

Quantitative and Qualitative Microbiological Profiles of the Apollo 10 and 11 Spacecraft

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Microbiological profiles were determined for surfaces of the command module, lunar module (ascent and descent stages), instrument unit, Saturn S-4B stage, and the spacecraft lunar module adapter of the Apollo 10 and 11 spacecraft. Average levels of contamination of the command module were 2.1×10^4 and 2.7×10^4 microorganisms per ft² for Apollo 10 and 11, respectively. With the exception of the exterior surfaces of the ascent stage of the lunar module and the interior surfaces of the command module, average levels of microbial contamination on all components of the Apollo 11 were found to be lower than those observed on Apollo 10. For each Apollo mission, approximately 2,000 colonies were picked from a variety of media and identified. The results showed that approximately 95% of all isolates were those considered indigenous to humans; the remaining were associated with soil and dust in the environment. However, the ratio of these two general groups varied depending on the degrees of personnel density and environmental control associated with each module.

The National Aeronautics and Space Administration requires that microbial contamination that is brought into contact with the surface of the moon by spacecraft be quantified and identified (NASA Policy Directive NPD 8020.7, paragraph 3.b., September 6, 1967, National Aeronautics and Space Administration, Washington, D.C.). In addition attempts are made to keep the level of contamination on spacecraft relatively low. There are two basic reasons for this policy. The first is concerned with protecting the moon from excessive microbial contamination, and the second is associated with problems of "back contamination" (3, 6).

The probability that the lunar surface has been contaminated with terrestrial microorganisms from unsterile spacecraft has increased with each landing mission. Consequently, it is necessary to determine the levels and types of microbial contaminants with each spacecraft to aid in the interpretation of biological data obtained from the moon in future experiments.

Material returned from the moon is quarantined for a period of time and subjected to a variety of tests to insure that no pathogenic or toxic agents are present. A variety of chemical and biological assays are performed to determine whether life exists or did exist at one time on the

moon. Since the lunar samples are not collected aseptically, there is a good possibility that they could be contaminated with terrestrial microorganisms from the spacecraft or the astronauts, or both. Detection of these contaminants by the various life detection systems used by investigators at the Lunar Receiving Laboratory and the Ames Research Center would represent false-positive tests. Not only could release of the samples be delayed, but, more importantly, the results of the exobiological tests would become ambiguous. This problem can be resolved, in part, if microbiological profiles of the spacecraft and crew have been determined before launch.

The primary objective of this study was to determine the levels and types of microbial contamination present on the Apollo 10 and 11 spacecraft before launch.

MATERIALS AND METHODS

Microbiological assays were conducted on the Apollo 10 and 11 spacecraft during assembly and testing. Sampling locations were selected on the interior and exterior surfaces of various spacecraft components. A prerequisite for sites was that they be representative surfaces of the entire spacecraft and be accessible throughout the sampling periods. The interior surfaces of the command module, lunar module ascent stage, instrument unit, Saturn S-4B stage, and spacecraft lunar module adapter were

¹ Deceased.

included in these tests as well as the exterior surfaces of the ascent and descent stages of the lunar module. The various spacecraft components were studied at three periods during assembly and testing. The command module was sampled at 14 and 7 days and 24 hr before launch. The sampling periods for the other spacecraft components were 14 and 7 days and 65 hr before launch. At each interval, 15 locations on each spacecraft component were sampled.

Sterile cotton swabs, moistened in sterile distilled water, were rubbed over the surfaces to be sampled, which were outlined with a sterile paper template (4 square inches). Surface areas smaller than 4 square inches were determined by direct measurement. Five swabs were returned to a sterile screw-cap test tube (25 by 150 mm) containing 25 ml of sterile buffered rinse solution with 0.02% (v/v) solution of polyoxyethylene sorbitan monooleate. The swab heads were broken off below the portion of the handles touched by the sampler. Tubes were taken immediately to the laboratory, agitated on a vortex mixer for 5 to 10 sec, placed in an ultrasonic bath (tank, LTH60-3; generator, A-300; Branson Instruments, Inc., Stamford, Conn.) containing a 0.3% (v/v) solution of polyoxyethylene sorbitan monooleate, and insonated for 2 min at 25 kHz (4, 7, 8).

After insonation, portions from each tube were plated with Trypticase Soy Agar (TSA; BBL) and also spread over the surface of blood-agar (TSA plus 5% horse blood), MacConkey Agar (BBL), and Mycophil Agar (BBL). Spore assays were performed by heat-shocking the remaining rinse fluid in each tube at 80 C for 15 min before plating. Brewer jars for anaerobic incubation were flushed three times with a gas mixture of nitrogen (80%), carbon dioxide (10%), and hydrogen (10%), filled a fourth time with the gas mixture, and connected to an electrical source for 45 min for catalytic removal of oxygen.

All laboratory procedures were performed in a horizontal laminar flow clean bench. Other details of the sampling procedure are described in *NASA Standard Procedures for the Microbiological Examination of Space Hardware* (4).

Plates were incubated at 32 C for 72 hr and colony counts were performed after 48 and 72 hr. For each Apollo mission, approximately 2,000 colonies were picked from culture plates, Gram-stained, and identified.

Micrococcaceae were classified by the scheme of Baird-Parker (1); aerobic sporeformers (*Bacillus* spp.) by the method of Smith, Gordon, and Clark (11); *Enterobacteriaceae* by the schemes of Edwards and Ewing (32); and the *Pseudomonas-Achromobacter-Flavobacterium* group and related gram-negative bacteria by the method described by Shewan, Hobbs, and Hodgkiss (10). *Bergey's Manual* (7th ed.) was used for classifying other groups of bacteria.

RESULTS AND DISCUSSION

Observed levels of microbial contamination are presented in Table 1. Although the levels of aerobic mesophilic microorganisms were lower on the instrument unit, Saturn S-4B, and spacecraft

lunar module adapter of both spacecraft than on the CM-106 and CM-107, the concentrations of bacterial spores and molds on these components were higher than on the two command modules. The covering of the instrument unit and Saturn S-4B area with hypergolic covers, plus the forcing of high volumes of air (1,300 to 2,100 ft³ per min) beneath the covers, could have caused the reduction in microbial contamination by means of desiccation and physical removal. In addition, the surface of the spacecraft lunar module adapter was vertical and would be expected to be contaminated with fewer microorganisms.

Table 2 lists data collected from lunar modules 4 and 5 (LM-4 and LM-5). The levels of microbial contamination on the interior surface of both lunar modules were about 1 to 1.5 logs higher per ft² than those detected on the exterior surfaces of both stages. However, the exterior surfaces of the ascent and descent stages of both the LM-4 and LM-5 showed higher percentages of spores and molds than were detected on the interior surfaces with large volumes of filtered air. The presence of fewer personnel in these areas probably accounted for the reduction in the levels of vegetative bacteria, resulting in a relatively low population and one which was composed of molds and sporeformers resistant to desiccation. With the exception of the interior surface of the command module (Table 1) and the exterior surface of the ascent stage of the lunar module (Table 2), levels of microbial contamination on all components of Apollo 11 were found to be about 1 log lower per ft² than those observed on Apollo 10.

A nonparametric statistical test (sign test) was used to determine whether TSA or blood-agar consistently recovered higher numbers of microorganisms from pooled samples of environmental swabs. The test was applied to four categories: Apollo 10 aerobes, Apollo 10 anaerobes, Apollo 11 aerobes, and Apollo 11 anaerobes. Each category consisted of 21 pairs of observations from 7 different spacecraft components. Only in the Apollo 10 aerobes category did recovery from TSA exceed recovery from blood-agar a significant number of times (16 of 21, $\alpha < 0.05$). Results from the other three categories indicated that neither medium was consistently superior. In addition, no differences were noted in the types of microorganisms recovered from both media.

A total of 1,991 and 2,041 bacterial colonies were picked and subsequently identified from the Apollo 10 and 11 spacecraft, respectively. There were 39 different types or groups isolated and identified for both spacecraft; six were detected only on Apollo 10 and two were detected only on the Apollo 11 spacecraft. Table 3 shows the types

TABLE 1. Comparison of the levels of microbial contamination detected on components of the Apollo 10 and 11 spacecraft

Source	Microorganisms per square foot ^a				Per cent ^b	
	Aerobic count ^c	Anaerobic count ^d	Aerobic spore count ^e	Anaerobic spore count ^f	Aerobic spores	Molds
Command module						
Apollo 10 (CM-106).....	2.1×10^4	1.3×10^4	1.7×10^2	21	0.80	0.02
Apollo 11 (CM-107).....	2.7×10^4	1.6×10^4	1.3×10^2	88	0.46	0.07
Instrument unit						
Apollo 10.....	1.5×10^4	2.7×10^3	1.9×10^3	269	12.94	3.95
Apollo 11.....	7.6×10^3	3.7×10^3	1.3×10^3	162	17.33	7.79
Saturn S-4B						
Apollo 10.....	2.1×10^4	3.3×10^3	3.1×10^3	321	14.66	1.97
Apollo 11 ^g	9.6×10^3	3.0×10^3	1.9×10^3	411	19.59	4.86
Spacecraft lunar module adapter						
Apollo 10.....	215	24	12	16	5.58	1.86
Apollo 11.....	83	24	65	24	78.31	4.82

^a Average of three final sampling periods; total area sampled was 180 square inches.

^b Percentage of total aerobic mesophilic microorganisms.

^c Samples not heat-shocked; aerobic incubation.

^d Samples not heat-shocked; anaerobic incubation.

^e Samples heat-shocked; aerobic incubation.

^f Samples heat-shocked; anaerobic incubation.

^g Total area sampled was 160 square inches.

TABLE 2. Comparative levels of microbial contamination detected on the lunar modules (ascent and descent stages) of the Apollo 10 and 11 spacecraft

Source	Microorganisms per square foot ^a				Per cent ^b	
	Aerobic count ^c	Anaerobic count ^d	Aerobic spore count ^e	Anaerobic spore count ^f	Aerobic spores	Molds
Ascent stage, interior						
LM-4 (Apollo 10).....	1.8×10^5	1.0×10^5	3.7×10^2	32	0.21	0.002
LM-5 (Apollo 11).....	8.2×10^4	3.1×10^4	3.3×10^2	64	0.41	0.03
Ascent stage, exterior						
LM-4 (Apollo 10).....	5.0×10^3	1.1×10^3	1.5×10^2	20	3.10	0.32
LM-5 (Apollo 11).....	5.1×10^3	1.2×10^3	1.8×10^2	36	3.50	2.68
Descent stage, exterior						
LM-4 (Apollo 10) ^g	1.6×10^4	1.1×10^4	5.1×10^2	54	3.13	1.08
LM-5 (Apollo 11).....	4.6×10^3	1.1×10^3	2.6×10^2	24	5.69	1.14

^a Average of three final sampling periods; total surface area sampled was 180 square inches.

^b Percentage of total aerobic mesophilic microorganisms.

^c Samples not heat-shocked; aerobic incubation.

^d Samples not heat-shocked; anaerobic incubation.

^e Samples heat-shocked; aerobic incubation.

^f Samples heat-shocked; anaerobic incubation.

^g Total area sampled was 140 square inches.

of aerobic mesophilic microorganisms isolated from each component of the Apollo spacecraft by using TSA. The distribution by types of microorganisms on components of Apollo 10 and 11 was remarkably similar.

The percentages of those microorganisms con-

sidered to be indigenous to humans (i.e., *Staphylococcus* spp., *Micrococcus* spp., and the *Corynebacterium-Brevibacterium* group) are shown in Fig. 1. The highest percentages were detected on the interior surfaces of the command and lunar modules. The other components of the spacecraft

TABLE 3. Percentages and types of microorganisms detected on the Apollo 10 and 11 spacecraft^a

Type	CM	LAI	LAE	LDE	SLA	IU	S-4B	All components of spacecraft
<i>Apollo 10</i>								
<i>Staphylococcus</i> spp.								
Subgroup I.....		9.5	1.6	8.3				4.8
Subgroup II.....	14.7	13.6	2.3	5.0		3.2	1.6	10.2
Subgroup III.....	3.5	3.7			5.9	4.8		2.7
Subgroup IV.....	8.4	4.6	3.9	4.2	11.8	4.8	6.2	5.7
Subgroup V.....	14.4	2.5	12.4	7.5	11.8	17.5	1.6	8.2
Subgroup VI.....	7.4	5.8	9.3	8.3		1.6	4.7	6.5
<i>Micrococcus</i> spp.								
Subgroup 1.....	7.7	14.1	0.8	5.8		3.2	3.1	8.6
Subgroup 2.....	7.0	3.7	3.9	5.8				4.3
Subgroup 3.....	2.1	4.9	5.4	3.3				3.4
Subgroup 5.....	0.7	1.6	0.8	0.8				1.0
Subgroup 7.....	4.9	5.3	4.6	12.5	5.9	4.8	4.7	5.8
Atypical micrococci.....		0.9						0.4
<i>Streptococcus-Enterococcus</i> group.....			0.8					0.1
<i>Streptococcus-Viridans</i> group.....		3.0						1.2
<i>Streptococcus-pyogenic</i> group.....		0.5						0.2
<i>Bacillus</i> spp.								
<i>B. brevis</i>	0.4	0.2						0.2
<i>B. cereus</i>	0.4							0.1
<i>B. circulans</i>	0.4		1.6		5.9	4.8	1.6	0.7
<i>B. coagulans</i>				1.7				0.2
<i>B. firmus</i>			0.8					0.1
<i>B. lentus</i>	0.4		4.6					0.6
<i>B. licheniformis</i>							3.1	0.2
<i>B. megaterium</i>							1.6	0.1
<i>B. pantothenicus</i>		0.2						0.1
<i>B. polymyxa</i>	0.4					1.6		0.2
<i>B. pulvifaciens</i>	0.4				5.9		1.6	0.3
<i>B. pumilus</i>							1.6	0.1
<i>B. sphaericus</i>			0.8					0.1
<i>Corynebacterium-Brevibacterium</i> group.....	2.1	3.5	20.2	11.7	17.6	19.0	23.4	8.2
<i>Alcaligenes</i> spp.....			0.8				1.6	0.2
<i>Flavobacterium</i> spp.....	0.4							0.1
Actinomycetes.....			1.6					0.2
Yeasts.....	0.4							0.1
Molds.....	0.4			0.8	5.9	4.8	3.1	0.7
No growth on subculture.....	23.9	22.2	24.0	24.2	29.4	30.2	40.6	24.7
<i>Apollo 11</i>								
<i>Staphylococcus</i> spp.								
Subgroup I.....	0.3	2.8						1.2
Subgroup II.....	16.0	13.9	11.1	10.3		17.2	3.2	13.2
Subgroup III.....	0.3	0.6						0.3
Subgroup IV.....	15.3	12.0	10.4	2.8			1.6	10.2
Subgroup V.....	14.7	5.0	3.7	9.6		7.8	6.3	8.1
Subgroup VI.....	8.3	11.5	4.4	2.8		4.7	1.6	7.9

^a Abbreviations: CM, command module; LAI, lunar module ascent stage; LAE, exterior surfaces of ascent stage of lunar module; LDE, exterior surfaces of descent stage of lunar module; SLA, spacecraft lunar module adapter; IU, instrument unit; S-4B, Saturn S-4B stage.

had higher levels of bacterial sporeformers, molds, and actinomycetes, which are associated with soil and dust. This qualitative picture also was observed with Apollo 7, 8, and 9 spacecraft (7). Only a few gram-negative microorganisms were

isolated (Table 3). The anaerobic counts (Tables 1 and 2) were due entirely to the growth of facultative bacteria. No strictly anaerobic bacteria or *Clostridia* spp. were detected.

The genera of molds isolated from Apollo 10

TABLE 3—Continued

Type	CM	LAI	LAE	LDE	SLA	IU	S-4B	All components of spacecraft
<i>Micrococcus</i> spp.								
Subgroup 1.....	12.0	12.8	9.6	9.6		4.7	4.8	10.9
Subgroup 2.....	5.0	3.5	3.0	4.1		4.7	19.0	4.8
Subgroup 3.....	0.3	2.0	4.4	0.7		1.6	3.2	1.7
Subgroup 5.....	2.7	1.3		1.4			4.8	1.6
Subgroup 7.....	5.7	5.4	8.9	6.9	25.0	3.1	12.7	6.4
Subgroup 8.....			0.7					0.1
Atypical micrococci.....	0.3	2.0	0.7	23.4				3.8
<i>Streptococcus</i> -Viridans group.....	0.3							0.1
<i>Bacillus</i> spp.								
<i>B.adius</i>				1.4				0.2
<i>B. brevis</i>				0.7				0.1
<i>B. cereus</i>						1.6		0.1
<i>B. circulans</i>			0.7		25.0			0.2
<i>B. coagulans</i>						1.6	3.2	0.2
<i>B. firmus</i>						1.6		0.1
<i>B. lentus</i>			0.7				3.2	0.2
<i>B. licheniformis</i>	0.3				25.0			0.2
<i>B. pantothenicus</i>		0.6	0.7			1.6		0.4
<i>B. polymyxa</i>	0.3					6.2	4.8	0.7
<i>B. pumilus</i>		0.6	0.7	2.1			1.6	0.7
<i>B. sphaericus</i>				0.7				0.1
Atypical <i>Bacillus</i> spp.....			2.2					0.2
<i>Corynebacterium</i> - <i>Brevibacterium</i> group.....	5.3	10.9	15.6	10.3		21.9	14.3	10.7
<i>Achromobacter</i> spp.....				0.7				0.1
<i>Alcaligenes</i> spp.....			6.7					0.8
Actinomycetes.....		0.2	0.7	0.7		1.6	4.8	0.6
Yeasts.....			1.5	0.7		6.2		0.6
Molds.....		0.4	1.5	2.8	25.0	1.6	6.3	1.2
No growth on subculture.....	12.7	14.2	11.8	8.3		12.5	4.8	12.1
Total no. isolated.....	585	891	264	265	21	127	127	2,280

and 11 are listed in Table 4 and are those commonly associated with soil and dust found in the environment. Although the table shows that a greater number of molds was isolated from Mycophil Agar than from TSA, this does not indicate that more mold colonies developed on Mycophil Agar. Identifications were made on all mold colonies that developed on Mycophil Agar but on only a percentage of the colonies which grew on TSA. To determine if extended incubation would increase the recovery of molds, colony counts on TSA were performed at 3, 7, 14, and 21 days. From a total of 362 culture plates selected from 12 sampling periods, only six plates showed an increase in the mold count and these increased by only one mold colony on each plate. These results indicated that there was no significant increase in the number of mold colonies after 72 hr of incubation at 32 C.

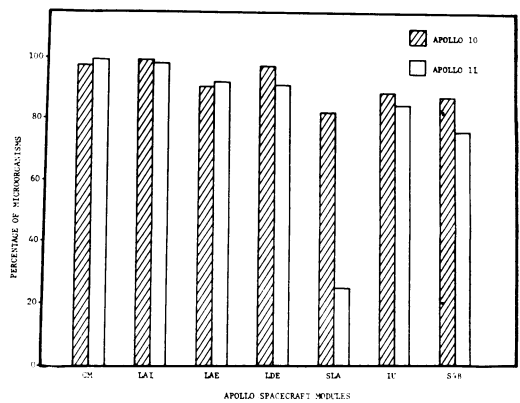


FIG. 1. Percentages of microorganisms considered to be indigenous to humans detected on the Apollo 10 and 11 spacecraft.

TABLE 4. *Genera of molds detected on the Apollo 10 and 11 spacecraft*

Genus	No. of isolates			
	Tryp- ticase Soy Agar	Blood agar	Mac- Conkey Agar	Mycoph- il Agar
<i>Helminthosporium</i>	5	0	0	5
<i>Aspergillus</i>	3	0	0	3
<i>Pithomyces</i>	2	0	0	4
<i>Curvularia</i>	3	2	0	3
<i>Alternaria</i>	4	1	2	6
<i>Spegazzinia</i>	0	0	0	2
<i>Fusarium</i>	0	0	2	2
<i>Phoma</i>	0	0	0	1
<i>Pyrenochaeta</i>	0	0	0	1
<i>Bipolaris</i>	1	0	0	4
<i>Drechslera</i>	0	0	0	4
<i>Scopulariopsis</i>	1	0	0	0
<i>Penicillium</i>	1	1	2	0
<i>Nigrospora</i>	2	0	0	0
<i>Mucor</i>	0	1	0	0
<i>Syncephalastrum</i>	1	0	0	0
Total.....	23	5	6	35

Contamination levels on most parts of the Apollo 10 and 11 spacecraft were similar to prior Apollo spacecraft and relatively high in comparison with some of the automated spacecraft [anchored interplanetary monitoring platform (A-IMP), Surveyor, and lunar orbiter (5)]. This is probably due to the fact that the automated spacecraft were tested and assembled in areas that had more environmental and personnel controls. For example, the A-IMP was assembled in a class 100 vertical laminar flow clean room with appropriate restraints on the types of personnel clothing and personnel density. This type of environment is several orders of magnitude cleaner than the areas used for assembling the

Apollo spacecraft. Consequently, it is not surprising that the contamination levels were higher on Apollo spacecraft.

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