MICROBIAL CONTAMINATION ASSOCIATED WITH THE APOLLO 6 SPACECRAFT DURING FINAL ASSEMBLY AND TESTING

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Abstract. The National Aeronautics and Space Administration (NASA) requires that microorganisms which could contaminate the surface of the moon as the result of lunar missions be enumerated and identified so that life forms in lunar materials returned to earth may be more easily recognized as being of native or terrestrial origin.

Assessment of microbial contamination in the intramural environments used for the assembly and test of the manned lunar spacecraft (Apollo) was made using fallout strips and air samplers. Microbial contamination on the surfaces of Apollo Command and Lunar Modules was determined by use of the swab-rinse method.

Preliminary results indicate that the levels of microbial contamination which accumulated on exposed stainless steel surfaces, as well as airborne microbial contamination in the high bay assembly areas, were similar to those encountered in the unmanned spacecraft assembly areas. However, higher levels of microbial contamination were detected on the Apollo spacecraft than on the unmanned lunar spacecraft.

1. Introduction

Samples of lunar material collected as part of the Apollo Program will be thoroughly analyzed for the presence of living organisms. To help prevent viable organisms of terrestrial origin from contaminating potential sampling sites, the National Aeronautics and Space Administration (NASA) has required that microbial contamination on spacecraft destined to impact or land on the moon be reduced to the lowest possible level. Studies on various unmanned spacecraft bound for the moon have defined levels of microbial contamination both on the spacecraft and within associated assembly and test environments (Olson *et al.*, 1968; Tritz *et al.*, 1967). These studies have been useful for pinpointing areas requiring improved contamination control as well as for providing data for a valid estimation of the distribution of viable terrestrial organisms transported to the moon.

The fact that manned lunar spacecraft consist of many more components and are exposed to more and different environmental conditions than unmanned lunar spacecraft created the need for a documentation of the levels and types of microbial contamination which accumulate on Apollo spacecraft during assembly and testing.

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This study was designed to determine levels of microbial contamination on the Apollo 6 spacecraft and in the intramural environment used for the assembly and testing of manned lunar spacecraft.

2. Materials and Methods

Assembly and testing of the Apollo spacecraft took place in three different environmental areas: The Manned Spacecraft Operations Building (MSOB), Vehicle Assembly Building (VAB), and Launch Complex 39A (Figure 1). The Lunar and Command Module High Altitude Test Chambers and Integrated Test Stands #1 and #2 were

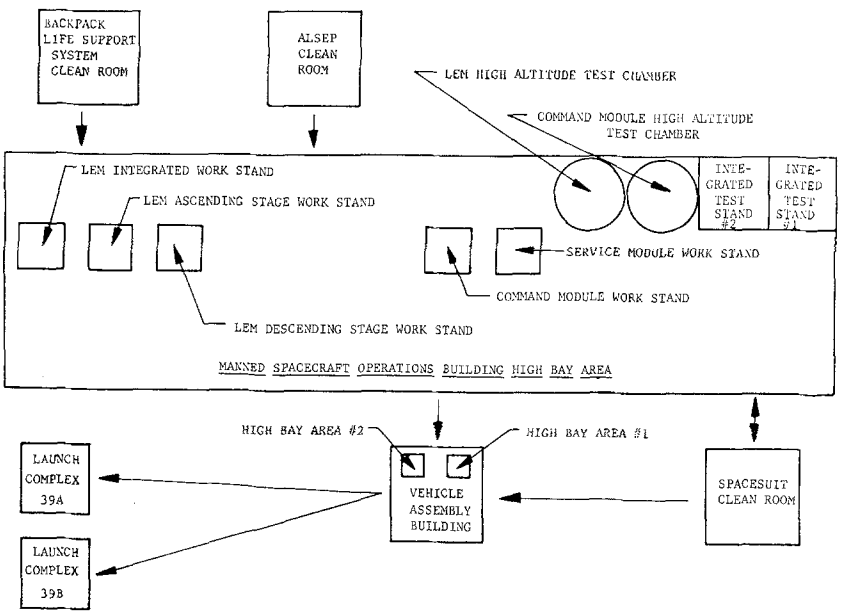

Fig. 1. Facilities associated with the Apollo Program.

located in the high bay area (105 ft). The low bay area (70 ft) contained the other work stands. The spacecraft was exposed to each environment for approximately 1 month. The Service and Command Modules (Spacecraft 20) were assembled and tested in their respective work stands and were then mated in the Integrated Test Stand #2. All of these procedures were performed in the MSOB. The Command and Service Modules, after mating, were transported to the VAB and mounted on the Apollo/Saturn 5 rocket. After final assembly and testing, the whole unit was moved to Launch Complex 39A where it remained until it was launched.

Trays containing approximately 60 sterile stainless steel strips (1″ × 2″) were exposed to the intramural air of various study areas in the MSOB. At weekly intervals six strips from each tray were retrieved and assayed. Each strip was placed in a bottle

containing 50 ml of sterile 1% peptone (Difco Laboratories, Detroit, Mich.*) water (pH 7.0). Bottles were placed in an ultrasonic tank (Branson Instruments, Inc., Stamford, Conn.) containing an 0.3% v/v solution of polyoxyethylene sorbitan monooleate (Tween 80) and insonated (exposed to ultrasonic energy) for 12 minutes (Puleo *et al.*, 1967a, b).

After insonation four 5-ml portions from each bottle were plated with trypticase soy agar (TSA, BBL, Division of BioQuest, Cockeysville, Md.). Two of the plates were incubated aerobically and two anaerobically in Brewer anaerobic jars. The Brewer jars were flushed with nitrogen gas three times, filled with hydrogen gas, and connected to an electrical source for 45 minutes. Incubation was at 32 C for 72 hours. The remaining liquid in each bottle was transferred to a sterile test tube and placed in a water bath at 80 C for 20 minutes. Tubes were removed and four 5-ml portions were plated and incubated.

Sampling locations in the interior of the Command Module were selected to include those areas which had the highest probability of being contaminated by touching or handling. A total of 20 samples was taken at each sampling period. The Command Module exterior (359 sq ft) was divided into four major areas which were the natural quadrants formed by passing two perpendicular planes through the apex and base of the cone. These quadrants were designated as Quadrant 1, 2, 3 and 4, followed by the appropriate positive or negative axis notation. At each sampling period five areas were swabbed in each quadrant, providing a total of 20 samples from the exterior surface of the Command Module.

The Service Module was divided into six sectors which were formed by passing imaginary planes down through the brackets which secure the Command and Service Modules together. Because of the size of the Service Module (13 ft diam, 18 ft high) the sectors were divided into upper and lower areas. Samples were taken from the upper exterior portions of the odd sectors and from the lower exterior portions of the even sectors. Interior samples also were taken from areas and equipment in each sector of the Service Module. At each sampling period a total of 40 samples was taken from the exterior and interior surfaces.

Surfaces to be sampled were outlined with sterile paper templates (4 sq in). Areas of components smaller than 4 sq in were determined by direct measurement. Sterile cotton swabs were immersed in sterile distilled water and rubbed over the surfaces. After sampling the head of each swab was placed in a sterile screw cap test tube and the handle was broken off below any portion that was touched by the sampler. Tubes were taken immediately to the laboratory where 5 ml of sterile 1% peptone water were added to each tube. The samples were insonated for 12 minutes.

One-milliliter portions were plated with TSA. The remaining liquid was heat-shocked at 80 C for 15 min and 1-ml portions plated with TSA. One unheated and one heat-shocked sample were incubated aerobically while the remaining two plates

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were incubated anaerobically in Brewer jars as described previously. Incubation was at 32 C for 72 hours.

All stainless steel fallout strips and swab samples were assayed in a horizontal laminar air flow clean bench.

In each area studied, air samples were obtained with slit samplers (Reyniers and Son, Chicago, Ill.) drawing 1 cu ft of air per minute and using TSA as the collecting medium. After a series of sequential hourly samples, plates were incubated aerobically at 32 C for 72 hours.

These sampling procedures are described further in 'NASA Standard Procedures for the Microbiological Examination of Space Hardware' (NASA, 1968).

3. Results

Air sampling results obtained in various locations in the MSOB are presented in Table I. The highest numbers of airborne viable particles were found at sites 1, 2, 9 and 10. Sites 1 and 2 were high bay areas and sites 9 and 10 were located near the Lunar Module work stands where considerable activity was going on during the sampling period. Table II shows results of air sampling in the Service and Command Module work stands with no personnel activity in the immediate area. These results are comparable to those obtained in similar environmental areas used for assembly of the Surveyor spacecraft. Levels of microbial contamination which accumulated on stainless steel strips located on the Service Module work stand, the Command Module work stand and the Integrated Work Stand #2 (high bay) are shown in Figure 2. Levels in the Command and Service Module work stands were equivalent to those obtained in one of the Surveyor assembly areas. The 'plateau phenomenon' (McDade *et al.*, 1965a, b; Portner *et al.*, 1965) also occurred in the three areas studied.

Microbial contamination isolated from exterior and interior surfaces of the Apollo Command Module as it was moved from one environment to another is shown in

TABLE I

Airborne viable particles per 5 cubic ft within the Manned Spacecraft Operations Building during assembly of Apollo 6

	Sampling sites[a]									
Time	1	2	3	4	5	6	7	8	9	10
0930	13.1	16.3	4.7	6.9	3.3	6.3	4.8	2.1	12.8	7.9
1030	15.3	17.5	5.4	6.3	3.6	8.9	3.5	3.8	14.6	8.1
1130	11.3	11.6	5.9	4.1	4.8	6.0	3.8	3.2	14.3	17.4
1230	17.9	19.1	4.3	3.8	3.0	7.2	3.5	3.1	10.9	7.0
1330	–	–	4.3	5.3	2.8	9.6	4.2	3.2	14.5	16.3
1430	–	–	4.8	4.5	3.8	13.0	5.3	5.3	11.2	9.0
Average	14.4	16.1	4.9	5.15	3.55	8.5	4.18	3.45	13.1	10.5

[a] Distance between sites was 50 ft.

Table III. An increase in the level of microorganisms, especially aerobic spores, on the exterior of the Command Module was observed as it passed among the various environmental areas. Table IV shows that the levels of microbial contamination found on the surfaces of the Apollo Service Module remained fairly constant throughout

TABLE II

Airborne viable particles per 5 cubic ft within two Apollo areas

	Location			
	Service Module work stand		Command Module work stand	
Time	Site 1	Site 2[a]	Site 1	Site 2[b]
0930	3.8	3.8	4.3	7.7
1030	2.9	–	4.6	7.2
1130	3.8	3.3	4.5	8.8
1230	10.4	4.9	3.7	6.2
1330	18.7	7.2	6.2	8.2
1430	14.1	8.1	5.5	8.3
Average	9.0	5.5	4.8	7.7

[a] Air sampler was 16 ft above floor level.
[b] Air sampler was 20 ft above floor level.

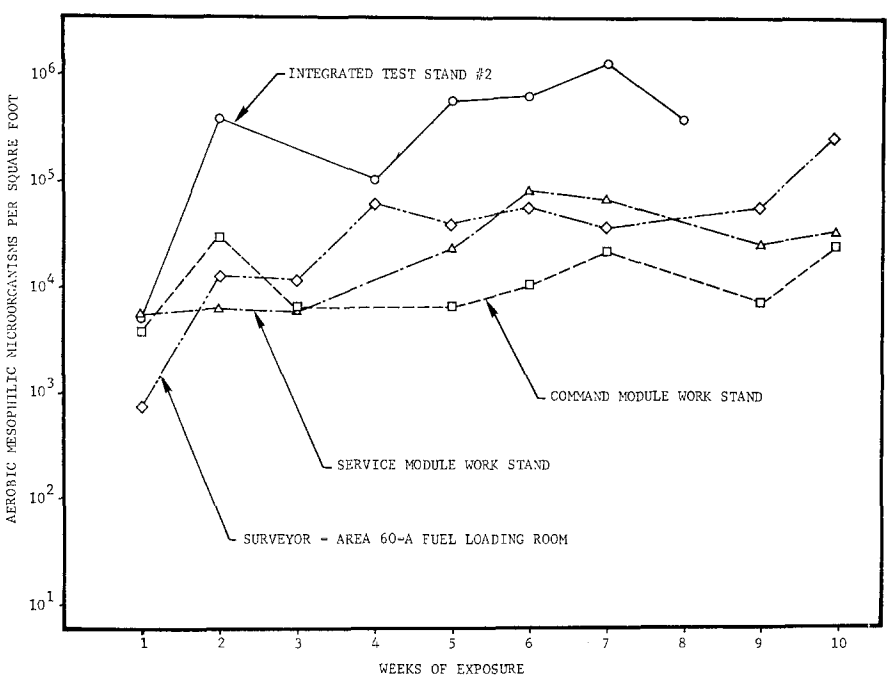

Fig. 2. Comparative levels of airborne microbial contamination which accumulated on stainless steel surfaces exposed in Apollo and Surveyor areas.

TABLE III

Microorganisms per sq ft isolated from exterior and interior surfaces of the Apollo 6 Command Module

Source	Date	Aerobes	Anaerobes	Aerobic spores	Anaerobic spores
Exterior[a]	12/1/67	6579	846	72	72
Exterior[a]	12/6/67	10530	1269	135	81
Exterior[a]	1/3/68	2277	477	558	99
Exterior[b]	1/26/68	4005	1894	117	63
Exterior[c]	2/15/68	13405	2552	382	32
Exterior[c]	2/29/68	31945	4320	503	104
Exterior[c]	3/22/68	11781	4104	612	279
Interior[b]	1/11/68	3182	6615	72	9
Interior[b]	1/26/68	10053	3609	90	9
Interior[c]	2/15/68	7093	4797	42	10

[a] Samples taken while Command Module was located in the Manned Spacecraft Operations Building.
[b] Samples taken while Command Module was located in the Vehicle Assembly Building.
[c] Samples taken while Command Module was located at Launch Complex 39A.

TABLE IV

Microorganisms per sq ft isolated from surfaces of the Apollo 6 Service Module

Date	Aerobes	Anaerobes	Aerobic spores	Anaerobic spores
12/1/67[a]	6983	2494	79	69
12/6/67[a]	8774	3462	114	78
1/3/68[a]	8217	3218	572	206
1/18/68[b]	7086	2114	390	150
2/1/68[b]	5054	1944	189	117
2/23/68[c]	6309	1845	216	63
3/8/68[c]	6714	2646	207	54

[a] Samples taken while Service Module was located in the Manned Spacecraft Operations Building.
[b] Samples taken while Service Module was located in the Vehicle Assembly Building.
[c] Samples taken while Service Module was located at Launch Complex 39A.

the sampling period. A comparison of microbial contamination detected on surfaces of the Apollo Command and Service Modules and three Surveyor spacecraft is presented in Table V. Aerobic mesophilic microorganisms and aerobic spores detected per square inch ranged from 2 to 8 and 3 to 8 times, respectively, higher than those found on the Surveyor spacecraft. Figure 3 shows levels of aerobic and anaerobic spores detected on the exterior of the Command Module as it was moved from one

TABLE V

Comparison of the microbial contamination detected on the surfaces of the Apollo 6 Command and Service Modules and Surveyors 5, 6 and 7

Spacecraft	Area sampled[a] (sq in)	Microorganisms detected per sq in			
		Aerobes	Anaerobes	Aerobic spores	Anaerobic spores
Apollo 6					
Command Module (Exterior)	544	78.6	15.2	2.3	0.7
Command Module (Interior)	228	46.8	34.8	0.5	0.07
Service Module	792	50.3	18.2	1.8	0.8
Surveyor 5	320	28.8	7.1	0.3	0.09
Surveyor 6	240	20.0	1.0	0.4	0.0
Surveyor 7	320	9.1	3.2	0.6	0.2

[a] Swab-rinse technique.

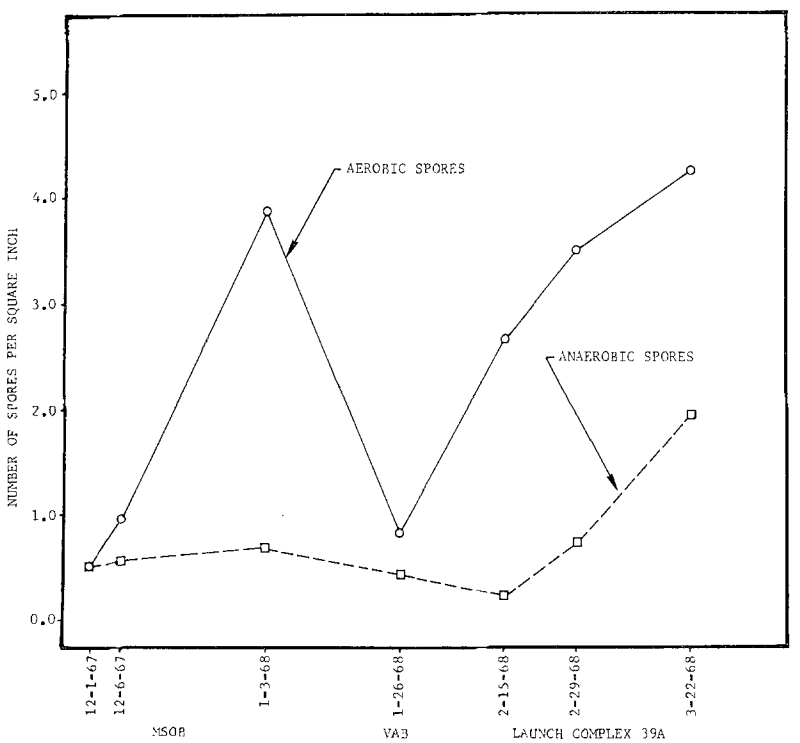

Fig. 3. Comparison of aerobic and anaerobic spores recovered from surface of Apollo 6 Command Module while located in three environmental areas.

environment to another. An increase in the number of spores was noted as the spacecraft was moved from the MSOB to the launch pad, while a decrease occurred when the spacecraft was sampled in the VAB. This reduction could have been due to the washing of the surface of the spacecraft prior to being moved to the VAB.

A representative number of microorganisms isolated from the Command and Service Modules were gram-stained and observed microscopically. When the spacecraft was in the MSOB the majority of these microorganisms were gram positive cocci (Table VI). There was an increase in the number of gram positive (including sporeformers) and gram negative rods when the spacecraft was moved to Launch Complex 39A.

TABLE VI

Staining and morphological characteristics of microorganisms isolated from the surfaces of the Apollo 6 Command and Service Modules

Spacecraft	No. of isolants	Staining and morphology	Percent
Apollo 6 Command and Service Modules[a]	207	Gram positive cocci	60.4
		Gram positive rods	10.1
		Gram negative rods	6.3
		Actinomycetes	1.0
		Yeasts	2.4
		Molds	19.8
Apollo 6 Command Module[b]	159	Gram positive cocci	56.0
		Gram positive rods	25.2
		Gram negative rods	13.2
		Actinomycetes	0.0
		Yeasts	0.6
		Molds	5.0

[a] Spacecraft was located at Manned Spacecraft Operations Building.
[b] Spacecraft was located at Launch Complex 39A.

4. Discussion

It is evident from the results obtained in this study that the levels of microorganisms on surfaces of the Apollo 6 spacecraft were higher than those detected on unmanned spacecraft. One of the major contributing factors was the environment in which the spacecraft was assembled and tested. Presumably because of the size of the MSOB (length 655 ft, width 84 ft) and the different types of activities in the high bay and low bay areas, the levels of microbial contamination varied within the building (Figure 2). A 1- to 2-log difference in surface contamination (fallout strips) was noted between the high bay and low bay areas. In addition air sampling revealed a high level of airborne viable particles in the high bay area.

Personnel clothing restraints also varied. Those working on the Service Module and the Command Module exterior areas wore three-quarter length laboratory coats and hats, while personnel working inside the Command Module wore a clean room uniform consisting of coveralls, shoe covers, and cap. This could account for the level of aerobic mesophilic microorganisms and spores in the interior of the Command Module being less than that found on the exterior surfaces.

As the spacecraft was moved from one assembly area to another and the environment was less controlled with respect to particulate contamination, numbers of microorganisms which accumulated on the exposed surfaces tended to increase. The reason that the increase was not so great as would normally be expected could have been due to the periodic washing of the exterior surfaces of the Command and Service Modules. While at the MSOB and VAB areas, the Command and Service Modules were covered by a plastic film.

The majority of the microorganisms found on the spacecraft were those considered indigenous to humans; however, a two-fold increase in the number of microorganisms associated with soil and dust (sporeformers) was noted when the spacecraft was at the launch complex.

In conclusion, this initial study of an Apollo spacecraft and the environments in which it was assembled and tested showed that there were higher levels of microbial contamination detected on the Apollo spacecraft than automated lunar spacecraft. This difference emphasizes the importance of personnel and environmental controls.

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