compare the heat shocked versus nonheat shocked data summarized in Table III to determine whether these were significant differences. Substituting the data into the statistical formulas indicates that there is no significant difference in the heat shocked versus nonheat shocked methods of treating the samples.

By inspection of the summary of data, Table III, there is an obvious difference because of the cleaning of both the panels and the tubes. After cleaning, both groups of data (panels and tubes) are significantly lower in biological load. In groups 4, 7, and 8, (Table III) there were so few colonies present after cleaning that the data could not be treated statistically.

It is possible to determine the sample size requirements using the data from Table III and substituting into the proper statistical equation. Based on the variability of the data a sample size of 17 would be required to be assured

TABLE III. SUMMARY OF BIOLOGICAL DATA
Numbers of Colonies Per Square Foot

Group	Mean Value	σ	2σ	3σ	LCL	UCL
1. Panels (Nonheat Shock)	3830	1979	3958	5937	1850	5808
2. Panels (Heat Shock)	2770	1335	2670	4005	1435	4104
3. Panels (Cleaned - Nonheat Shock)	192	2813	5626	8439	0	3045
4. Panels (Cleaned - Heat Shock)	Insufficient data for analysis					
5. Tubes (Nonheat Shock)	3222	1417	2834	4251	1805	4639
6. Tubes (Heat Shock)	2444	1310	2620	3930	1134	3754
7. Tubes (Cleaned - Heat Shock)	Insufficient data for analysis					
8. Tubes (Cleaned - Nonheat Shock)	Insufficient data for analysis					

Where: Mean value is average of individual values

σ = 67 percent of all data in mean value calculations

2σ = 95 percent of all data in mean value calculations

3σ = 99.3 percent of all data in mean value calculations

LCL is Lower Control Limit

UCL is Upper Control Limit

that significant differences between two hypotheses, such as heat shocked versus nonheat shocked treatment of samples, were not due to chance causes in more than 5 percent of a series of test runs. Also, significant differences would exist but not be found in only 5 percent of a series of test runs.

Because the sample size of 10 was chosen for this experiment, it is obvious that any conclusions concerning heat shocked versus nonheat shocked methods will have somewhat less confidence than 95 percent - 5 percent. This

does not mean that the data are inconclusive but rather indicates that more data are required to further refine the conclusion to the desired confidence level.

On the other hand, the sample size of 10 was more than adequate to determine significant changes at the 95 percent confidence level when considering the effect of cleaning on the biological level. Here again additional data are required, but in this comparison the reason is the low level of contamination after cleaning.

CONCLUSIONS

No significant difference is assigned to the heat shocked versus nonheat shocked methods for assaying panels and tubes.

Cleaning produces a significant reduction in contamination level.

The sample size of 10 used in these (2232-1) experiments was adequate to determine significant changes in contamination levels because of the normal cleaning processes. A better determination of the sample size requirements after cleaning will require additional data because of the low levels of data.

These cleaning procedures did not sterilize the spacecraft components.

Laboratory procedures and techniques used to contaminate and assay the spacecraft components were effective and may be applied to measure the biological reductions resulting from nonviable cleaning procedures.

George C. Marshall Space Flight Center,
National Aeronautics and Space Administration,
Huntsville, Alabama, June 8, 1967.

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Manufacturing Engineering Laboratory

The information in this report has been reviewed for security classification. Reveiw of any information concerning Department of Defense or Atomic Energy Commission programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

This document has also been reviewed and approved for technical accuracy.

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