APPLIED MICROBIOLOGY, Dec. 1973, p. 838-845
Copyright © 1973 American Society for Microbiology
Vol. 26, No. 6
Printed in U.S.A.

Microbiological Profiles of Four Apollo Spacecraft

J. R. PULEO, G. S. OXBORROW, N. D. FIELDS, C. M. HERRING, AND L. S. SMITH

Environmental Microbiology Section, Ecological Investigations Program, Center for Disease Control, Cape Canaveral, Florida 32920

Received for publication 11 July 1973

Selected surfaces from the Command Module, Lunar Module (ascent and descent stages), Instrument Unit, Saturn S-4B engine, and Spacecraft Lunar Module Adapter comprised the various components of four Apollo spacecraft which were assayed quantitatively and qualitatively for microorganisms. In addition, the first Lunar Roving Vehicle was assayed. Average levels of microbial contamination (10^4 per square foot of surface) on the Command Module, Instrument Unit, and Saturn S-4B engine were relatively consistent among spacecraft. The first postflight sampling of interior surfaces of the Command Module was possible due to elimination of the 21-day back-contamination quarantine period. Results of the pre- and postflight samples revealed increases in the postflight samples of 3 logs/inch^2. A total of 5,862 microbial isolates was identified; 183 and 327 were obtained from the Command Module at preflight and postflight sampling periods, respectively. Although the results showed that the majority of microorganisms isolated were those considered to be indigenous to humans, an increase in organisms associated with soil and dust was noted with each successive Apollo spacecraft.

Microbiological profiles of automated and manned spacecraft are being determined on a continuous basis due to national and international agreements which stipulate that microorganisms which have a potential of being transported in a viable state to the surface of the moon be enumerated and identified, and an inventory of the levels of contamination at each landing site be maintained (13). In addition, because the existence of life on other planets is possible, scientific investigations for determining the possibility of extraterrestrial life forms must not be jeopardized. Contamination of Mars and other planets of biological interest with terrestrial microorganisms will be controlled to the extent that the probability of contamination will be 1 in 1,000 (10^{-3}) (5, 10, 11, 15). Dry-heat sterilization of the spacecraft will be employed to achieve these objectives. The assessment of microbial contamination levels, especially bacterial spores on space hardware, will be one of the essential controlling parameters in the sterilization of interplanetary spacecraft because the number of bacterial spores present on the spacecraft, as determined by microbiological assays, will determine the extent and character of the dry-heat sterilization cycle (2, 3, 10, 11).

The objective of this study was to determine and compare the levels and types of microorganisms on various components of four Apollo spacecraft.

MATERIALS AND METHODS

Microbiological assays were conducted on Apollo spacecraft during assembly and testing, and 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 Command Module (CM), Lunar Module ascent stage, Instrument Unit (IU), Saturn S-4B stage (S-4B), and Spacecraft Lunar Module Adapter (SLA) were interior surfaces studied. Exterior surfaces included the ascent and descent stages of the Lunar Module, and, for Apollo 15, the first Lunar Roving Vehicle (LRV). The various spacecraft components were studied at three periods during assembly and testing. The CM was sampled at 14 days, 7 days, and 24 h, and other spacecraft components were sampled at 14 days, 7 days, and 57 h, respectively, 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 or aluminum 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% (vol/vol) solution of Tween 80 (polyoxyethylene sorbitan monooleate, Hilltop Research, Inc., Miamiville, Ohio). The swab heads were broken off below the portion of the handles touched by the sampler. Tubes were taken

TABLE 1. *Comparison of the levels of microbial contamination detected on components of the Apollo 12, 13, 14, and 15 spacecraft*

Source	No. microorganisms per square foot[a]				Percent[b]	
	Aerobes[c]	Anaerobes[d]	Aerobic spores[e]	Anaerobic spores[f]	Aerobic spores	Molds
Command Module						
Apollo 12	2.9×10^4	1.4×10^4	4.0×10^1	2.4×10^1	0.14	0.00
Apollo 13	4.1×10^4	1.8×10^4	5.2×10^1	1.2×10^1	0.13	0.02
Apollo 14	7.1×10^4	2.8×10^4	1.7×10^2	6.0×10^1	0.24	0.005
Apollo 15	4.7×10^4	2.0×10^4	2.1×10^2	4.2×10^1	0.37	0.03
Instrument Unit						
Apollo 12	2.0×10^4	4.6×10^3	6.3×10^2	2.6×10^2	3.16	0.84
Apollo 13	1.0×10^4	1.7×10^3	5.4×10^2	1.4×10^2	5.46	2.05
Apollo 14	3.1×10^4	8.5×10^3	1.2×10^3	2.5×10^2	3.73	2.19
Apollo 15	2.8×10^4	3.5×10^3	1.4×10^3	3.3×10^2	4.89	1.78
Saturn S-4B						
Apollo 12	3.0×10^4	4.1×10^3	1.1×10^3	1.9×10^2	3.69	0.20
Apollo 13	1.3×10^4	2.1×10^3	1.0×10^3	2.0×10^2	7.92	1.02
Apollo 14	4.8×10^4	8.6×10^3	1.7×10^3	3.8×10^2	3.63	1.41
Apollo 15	5.2×10^4	9.7×10^3	2.1×10^3	3.8×10^2	4.02	1.32
Spacecraft Lunar Module Adapter						
Apollo 12	2.8×10^1	1.6×10^1	1.6×10^1	3.2×10^1	57.10	0.00
Apollo 13	1.6×10^1	8.0×10^0	4.0×10^0	0.0	25.00	0.00
Apollo 14	1.8×10^2	5.6×10^1	2.0×10^1	0.0	10.90	0.00
Apollo 15[g]	4.2×10^1	6.0×10^0	1.2×10^1	0.0	28.57	0.00
Ascent stage (interior)						
Apollo 12	4.9×10^4	1.3×10^4	7.2×10^1	2.4×10^1	0.15	0.16
Apollo 13	3.7×10^4	9.0×10^3	7.6×10^1	3.6×10^1	0.20	0.02
Apollo 14	1.1×10^5	5.5×10^4	3.1×10^2	6.0×10^1	0.29	0.02
Apollo 15	3.4×10^4	1.5×10^4	6.0×10^2	2.1×10^2	1.79	0.17
Ascent stage (exterior)						
Apollo 12	2.0×10^3	7.2×10^2	5.6×10^1	2.4×10^1	2.75	0.39
Apollo 13	2.7×10^3	6.1×10^2	3.6×10^1	1.2×10^1	1.33	0.74
Apollo 14	2.1×10^4	3.3×10^3	1.8×10^2	4.0×10^1	0.88	0.34
Apollo 15[g]	8.0×10^3	1.7×10^3	6.7×10^2	8.4×10^1	8.29	0.97
Descent stage (exterior)						
Apollo 12	1.1×10^4	5.2×10^3	1.4×10^2	2.8×10^1	1.25	0.44
Apollo 13	3.4×10^4	2.5×10^4	6.8×10^1	1.2×10^1	0.20	0.08
Apollo 14	1.1×10^5	6.0×10^4	2.3×10^2	4.0×10^1	0.21	0.04
Apollo 15[g]	2.1×10^4	9.2×10^3	3.3×10^2	1.3×10^2	1.58	0.35
Lunar Roving Vehicle 1						
Apollo 15	1.4×10^3	4.2×10^2	9.2×10^1	3.2×10^1	6.39	3.61

[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] Average of two sampling periods; total area sampled was 120 square inches.

TABLE 2. *Comparison of the numbers and types of microorganisms detected on four Apollo spacecraft*

Microorganism	Apollo 12	Apollo 13	Apollo 14	Apollo 15[a]
Staphylococcus spp.				
Subgroup I	1	0	11	30
Subgroup II	334	152	161	163
Subgroup III	29	2	105	10
Subgroup IV	229	51	85	85
Subgroup V	155	34	110	194
Subgroup VI	217	48	118	106
Micrococcus spp.				
Subgroup 1	161	124	22	40
Subgroup 2	82	80	2	27
Subgroup 3	77	75	11	8
Subgroup 4	1	0	0	2
Subgroup 5	20	4	7	15
Subgroup 6	3	0	0	0
Subgroup 7	283	220	121	45
Subgroup 8	0	0	1	1
Streptococcus-Viridans group	0	1	0	0
Bacillus spp.				
B. alvei	2	0	1	0
B. badius	5	0	1	10
B. brevis	1	0	0	10
B. cereus	7	4	4	11
B. circulans	27	5	15	19
B. coagulans	7	4	20	19
B. firmus	9	4	5	2
B. laterosporus	1	0	0	0
B. lentus	8	4	4	14
B. licheniformis	3	1	2	20
B. macerans	0	0	2	3
B. megaterium	0	0	0	4
B. pantothenticus	11	3	0	4
B. polymyxa	4	2	10	10
B. pulvifaciens	5	2	0	1
B. pumilus	0	0	3	0
B. sphaericus	5	2	13	3
B. subtilis	6	1	3	8
Corynebacterium-Brevibacterium group	172	159	158	134
Alcaligenes spp.	2	0	0	0
Flavobacterium spp.	8	0	0	0
Actinomycetes	3	2	7	3
Streptomycetes	1	1	4	0
Yeasts	13	18	2	16
Molds	36	14	31	36
Atypical *Micrococcus* spp.	28	2	36	24
Atypical *Bacillus* spp.	2	1	30	77
No growth on subculture	75	0	12	18
No. isolated	2,037	1,020	1,123	1,172

[a] Does not include microorganisms isolated from pre- and postflight samples.

immediately to the laboratory, agitated on a Vortex mixer for 5 to 10 s, placed in an ultrasonic bath (tank LTH60-3; generator, A-300; Branson Instruments, Inc., Stamford, Conn.) containing a 0.3% (vol/vol) Tween 80, and insonated for 2 min at 25 kHz (14, 18, 19). Randomly, sterile swabs were moistened in sterile distilled water and then returned to sterile screw-cap tubes containing sterile buffered rinse solution with 0.02% (vol/vol) solution of Tween 80. These swabs were then assayed as described above and served as controls.

After insonation, replicate portions from each tube were plated with Trypticase soy agar (TSA; BBL). For Apollo 12, portions also were spread over the surface of blood agar (TSA plus 5% defibrinated sheep 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 and plating with TSA. 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.

The CM interior surfaces of Apollo 15 were sampled at approximately 9 h (preflight) prior to launch and also after the mission (postflight) when the CM was taken on board the recovery vessel. The sampling procedures were similar to the above, except that each cotton swab was placed into 10 ml of sterile veal infusion broth. The postflight samples were kept at 4 C, transported to the Planetary Quarantine Laboratory at Cape Kennedy, Fla., and assayed within 30 h after being taken. In addition to plating on TSA, portions were spread over the surfaces of blood agar and blood agar enriched with vitamin K and hemin. All media except TSA were incubated at 37 C under aerobic, anaerobic, and CO_2 conditions. The TSA culture plates were incubated at 32 C under aerobic conditions.

All laboratory procedures were performed in a horizontal laminar flow clean bench (7) to eliminate background contamination. Other details of the sampling procedure have been described previously (14).

Plates were incubated at 32 C and colony counts were performed after 48 and 72 h. For each Apollo mission, 1,000 to 2,000 colonies were picked randomly from culture plates, gram stained, and identified. All isolates were subsequently lyophilized and stored for future reference.

Micrococcaceae were classified by the scheme of Baird-Parker (1), aerobic sporeformers (*Bacillus* spp.) were classified by the method of Smith et al. (24), *Enterobacteriaceae* were classified by the schemes of Edwards and Ewing (4), and the *Pseudomonas-Achromobacter-Flavobacterium* group and related gram-negative bacteria were classified by the method described by Shewan et al. (23). *Bergey's Manual* (7th ed.) was used for classifying other groups of bacteria.

RESULTS AND DISCUSSION

Comparison of the levels of microbial contamination detected on the four Apollo spacecraft is shown in Table 1. Aerobic mesophilic

TABLE 3. *Percentage of microorganisms considered to be indigenous to humans on Apollo 12, 13, 14, and 15*

Apollo[a]	CM	LAI	LAE	LDE	SLA	IU	LRV-1	S-4B	Total spacecraft
12	98	97	89	94	100	79		80	94
13	99	97	98	96	100	77		83	95
14	94	94	89	83	94	64		51	86
15	94	88	67	72	0	49	70	52	78

[a] Abbreviations: CM, Command Module (interior); LAI, Lunar Module, interior ascent stage; LAE, Lunar Module, exterior ascent stage; LDE, Lunar Module, exterior descent stage; SLA, Spacecraft Lunar Module Adapter; IU, Instrument Unit; LRV-1, Lunar Roving Vehicle-1; S-4B, Saturn S-4B.

TABLE 4. *Genera of molds detected on Apollo spacecraft*

Genera	No. of isolates			
	Apollo spacecraft			
	12	13	14	15
Alternaria	2	1	3	1
Aspergillus	2	0	0	0
Nidulans group	0	0	0	3
Niger group	0	0	0	5
Versicolor group	11	0	0	1
Aureobasidium	1	0	0	0
Bipolaris	3	0	8	6
Cephalosporium	1	1	0	1
Chaetomium	0	0	2	0
Curvularia	4	4	6	10
Drechslera	0	1	4	0
Epicoccum	0	0	0	1
Fusarium	2	0	1	2
Nigrospora	1	0	1	0
Paecilomyces	0	2	0	0
Penicillium	7	1	5	2
Phoma	1	1	0	0
Pithomyces	0	1	1	0
Pleospora	0	1	0	0
Pyrenochaeta	0	0	0	1
Rhizopus	0	0	0	1
Scopulariopsis	1	1	0	1
Unidentified ascomycete				1

TABLE 5. *Comparison of the pre- and postflight microbiological results of the individual surface sites sampled in Apollo 15 command module*

Areas sampled	Mean no. microorganisms per square inch[a]	
	Preflight	Postflight
Girth shelf, right	7.5×10^1	4.5×10^1
Girth shelf, left	1.3×10^2	1.9×10^3
Waste disposal rim (compartment no. 5)	8.8×10^1	1.7×10^4
Top flight recorder (flight tape recorder)	1.3×10^2	5.0×10^0
Reaction jet control (on-off)	5.0×10^1	6.0×10^1
Exposed floor by hatch	1.5×10^2	TNTC[b]
Ordeal cable stowage (top)	5.8×10^1	2.1×10^4
Vertical couch support beam, right	2.5×10^0	9.8×10^1
Vertical couch support beam, left	0.0	1.8×10^2
Horizontal couch support beam, right	3.3×10^2	2.0×10^2
Horizontal couch support beam, left	1.5×10^2	Sample not taken
Ledge below left window	2.3×10^1	2.6×10^4
Right control handle	0.0	2.9×10^2
Left control handle	0.0	4.0×10^1
Drink gun[c]	0.0	5.2×10^3

[a] Aerobic mesophilic count.
[b] TNTC, Too numerous to count.
[c] Total number of microorganisms recovered from sample.

microorganisms per square foot of surface for each of the component parts were relatively consistent for the four Apollo spacecraft. Although the levels of total microorganisms were similar for all CM, IU, and S-4B, the concentrations of bacterial spores and molds on the latter two components were higher than on the CM, which was consistent with what was found on previous Apollo spacecraft (21). The highest percentage of bacterial spores was detected on the surfaces of the SLA, although this component had the lowest number of microorganisms. The constant flushing of the SLA with high volumes of filtered air might have reduced the vegetative microbial population due to physical removal or desiccation, resulting in a relatively high percentage of sporeformers. The vertical configuration of the surfaces also would account for lower total levels of microbial contaminants. Microbial contamination of the four Lunar Modules revealed that higher levels of microorganisms (approximately 1 log per square foot) were found on the Lunar Modules of Apollo 14 than on Lunar Modules of Apollo 12, 13, and 15. The percentage of bacterial spores on the Lunar Modules of Apollo 15 was greater than on the other Lunar Modules. The Lunar Roving Vehicle (LRV-1) showed a higher percentage of bacterial spores than all the Lunar Modules, with the exception of the exterior surfaces of the ascent stage of Apollo 15. The greatest percentage of molds also was detected on the LRV-1.

A total of 5,862 microbial colonies were picked and identified from the four Apollo spacecraft. Table 2 shows the types of aerobic mesophilic microorganisms isolated from each of the Apollo spacecraft by using TSA. The distribution by types of microorganisms on the four Apollo spacecraft was remarkably similar. Vegetative microorganisms of human origin such as *Staphylococcus* spp., *Micrococcus* spp., and the *Corynebacterium-Brevibacterium* group accounted for the vast majority of microbial contamination detected.

This pattern is consistent with previous Apollo spacecraft (17, 20, 21, 22). The percentage of these microbial types (i.e., indigenous to humans) as detected on the various components of the Apollo spacecraft is shown in Table 3. The highest percentages were found on the interior surfaces of the Command and Lunar Modules. The levels of microorganisms associated with soil and dust (bacterial sporeformers, molds, and actinomycetes) have increased with each Apollo spacecraft. Normally, these types of microorganisms reflect the degree of environmental and personnel controls employed, and when environmental controls are relaxed there is a marked increase in the types of microorganisms originating from soil and dust.

Nineteen genera of molds were isolated from Apollo spacecraft (Table 4), with *Aspergillus*, *Bipolaris*, *Curvularia*, and *Penicillium* being the predominant.

The elimination of the 21-day back-contamination quarantine period of the Apollo 15 mission made possible the first opportunity to take postflight microbiological samples on the interior surfaces of the CM. In addition to the 14-day, 7-day, and 24-h sampling of the CM, a 9-h (preflight) sample was taken by a member of the astronaut back-up crew. The postflight sampling was conducted by the flight surgeon on board the recovery vessel. Samples were

TABLE 6. *Types of microorganisms detected on preflight from CM of Apollo 15 on various media*[a]

Microorganism	Incubation conditions						
	Aerobic			CO_2		Anaerobic	
	TSA	BA	BA-S	BA	BA-S	BA	BA-S
Staphylococcus spp.							
Subgroup I	+	−	−	−	−	−	−
Subgroup II	+	+	+	+	+	+	+
Subgroup III	+	−	−	−	−	−	+
Subgroup IV	+	+	−	+	+	+	+
Subgroup V	+	+	+	+	+	+	+
Subgroup VI	+	−	+	+	−	+	+
Micrococcus spp.							
Subgroup 1	+	−	+	+	−	−	−
Subgroup 2	−	−	−	−	+	−	−
Subgroup 3	+	−	−	−	−	−	−
Subgroup 7	+	+	+	+	+	+	−
Bacillus spp.							
B. circulans	+	−	−	−	−	−	−
B. lentus	+	−	−	−	−	+	−
Corynebacterium-Brevibacterium group	+	−	+	+	+	−	+
Yeasts	+	−	−	−	−	−	−
Molds	+	−	−	−	−	−	−
Atypical *Micrococcus* spp.	+	+	+	+	+	−	−
Atypical *Bacillus* spp.	+	+	−	−	−	+	−
No. isolated	96	14	13	15	16	15	14

[a] Abbreviations: TSA, Trypticase soy agar; BA, blood agar; BA-S, blood agar enriched with vitamin K and hemin.

taken from the same locations as for preflight, and a comparison of the quantitative results is shown in Table 5. The levels of microorganisms increased in some areas by 3 logs per square inch.

A total of 1,682 microorganisms were isolated and identified from the Apollo 15 spacecraft. Of these isolates, 183 and 327 were obtained from the interior surfaces of the CM at preflight and postflight sampling periods, respectively. Tables 6 and 7 list the types of microorganisms detected in the CM from pre- and postflight samples employing various media and incubation methods. These microorganisms were isolated from TSA, blood agar, and enriched blood agar. All media except TSA were incubated at 37 C under aerobic, anaerobic, and CO_2 conditions as requested by the Manned Space Center (MSC). Three types of microorganisms (*Streptococcus-Viridans* group, *Peptostreptococcus* spp., and *Lactobacillus* spp.) were detected only on postflight samples. For the identification data to be meaningful to MSC, colonies resulting from postflight samples were selected by the MSC protocol, i.e., every different colonial type on every culture plate was picked. The standard method used in the Planetary Quarantine Laboratory employs a template with randomly selected points for picking colonies only from aerobic TSA plates. With the exception of the three types of microorganisms detected from postflight sample plates incubated under special conditions (CO_2 or anaerobic), all other types were detected on aerobic TSA plates. Nine types of microorganisms were detected on TSA which were not detected on the other

TABLE 7. *Types of microorganisms detected on postflight from CM of Apollo 15 on various media*[a]

Microorganisms	Incubation conditions						
	Aerobic			CO_2		Anaerobic	
	TSA	BA	BA-S	BA	BA-S	BA	BA-S
Staphylococcus spp.							
Subgroup I	+	+	+	−	+	+	+
Subgroup II	+	+	+	+	+	+	+
Subgroup III	+	+	+	+	+	+	+
Subgroup IV	+	+	+	+	−	+	−
Subgroup V	+	+	+	+	+	+	+
Subgroup VI	+	−	−	+	+	+	+
Micrococcus spp.							
Subgroup 1	+	+	+	−	+	−	−
Subgroup 2	+	−	−	−	−	−	−
Subgroup 3	+	−	−	−	−	−	−
Subgroup 7	+	−	+	−	+	−	+
Streptococcus-Viridans group	−	−	−	+	−	−	−
Peptostreptococcus spp.	−	−	−	−	−	+	−
Bacillus spp.							
B. lentus	+	−	−	−	−	−	−
B. sphaericus	+	−	−	−	−	−	−
Corynebacterium-Brevibacterium group	+	+	+	+	+	+	+
Lactobacillus spp.	−	−	−	−	−	−	+
Actinomycetes	+	+	−	−	−	−	−
Atypical *Micrococcus* spp.	+	−	−	−	−	−	−
Atypical *Bacillus* spp.	+	+	−	−	−	−	−
No. isolated	174	19	17	18	22	35	42

[a] Abbreviations: TSA, Trypticase soy agar; BA, blood agar; BA-S, blood agar enriched with vitamin K and hemin.

media. No gram-negative microorganisms were isolated from pre- or postflight samples. This was not surprising since this group of bacteria is very sensitive to drying and normally is not found on spacecraft surfaces.

Analysis of the types of microorganisms isolated from the various sites on the surfaces of the CM of Apollo 15 from pre- and postflight samples revealed that no apparent recovery pattern existed. Any microorganism which was isolated was equally likely to be found on any given site.

It is evident from the results obtained that the levels and types of microorganisms on surfaces of Apollo spacecraft remain relatively constant among spacecraft (20–22) but greater than some of the automated (6, 9, 16) spacecraft. This was to be expected since Apollo spacecraft are assembled and tested in environmental areas (20) which do not have the same degrees of environmental and personnel controls exerted in those areas used for automated spacecraft (25). If the total number of microorganisms was used as a criterion for evaluating spacecraft microbial cleanliness, the Apollo 14 spacecraft was clearly the most contaminated Apollo spacecraft flown to date. However, if numbers of microorganisms indigenous to soil were used as the standard, the Apollo 15 would be the most contaminated. It has been shown that man is the chief source of microbial contamination to which spacecraft are exposed when assembled and tested in rigidly controlled environments such as high-quality conventional clean rooms (8, 12) and, to a greater extent, in laminar flow clean rooms. When environmental constraints are non-existent or nominal, the percentage of soil microorganisms in the total microbial population increases. This might explain the increase of these microorganisms on the Apollo spacecraft with each mission.

ACKNOWLEDGMENTS

We thank John W. Brandsberg of the Center for Disease Control, Ecological Investigations Program, Kansas City, Kan., who identified the mold isolates.

This investigation was supported by the National Aeronautics and Space Administration under contract W-13,062.

LITERATURE CITED

1. Baird-Parker, A. C. 1966. Methods for classifying staphylococci and micrococci, p. 59–64. *In* B. M. Gibbs and F. A. Skinner (ed.), Identification methods for microbiologists, part A. Academic Press Inc., New York.
2. Bond, W. W., M. S. Favero, N. J. Petersen, and J. H. Marshall. 1970. Dry-heat inactivation kinetics of naturally occurring spore populations. Appl. Microbiol. **20**:573–578.
3. Bond, W. W., M. S. Favero, N. J. Petersen, and J. H. Marshall. 1971. Relative frequency distribution of D_{125C} values for spore isolates from the Mariner-Mars 1969 spacecraft. Appl. Microbiol. **21**:832–836.
4. Edwards, P. R., and W. H. Ewing. 1962. Identification of *Enterobacteriaceae*. Burgess Publishing Co., Minneapolis.
5. Favero, M. S. 1968. Problems associated with the recovery of bacterial spores from space hardware, p. 88–98. *In* C. J. Corum (ed.), Developments in industrial microbiology, vol. 9. American Institute of Biological Science, Washington, D.C.
6. Favero, M. S. 1971. Microbiological assay of space hardware. Environ. Biol. Med. **1**:27–36.
7. Favero, M. S., and K. R. Berquist. 1968. Use of laminar air-flow equipment in microbiology. Appl. Microbiol. **16**:182–183.
8. Favero, M. S., J. R. Puleo, J. H. Marshall, and G. S. Oxborrow. 1966. Comparative levels and types of microbial contamination detected in industrial clean rooms. Appl. Microbiol. **14**:539–551.
9. Fields, N. D., J. R. Puleo, B. Moore, and R. C. Graves. 1968. Surveyor spacecraft evaluation of microbial contamination levels. Proceedings of the Seventh Annual Technical Meeting. American Association for Contamination Control, Chicago.
10. Hall, L. B. 1968. Recent developments in planetary quarantine, p. 19–29. *In* C. J. Corum (ed.), Developments in industrial microbiology, vol. 9. American Institute of Biological Science, Washington, D.C.
11. Hall, L. B., and C. W. Bruch. 1965. Procedures necessary for the prevention of planetary contamination, p. 48–62. *In* M. Florkin (ed.), Life sciences and space research, vol. III. Amsterdam.
12. McDade, J. J., M. S. Favero, and L. B. Hall. 1967. Sterilization requirements for space exploration. J. Milk Food Technol. **30**:179–185.
13. National Aeronautics and Space Administration. September 6, 1967. NASA policy directive NPD 8020.7, paragraph 3.b. National Aeronautics and Space Administration, Washington, D.C.
14. National Aeronautics and Space Administration. October 1968. NASA standard procedures for the microbiological examination of space hardware. NHB 5340.1A. National Aeronautics and Space Administration, Washington, D.C.
15. National Aeronautics and Space Administration. August 1, 1972. NASA policy directive NPD 8020.10A, paragraph 3.b. National Aeronautics and Space Administration, Washington, D.C.
16. Olson, R. L., R. H. Green, and G. J. Tritz. 1968. Progressive biological monitoring on lunar orbiters, p. 99–104. *In* C. J. Corum (ed.), Developments in industrial microbiology, vol. 9. American Institute of Biological Science, Washington, D.C.
17. Oxborrow, G. S., and J. R. Puleo. 1970. Microbiological studies of spacecraft. Lab. Med. **1**:17–20.
18. Puleo, J. R., M. S. Favero, and N. J. Petersen. 1967. Use of ultrasonic energy in assessing microbial contamination on surfaces. Appl. Microbiol. **15**:1345–1351.
19. Puleo, J. R., M. S. Favero, and G. J. Tritz. 1967. Feasibility of using ultrasonics for removing viable microorganisms from surfaces. Contam. Contr. **6**:58–67.
20. Puleo, J. R., N. D. Fields, B. Moore, and R. C. Graves. 1970. Microbial contamination associated with the Apollo 6 spacecraft during final assembly and testing. Space Life Sci. **2**:48–56.
21. Puleo, J. R., G. S. Oxborrow, N. D. Fields, and H. E. Hall. 1970. Quantitative and qualitative microbiological profiles of the Apollo 10 and 11 spacecraft. Appl. Microbiol. **20**:384–389.
22. Puleo, J. R., G. S. Oxborrow, and R. C. Graves. 1969. Microbial contamination detected on the Apollo 9 spacecraft, p. 80–83. Proceedings of the Eighth Annual Technical Meeting. American Association for Contamination Control, New York.
23. Shewan, J. M., G. Hobbs, and W. Hodgkiss. 1960. A

determinative scheme for the identification of certain gram-negative bacteria with special reference to the *Pseudomonadaceae*. J. Appl. Bacteriol. **23**:379-390.

24. Smith, N. R., R. E. Gordon, and F. E. Clark. 1952. Aerobic sporeforming bacteria. Agriculture Monograph no. 16. U.S. Department of Agriculture, U.S. Government Printing Office, Washington, D.C.

25. Tritz, G. J., N. D. Fields, and B. Moore. 1967. Comparative levels of microbial contamination in clean rooms used for the assembly of lunar spacecraft, p. 149-152. Proceedings of the Sixth Annual Technical Meeting. American Association for Contamination Control, Washington, D.C.