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1. Studies were continued on the evaluation of the Sandia Corporation's vacuum probe for sampling surface contamination. The probe technique was compared with the standard strip assay procedure for recovering Bacillus subtilis var. niger spores and naturally occurring microorganisms in dust from stainless steel strips. The dust was prepared by sifting a fresh soil sample, collecting the fraction that passed a 125 μ screen, and drying this fraction at 50 C for 48 hours to reduce the vegetative microbial population. The method of depositing the contaminated dust on strips was described in Report No. 22. A summary of all comparisons made to date is presented in Table 1. Although no explanation can be offered for the differences in recovery rates for the three sets of experiments, all rates were considered relatively high compared with those achieved with other sampling procedures currently in use. The relatively good agreement between the coefficients of variation for the probe and the standard strip assay procedure in each set suggested that the probe accurately reflected the inherent variation in the deposition of fallout contamination.

   The vacuum probe was developed as a possible replacement for the swab-rinse procedure. Accordingly, a series of experiments was conducted to compare the vacuum probe technique with the swab rinse procedure for recovering microorganisms from surfaces. Sixty-two sampling sites on a variety of horizontal surfaces throughout the laboratory were selected randomly. At each site an area of four square inches was sampled using a swab. Similarly, a contiguous four square inch area was sampled using the vacuum probe. A ratio of the contamination level detected by the probe to that detected by the swab was calculated for each site. The mean of these 62 ratios was 3.1:1 with a standard deviation of 2.5:1. Overall, the probe and swab recovered 30,448 and 13,285 microorganisms respectively from the 248 in^2 sampled. This resulted in an overall recovery ratio of 2.1:1 in favor of the vacuum probe.

   The nature of the experiments to evaluate the effectiveness of the vacuum probe had restricted its use to small surface areas and consequently short running times. An experiment was conducted to determine whether prolonged passage of air through the probe and filter resulted in a reduction in the number of microorganisms recovered. From a tray containing 28 strips contaminated with naturally occurring airborne microorganisms 14 strips were selected and vacuumed in the usual manner of running the probe for less than 1 minute. After each of the remaining 14 strips was vacuumed the probe was allowed to run in a laminar flow clean bench for 10 minutes. This simulated prolonged use of the probe without the deposition of additional microorganisms in the probe or on the filter. Assays of the probes and filters resulted in the recovery of a mean of 327 microorganisms from each probe that had been run for one minute and 331 microorganisms from each probe that had been run for 10 minutes. While these data reveal no evidence of a marked reduction in recovery of microorganisms because of prolonged exposure to airflow, the rather small sample

size suggested that conclusions based on this experiment should be considered tentative. There was, in fact, no evidence to demonstrate that even one minute of exposure to this airflow may not be lethal to certain types of microorganisms. This aspect will be investigated further during the next quarter.

Because surface contamination may result from handling as well as from airborne deposition, an experiment was conducted to evaluate the effectiveness of the vacuum probe in removing this type of contamination. Forty sterile stainless steel strips were contaminated by having one side of each strip touched by four finger tips from each hand of an individual. Each strip was touched by only one individual. The contaminated surface was vacuumed and an assay was performed on the membrane filter and the probe cone and tip. The strip was then subjected to the standard strip assay procedure to determine the number of microorganisms remaining on the surface. The number of microorganisms obtained by summing the results of these two assays was considered to be the total number on the strip. The number recovered by the probe expressed as a percentage of the total number on the strip represents the effectiveness of the probe as a sampling device. The mean percentage of removal was 55 with a standard deviation of 27. This value compared unfavorably with a percentage of removal value for airborne deposition of 98 and a standard deviation of 2. Because of the great variability encountered in the levels of handling contamination no attempt was made to assess the effectiveness of total recovery of such contamination using the probe. It was evident, however, that total recovery would be relatively poor as long as efficient removal was not being accomplished.

In summary, the probe proved to be an effective sampling device for surface microbial contamination resulting from airborne fallout. It was less effective on contamination resulting from handling. Arrangements have been made to field test the vacuum probe on spacecraft at Cape Kennedy. These tests will provide additional data for the comparison of the probe and swab rinse procedures.

2. A mathematical procedure has been developed at the Sandia Corporation for determining the surface area that must be sampled to estimate the level of microbial contamination on the Apollo spacecraft. One of the terms of this mathematical expression that must be known is the mean number of viable microorganisms per viable clump of naturally occurring microbial contamination resulting from airborne fallout. Four experiments at the Phoenix Field Station and two experiments at the Apollo spacecraft assembly area at Cape Kennedy were conducted to determine this value. Each experiment consisted of exposing a tray of 44 sterile stainless steel strips to naturally occurring airborne contamination for a period of 48 to 96 hours. Every other strip was removed from the tray, placed in a petri plate and gently covered with TSA. The remaining strips were assayed for microbial contamination using the standard strip assay procedure in which ultrasonics is employed to remove microbial contaminants and to break up clumps of microbial cells. Colonies on the plated strips were counted at

48 hours and recorded as the number of microbial clumps per strip. Plate counts from the standard assay procedure were made at 48 hours and recorded as the number of viable microorganisms per strip. The number of viable microorganisms per viable clump was calculated for each experiment by dividing the mean number of viable microorganisms per strip by the mean number of clumps on each direct plated strip. Table 2 presents the results of these experiments.

3. The vertical laminar flow bioclean room is being used routinely for a variety of experiments and assays. Prior to its routine use air samples were collected to assure that no residual microbial contamination, which possibly remained on filters, protective screens, walls, and two clean benches, was entering the air flow. Each air sample consisted of 60 ft^3 of air drawn through a Reynier slit sampler at the rate of 1 ft^3/min. The samplers were positioned at the center of each of 72 floor grills (2 x 2 ft.). Nine of the first 72 samples showed the presence of at least 1 viable particle per 60 ft^3 (11 viable particles in all). The remaining 63 samples were negative. The positive samples occurred near one clean bench. After additional cleaning of this bench all subsequent samples were negative.

   With 3 to 4 people working in the room using no personnel clothing constraints such as suits, head covers or shoe covers, 30 samples were taken at work bench level as close to the individuals as possible. Only 7 viable particles were collected from 1800 ft^3 of air for a concentration of 4 viable particles per 1000 ft^3 of air. From 960 ft^3 of air sampled at floor level under the chairs of these individuals, a total of 44 viable particles was collected for a concentration of 46 viable particles per 1000 ft^3 of air. Results of smoke tests showed that contamination shed by personnel was most likely to occur at the air intakes of two clean benches located at opposite ends of the bioclean room. Twelve samples taken at these locations showed 272 viable particles per 1000 ft^3 of air. These values demonstrate that (1) the concentration of microbial contamination in the air of a vertical laminar flow room remains remarkably low even in the presence of personnel activity and no personnel clothing constraints, and (2) air sampling in a vertical laminar flow room is meaningful only at the location of the sampler intake, e.g., marked differences in contamination levels can be found at locations only inches apart.

   The bioclean room was operated routinely from Monday morning to Friday afternoon of each week and was turned off over the weekend. To determine whether microbial contamination generated during the week and possibly trapped in the floor prefilter would be resuspended in increasingly higher concentrations during the weekends, air samples were taken on each Monday morning before the room was placed in operation. It was evident (Table 3) that no consistent increase took place with time.

4. Characterization of the bacterial spore flora of soil sample X (Phoenix) was continued. Previous experiments showed that the slopes of both the first and second phases of dry heat survivor curves for

naturally occurring aerobic and anaerobic spores at 125 C were essentially the same (Fig. 1 in Report No. 22). This suggested that most of the spore population was probably facultative with respect to oxygen requirements. To substantiate this hypothesis representative isolates were obtained from heated (2 hrs at 125 C) or unheated soil sample X by picking colonies from plates of TSA which had been incubated aerobically or anaerobically. Of 13 aerobic and 17 anaerobic isolates, all proved to be facultative when incubated both aerobically and anaerobically.

Studies on the effect of subculturing on the dry heat resistance of bacterial spores isolated from soil and the heat resistance of spores isolated from space hardware were continued. The experimental procedures remained the same (Report No. 19), with the exception that all manipulations including heating (dry heat oven) were conducted within a vertical laminar flow clean room (42% $\pm$ 2% R.H., 70 $\pm$ 2 F). In order to test the effects of different culture media on the dry heat resistance of spores, crops of B. subtilis var. niger spores and isolate XA (Report No. 21) were prepared on four different media employing an active culturing technique. The media used were (1) TAM Sporulation Agar (Difco) supplemented with 80 ppm calcium chloride and 20 ppm magnesium sulfate; (2) TAM Sporulation Agar supplemented with a soil extract (prepared by boiling 100 g soil in 100 ml distilled water; 100 ml of the filtrate was used to make 100 ml of the final medium); (3) A-K Medium #2 (Difco) supplemented with 80 ppm calcium chloride and 20 ppm magnesium sulfate; and (4) Charcoal Agar (Difco). Cleaned spore crops were stored in 95% ethanol at 4 C until used. Results are shown in Table 4. As reported last quarter, preliminary results with B. subtilis var. niger spores had indicated no significant variation in D_{125C} values among the sporulation media used. However, spores of isolate XA showed a marked variation in dry heat resistance depending on which sporulation medium was employed. TAM supplemented with a soil extract produced spores which were most resistant to dry heat. Further evidence of dry heat resistance being dependent upon the sporulation medium is shown in Table 5. Spores of isolates CK-4 and CK-5 were more resistant to dry heat when TAM rather than A-K Medium #2 was used as the sporulation medium. However, spores of isolate CK-2 produced on these two media did not show any significant differences in dry heat resistance.

Variations in death rate of a particular spore isolate can be attributed not only to cultural conditions but also to the method and/or location in which a dry heat assay is performed. An ethanol suspension of B. subtilis var. niger spores (3×10^9/ml) was received from a cooperating laboratory (Sandia Corporation) and applied in 0.1 ml amounts to 1 x 1 in. stainless steel strips. Dry heat resistance tests were performed using the method described in Report No. 19 with the exception that the stainless steel strips were insonated in 125 ml flasks containing 50 ml of buffered distilled water. This method was similar to that used by the Sandia Corporation. Results of both laboratories are shown in Figure 1. It was evident that the "Sandia" crop of B. subtilis var. niger was more resistant than the

"standard" crop (D_{125C} value = 15 minutes). In addition, the Sandia group obtained a diphasic survivor curve whereas our group obtained a monophasic survivor curve. Although the reason for this variation is not known it is reasonable to assume that the difference in heating systems may be a significant factor.

Prevailing controversy regarding the calculation of spacecraft sterilization cycles rests, in part, with the choice of B. subtilis var. niger spores as a standard "conservative" or even "worst case" estimate of dry heat resistance of actual spacecraft contaminants. The experimental results described above depict some of the possible dangers involved in selection of a standard reference organism to be used in calculating a fixed sterilization cycle. First, different methods of culture and assay of a "standard" organism (in this case various crops of B. subtilis var. niger spores) have yielded D_{125C} values on exposed surfaces ranging from 8 to 36 min. Second, there is little indication that pure B. subtilis var. niger spores represent a valid estimate of dry heat resistance of the mixed, naturally occurring spore populations on spacecraft (Report No. 22). During the next quarter emphasis will be placed on determining the kinetics of dry heat inactivation of naturally occurring spores collected from flight spacecraft in residence at Cape Kennedy.

5. Spacecraft Bioassay Laboratory

Studies were continued in the Apollo facilities to determine the levels of microbial contamination in the intramural environments of the Manned Spacecraft Operations Building (MSOB), Back Pack Life Support Clean Room, and the Spacesuit Clean Room. Figure 2 shows the results obtained in the Command and Service Module work stands. The levels of microbial contamination accumulating on stainless steel strips remained essentially the same throughout a 12-week period and were in the range of 10^4 and 10^5 aerobic mesophilic microorganisms per square foot. Aerobic mesophilic microorganisms and spores detected in the Integrated work stand #2 are shown in Figure 3. The levels of microbial contamination appeared to stabilize after 3 weeks and did not increase significantly during the 11-week period. These levels of microbial contamination were similar to those observed in the Command and Service Module work stands. The number of aerobic spores was usually within one log of the number of aerobic mesophiles. Figure 4 shows the degree of fallout contamination in the Lunar Module (LM) work stands. Contamination levels were slightly higher in the LM Polarity Fixture work stand than those observed in the LM Ascent and Descent work stands. Increased personnel activity in this area could have accounted for this difference. Site 1 was at the LM-3 Ascent Stage work level, and Site 2 at the LM-3 Descent Stage work level of the Polarity Fixture work stand. Levels of fallout contamination for the Spacesuit Clean Room and Back Pack Clean Rooms are seen in Figures 5 and 6. Contamination levels in these clean rooms were slightly higher than had been observed previously and probably were due to concomitant increase in activities.

The Apollo 7 spacecraft (SCM-101) was studied in all of the intramural environments to which it was exposed during assembly and testing. The spacecraft was sampled for microbial contamination while at the Manned Spacecraft Operations Building (MSOB) and at Launch Complex 34. The levels of microbial contamination present on the Command and Service Modules, and on the Spacecraft Lunar Module Adapter (SLA) are presented in Table 6. The levels of aerobic and anaerobic mesophilic microorganisms were basically the same on the Command and Service Modules although one was an interior surface and the other an exterior surface. Levels of aerobic spores isolated from the Service Module were one log higher than those recovered from the Command Module. This could be explained by the differences in the environments to which each module was subjected during assembly and testing. The interior of the Command Module received HEPA-filtered air, and clean room procedures were observed by personnel working in the cabin; on the other hand the Service Module was exposed to environmental conditions of the MSOB, which is rated as a Class 100,000 clean room. An increase of approximately one log in the aerobic spore count occurred in the interior of the Command Module after it was sampled at Launch Complex 34. The interior surfaces of the SLA contained very low levels of microbial contamination. This was probably because its surfaces were vertical and there was low personnel activity in the SLA.

Studies were initiated on the Lunar Module-3 (LM-3). Microbiological samples were taken from the interior and exterior surfaces of the Ascent Stage, and from the exterior surfaces of the Descent Stage. Table 7 shows that levels of microorganisms recovered from the interior surfaces of the LM-3 were higher than those found on the interior surfaces of the Command Module. There appeared to be an increase in the number of microorganisms at each sampling period. Levels of aerobic spores were higher than those found on the interior surfaces of the Command Module. Levels of aerobic microorganisms on the Ascent and Descent Stages were essentially the same (Table 8). An increase in the contamination level was observed when the mated Ascent and Descent Stages were moved to the Altitude Test Chamber. Aerobic spore counts on the Ascent Stage were higher than those on the Descent Stage while they were located on the Polarity work stand, but both counts were nearly identical after the spacecraft were moved into the Altitude Test Chamber.

The number and percentage of molds isolated from the interior surfaces of the Command Module and the SLA, and the exterior surfaces of the Service Module are shown in Table 9. The level of mold contamination on the Command (0-.18%) and Service Modules (.46-1.33%) was relatively low. The initial sample taken from the SLA interior revealed a high level (44%) of mold contamination with each successive sampling resulting in lower mold counts. At the final sampling, taken while at Launch Complex 34, no molds were recovered. Low personnel densities inside the SLA while at the MSOB, and purging with filtered air while at Launch Complex 34 could have been responsible for these results. Table 10 shows the level of mold contamination detected on the surfaces of the Apollo Lunar Module-3 (LM-3). The interior surfaces of the Ascent Stages were found to contain very low levels of mold contamination.

This may have been due to the use of filtered air, control of personnel density, and the employment of clean room procedures in the LM-3 cabin. Levels of mold contamination found on the exterior surfaces of the Ascent and Descent Stages were higher than those observed on the interior surfaces.

A comparative study of the number of microorganisms on vertical and horizontal surfaces was continued. Results (Table 11) reconfirmed previous studies which indicated that more microorganisms accumulate on horizontal surfaces than vertical surfaces.

Table 12 shows the levels and types of aerobic mesophilic microorganisms isolated from the Apollo Command and Service Modules (CSM-101) and the SLA. No Bacillus spp. (sporeformers) were recovered from the interior of the Command Module. Most (ca. 96%) of the microorganisms were Staphylococcus epidermidis and Micrococcus spp. The Service Module showed about 6% of Bacillus spp., and the majority (ca. 80%) of microorganisms were Staphylococcus spp. and Micrococcus spp. The microorganisms found on the SLA were equally divided between Micrococcus spp. and molds. Also, more Bacillus spp. were isolated from the SLA than the Command and Service Modules. The levels and types of aerobic mesophilic microorganisms isolated from the Apollo Lunar Module-3 (LM-3) are shown in Table 13. The majority (ca. 88%) of microorganisms detected were Staphylococcus spp. and Micrococcus spp.

A comparison of the types of microorganisms isolated from the Apollo spacecraft (CSM-101) and the Lunar Module-3 in relation to humans and the environment is presented in Table 14. Microorganisms indigenous to humans (skin, hair, etc.) accounted for 90 to 100% of the total microorganisms recovered. In the case of the SLA the numbers of microorganisms associated with humans and with the environment were essentially equal.

A preliminary study was initiated to determine if extended incubation would increase the recovery of molds from the surfaces of space hardware. Swab samples taken from various Apollo spacecraft were assayed according to NASA Standard Procedures, and incubated at 32 C for 21 days. Culture plates (trypticase soy agar) were read at 2, 3, 7, 14 and 21 days. Five tests, which included a total of 165 culture plates have been conducted to date. The results showed that there was no significant increase in the number of mold colonies after 72 hours of incubation at 32 C. This study is still in progress and final results will be reported next quarter.

TABLE 1. COMPARISON OF THE VACUUM PROBE PROCEDURE WITH THE STANDARD STRIP ASSAY PROCEDURE IN RECOVERING MICROBIAL CONTAMINATION FROM SURFACES OF STAINLESS STEEL STRIPS.

Type of contamination	No. strips assayed by each method	Vacuum probe		Standard strip assay		Probe* recovery rate
		Mean no. microorganisms recovered	Coefficient of variation	Mean no. microorganisms recovered	Coefficient of variation	
Naturally occurring microorganisms in air - fallout	40	320	59%	335	57%	96%
B. subtilis var. *niger* spores on aluminum oxide dust - simulated fallout	106	283	20%	325	18%	87%
Naturally occurring microorganisms on soil dust - simulated fallout	23	82	62%	102	51%	80%

*Mean number of microorganisms recovered using the vacuum probe procedure expressed as a percentage of the number recovered using the standard strip assay.

TABLE 2. NUMBER OF VIABLE MICROORGANISMS PER VIABLE CLUMP OF NATURALLY OCCURRING MICROBIAL CONTAMINATION RESULTING FROM AIRBORNE FALLOUT.

Location	Mean No. Viable Clumps Per Strip	Mean No. Viable Microorganisms Per Strip	Mean No. Microorganisms Per Viable Clump
Phoenix Field Station Room 114	18	83	4.7
Phoenix Field Station Room 114	23	112	4.9
Phoenix Field Station Room 213	10	92	9.2
Phoenix Field Station Room 213	17	141	8.3
Cape Kennedy, MSOB	20	193	9.7
Cape Kennedy, MSOB	22	252	11.5
Overall	18	146	8.1

TABLE 3. CONCENTRATION OF AIRBORNE MICROBIAL CONTAMINATION IN THE BIOCLEAN ROOM PRIOR TO OPERATION ON MONDAY MORNING.

Date	Cubic Feet of Air Sampled	Viable Particles per 100 Cubic Feet of Air
June 24	120	64
July 22	360	81
August 5	120	73
August 19	240	66
August 26	240	138
September 9	120	16
September 16	180	19

TABLE 4. COMPARATIVE D_{125C} VALUES OF *BACILLUS SUBTILIS* VAR. *NIGER* AND ISOLATE XA SPORES GROWN ON VARIOUS MEDIA.

Organism	Sporulation Medium	D_{125C} (Min.)
B. subtilis var. *niger*	TAM[1]	15
B. subtilis var. *niger*	TAM-S[2]	18
B. subtilis var. *niger*	AK #2[1]	15
B. subtilis var. *niger*	Charcoal Agar[3]	13
XA	TAM	46
XA	TAM-S	82
XA	AK #2	27
XA	Charcoal Agar	37

[1]Difco. Supplemented with 80 ppm calcium chloride and 20 ppm magnesium sulfate.

[2]Prepared with a boiling water-soil extract: clear filtrate of 100 g soil/100 ml distilled water to make 100 ml medium.

[3]Difco.

TABLE 5. DEATH RATE OF SPORE ISOLATES FROM SOIL AND SPACECRAFT AT 125 C.

Isolate	Source	Sporulation Medium[1]	D_{125C} (Min.)	D_{125C} of 2nd Phase Parent Soil Suspension (Min.)[2]
G-2	G-soil[3]	AK #2	109	38
G-3	G-soil[3]	AK #2	25	38
G-5	G-soil	AK #2	21	38
J-1	J-soil[4]	AK #2	49	36
J-2	J-soil[4]	AK #2	36	36
M-1	M-soil[5]	AK #2	73	42
XA	X-soil[6]	TAM	46	126
CK-2	Surveyor 6	TAM	15	-
CK-2	Surveyor 6	AK #2	14	-
CK-4	Surveyor 6	TAM	46	-
CK-4	Surveyor 6	AK #2	19	-
CK-5	Surveyor 6	TAM	30	-
CK-5	Surveyor 6	AK #2	20	-
CK-6	Surveyor 6	AK #2	8	-
CK-8	Surveyor 6	AK #2	10	-
CK-9	Surveyor 6	AK #2	5	-
CK-10	Surveyor 6	AK #2	10	-
CK-12	Surveyor 6	AK #2	10	-
CK-13	Surveyor 6	AK #2	7.5	-
CK-14	Surveyor 6	AK #2	7.5	-
CK-17	Surveyor 6	AK #2	3.6	-
CK-19	Apollo 20	AK #2	15	-
CK-21	Apollo 20	AK #2	2.5	-
CK-22	Apollo 20	AK #2	11	-
CK-23	Apollo 20	AK #2	27	-
CK-24	Apollo 20	AK #2	1.4	

[1]Both media were supplemented with 80 ppm calcium chloride and 20 ppm magnesium sulfate.

[2]The D_{125C} value given is the largest value exhibited by the diphasic soil. See Report No. 20.

[3]McDonnell-Douglas; St. Louis, Missouri.

[4]Langley Research Center; Hampton, Virginia.

[5]Marshall Space Flight Center; Huntsville, Alabama.

[6]Phoenix, Arizona.

TABLE 6. MICROBIAL CONTAMINATION DETECTED ON THE APOLLO COMMAND AND SERVICE MODULES (CSM-101) AND SPACECRAFT LUNAR MODULE ADAPTER (SLA).

Source	Date Sampled	Area Sampled[1] (Sq. In.)	Microorganisms Per Square Foot			
			Aerobes	Anaerobes	Aerobic Spores	Anaerobic Spores
Command Module[2]	6-06-68	120	12,306	5,897	36	30
	6-27-68	120	17,562	4,914	12	12
	8-19-68[5]	120	27,066	5,376	408	30
Service Module[3]	6-03-68	140	1,542	422	118	42
	6-17-68	140	20,232	2,376	226	46
	7-30-68	120	13,938	8,467	703	0
SLA[4]	6-20-68	128	51	28	0	6
	7-09-68	128	264	225	62	28
	7-23-68	128	68	17	17	17
	9-05-68	128	45	29	29	17

[1]Swab-rinse technique.

[2]Samples taken from interior surfaces of Command Module while located in the Manned Spacecraft Operations Building (MSOB).

[3]Samples taken from exterior surfaces of Service Module while located in the Manned Spacecraft Operations Building.

[4]Samples taken from interior surfaces of SLA while located in the Manned Spacecraft Operations Building.

[5]Samples taken from interior surfaces while located at Launch Complex 34.

TABLE 7. MICROBIAL CONTAMINATION DETECTED ON THE INTERIOR SURFACES OF THE LUNAR MODULE-3 (LM-3).

Source	Date Sampled	Area Sampled[1] (Sq. In.)	Microorganisms Per Square Foot			
			Aerobes	Anaerobes	Aerobic Spores	Anaerobic Spores
Lunar Module-3	7-02-68[2]	116	28,101	16,647	87	37
	7-18-68[2]	84	17,803	7,526	231	43
	7-31-68[2]	112	47,131	9,144	1,080	919
	8-19-68[2]	96	100,901	35,467	143	45
	8-28-68[3]	108	65,707	29,030	274	46

[1]Swab-rinse technique.

[2]Samples taken while Lunar Module-3 was located in Polarity Fixture Work Stand in the Manned Spacecraft Operations Building (MSOB).

[3]Samples taken while Lunar Module-3 was located in the Altitude Test Chamber in the Manned Spacecraft Operations Building.

TABLE 8. LEVELS OF MICROBIAL CONTAMINATION DETECTED ON THE EXTERIOR SURFACES OF THE LUNAR MODULE-3 (LM-3) ASCENT AND DESCENT STAGES.

Source	Date Sampled	Area Sampled[1] (Sq. In.)	Microorganisms Per Square Foot			
			Aerobes	Anaerobes	Aerobic Spores	Anaerobic Spores
LM-3: Ascent Stage	7-03-68[2]	116	19,744	3,997	248	155
	7-17-68[2]	120	12,816	2,232	132	30
	8-01-68[2]	120	1,843	346	43	0
	8-14-68[2]	120	6,408	2,333	101	43
	8-28-68[3]	120	53,698	20,333	259	86
LM-3: Descent Stage	7-03-68[2]	116	7,703	2,886	75	25
	7-17-68[2]	88	15,357	2,790	49	33
	8-01-68[2]	112	10,411	2,002	65	26
	8-14-68[2]	120	8,770	2,434	30	42
	8-28-68[3]	92	48,571	12,686	212	32

[1]Swab-rinse technique.

[2]Samples taken while Lunar Module-3 was located in the Polarity Fixture Work Stand in the Manned Spacecraft Operations Building (MSOB).

[3]Samples taken while Lunar Module-3 was located in the Altitude Test Chamber in the Manned Spacecraft Operations Building.

TABLE 9. MOLD CONTAMINATION DETECTED ON SURFACES OF THE APOLLO COMMAND AND SERVICE MODULES (CSM-101) AND SPACECRAFT LUNAR MODULE ADAPTER (SLA).

Source	Experiment Number	Date Sampled	Area Sampled[1] (Sq. In.)	Number of Molds	Number Per Sq. Inch	Percent[2]
Command Module (Interior)	1	6-06-68	120	5	0.04	0.08
	2	6-27-68	120	0	0.00	0.00
	3	8-19-68	120	40	0.33	0.18
Service Module (Exterior)	1	6-03-68	140	20	0.14	1.33
	2	6-17-68	140	90	0.64	0.46
	3	7-30-68	120	115	0.96	0.99
SLA (Interior)	1	6-20-68	128	20	0.16	44.44
	2	7-09-68	128	55	0.43	23.40
	3	7-23-68	128	10	0.08	16.66
	4	9-05-68	128	0	0.00	0.00

[1]Swab-rinse technique.

[2]Percentage of total aerobic mesophilic microorganisms.

TABLE 10. MOLD CONTAMINATION DETECTED ON SURFACES OF THE APOLLO LUNAR MODULE-3 (LM-3).

Source	Sample Number	Date Sampled	Area Sampled[1] (Sq. In.)	Number of Molds	Number Per Sq. Inch	Percent[2]
Ascent Stage (Interior)	1	7-02-68	116	20	0.17	0.09
	2	7-18-68	84	25	0.30	0.24
	3	7-31-68	112	0	0.00	0.00
	4	8-19-68	96	10	0.10	0.01
	5	8-28-68	108	5	0.05	0.01
Ascent Stage (Exterior)	1	7-03-68	116	30	0.26	0.19
	2	7-17-68	120	325	2.70	3.04
	3	8-01-68	120	5	0.04	0.33
	4	8-14-68	120	35	0.29	0.66
	5	8-28-68	120	225	1.88	0.50
Descent Stage (Exterior)	1	7-03-68	116	50	0.43	0.81
	2	7-17-68	88	65	0.74	0.69
	3	8-01-68	112	70	0.63	0.86
	4	8-14-68	120	50	0.42	0.68
	5	8-28-68	92	120	1.30	0.39

[1]Swab-rinse technique.

[2]Percentage of total aerobic mesophilic microorganisms recovered.

TABLE 11. COMPARISON OF THE LEVELS OF MICROORGANISMS DEPOSITED ON HORIZONTAL (H) AND VERTICAL (V) SURFACES OF THE COMMAND AND SERVICE MODULES (CSM-101) AND LUNAR MODULE-3.

Source	Area Sampled* (Sq. In.)	Surface Position	Aerobes Per Sq. In.	Aerobic Spores Per Sq. Inch
Command Module	204	H	201	1.5
	156	V	124	0.5
Service Module	224	H	88	3.2
	176	V	75	1.2
Lunar Module-3	396	H	433	2.8
(Interior)	96	V	157	1.8
Lunar Module-3	364	H	163	1.3
(Ascent)	232	V	89	0.8
Lunar Module-3	344	H	192	0.8
(Descent)	184	V	21	0.2

*Swab-rinse technique.

TABLE 12. LEVELS AND TYPES OF AEROBIC MESOPHILIC MICROORGANISMS ISOLATED FROM THE APOLLO COMMAND AND SERVICE MODULES (CSM-101) AND SPACECRAFT LUNAR MODULE ADAPTER (SLA).

Microorganisms	Command Module (Interior)		Service Module (Exterior)		SLA (Interior)		Total	
	No.	%	No.	%	No.	%	No.	%
Staphylococcus epidermidis	121	39.4	72	23.6	3	9.4	196	30.4
Staphylococcus aureus	8	2.6	1	0.3	0	0	9	1.4
Micrococcus spp.	174	56.7	172	56.4	10	31.2	356	55.3
Corynebacterium Brevibacterium Group	2	0.7	15	4.4	2	6.3	19	3.0
Miscellaneous Gram negative rods	0	0	4	1.3	0	0	4	0.6
Bacillus spp.	0	0	18	5.9	4	12.5	22	3.4
Molds	0	0	11	3.6	13	40.6	24	3.7
Sarcina spp.	1	0.3	0	0	0	0	1	0.2
Graffkya spp.	1	0.3	1	0.3	0	0	2	0.3
Streptococcus spp.	0	0	2	0.7	0	0	2	0.3
Yeasts	0	0	9	3.0	0	0	9	1.4
TOTAL	307	100.0	305	100.0	32	100.0	644	100.0

TABLE 13. TYPES OF AEROBIC MESOPHILIC MICROORGANISMS ISOLATED FROM THE APOLLO LUNAR MODULE-3 (LM-3).

Microorganisms	LM-3 (Ascent) (Exterior) No.	%	LM-3 (Descent) (Exterior) No.	%	LM-3 (Ascent) (Interior) No.	%	Total No.	%
Staphylococcus epidermidis	44	16.8	48	14.4	138	31.7	230	22.6
Staphylococcus aureus	2	0.8	1	0.3	0	0	3	0.3
Micrococcus spp.	187	71.4	221	68.6	258	59.4	666	65.4
Corynebacterium Brevibacterium Group	5	1.9	12	3.7	5	1.1	22	2.2
Miscellaneous Gram negative rods	0	0	1	0.3	4	0.9	5	0.5
Bacillus spp.	10	3.8	12	3.7	6	1.4	28	2.7
Molds	9	3.4	19	5.4	11	2.5	39	3.8
Sarcina spp.	0	0	0	0	0	0	0	0
Graffkya spp.	4	1.5	6	1.9	13	3.0	23	2.2
Streptococcus spp.	0	0	0	0	0	0	0	0
Yeasts	1	0.4	2	0.7	0	0	3	0.3
TOTAL	262	100.0	322	100.0	435	100.0	1019	100.0

TABLE 14. MICROORGANISMS ISOLATED FROM APOLLO SPACECRAFT IN RELATION TO PERSONNEL AND ENVIRONMENT.

Source	Microorganisms Associated with Personnel[1]		Microorganisms Associated with Environment[2]	
	No.	%	No.	%
Command Module (CSM-101) (Interior)	307	100.0	0	0
Service Module (CSM-101) (Exterior)	276	90.5	29	9.5
SLA (Interior)	15	46.9	17	53.1
LM-3 Ascent Stage (Interior)	418	96.1	17	3.9
LM-3 Ascent Stage (Exterior)	243	92.7	19	7.3
LM-3 Descent Stage (Exterior)	291	90.4	31	9.6
Combined Spacecraft Components	1550	93.2	113	6.8

[1]Microorganisms associated with human skin, hair and respiratory tract (i.e., Staphylococcus spp., Micrococcus spp. Corynebacterium-Brevibacterium group, etc.).

[2]Microorganisms associated with soil and dust (i.e., Bacillus spp. and molds).

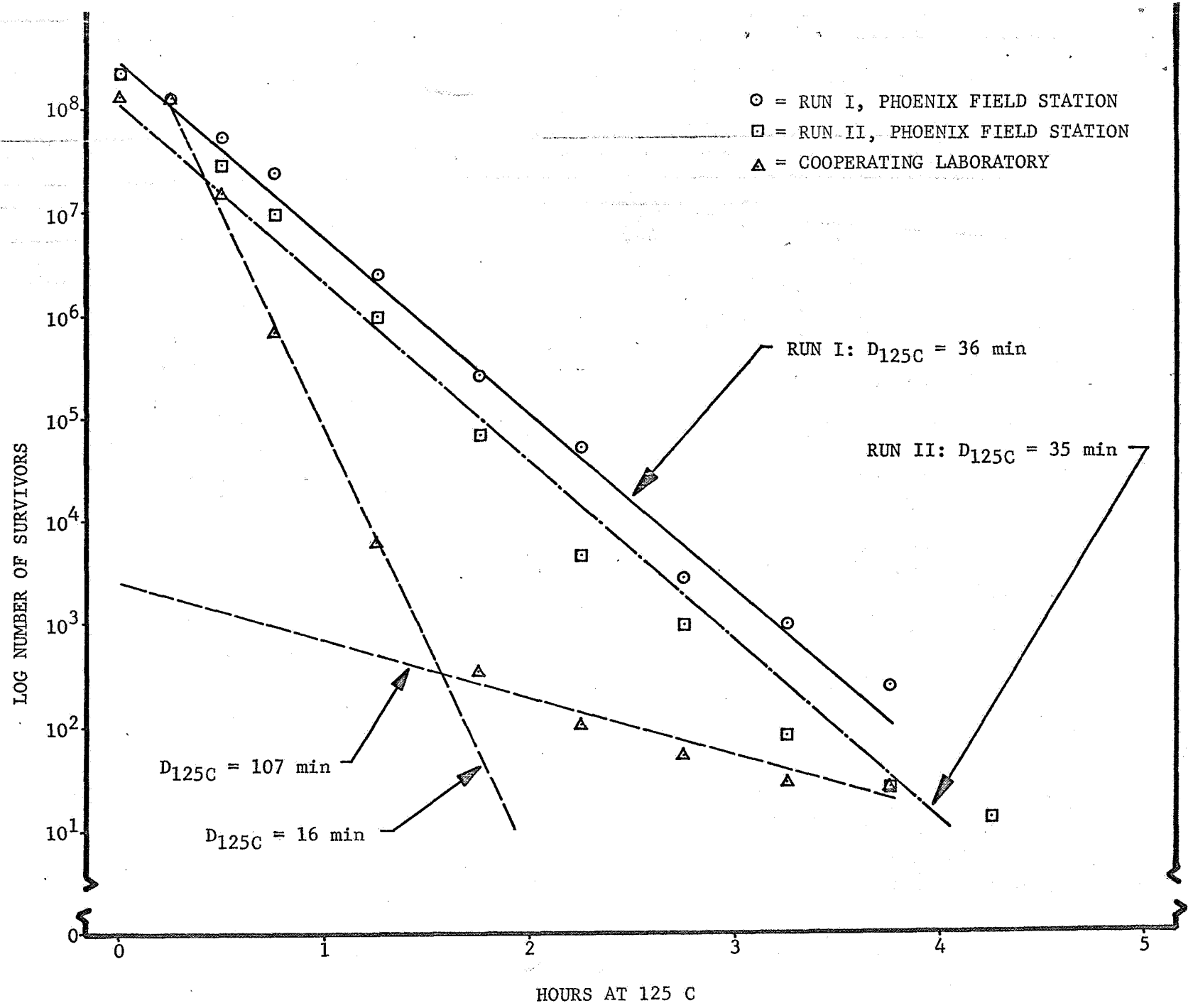

FIG. 1. COMPARATIVE DRY HEAT SURVIVOR CURVES OF BACILLUS SUBTILIS VAR. NIGER (SANDIA CORPORATION).

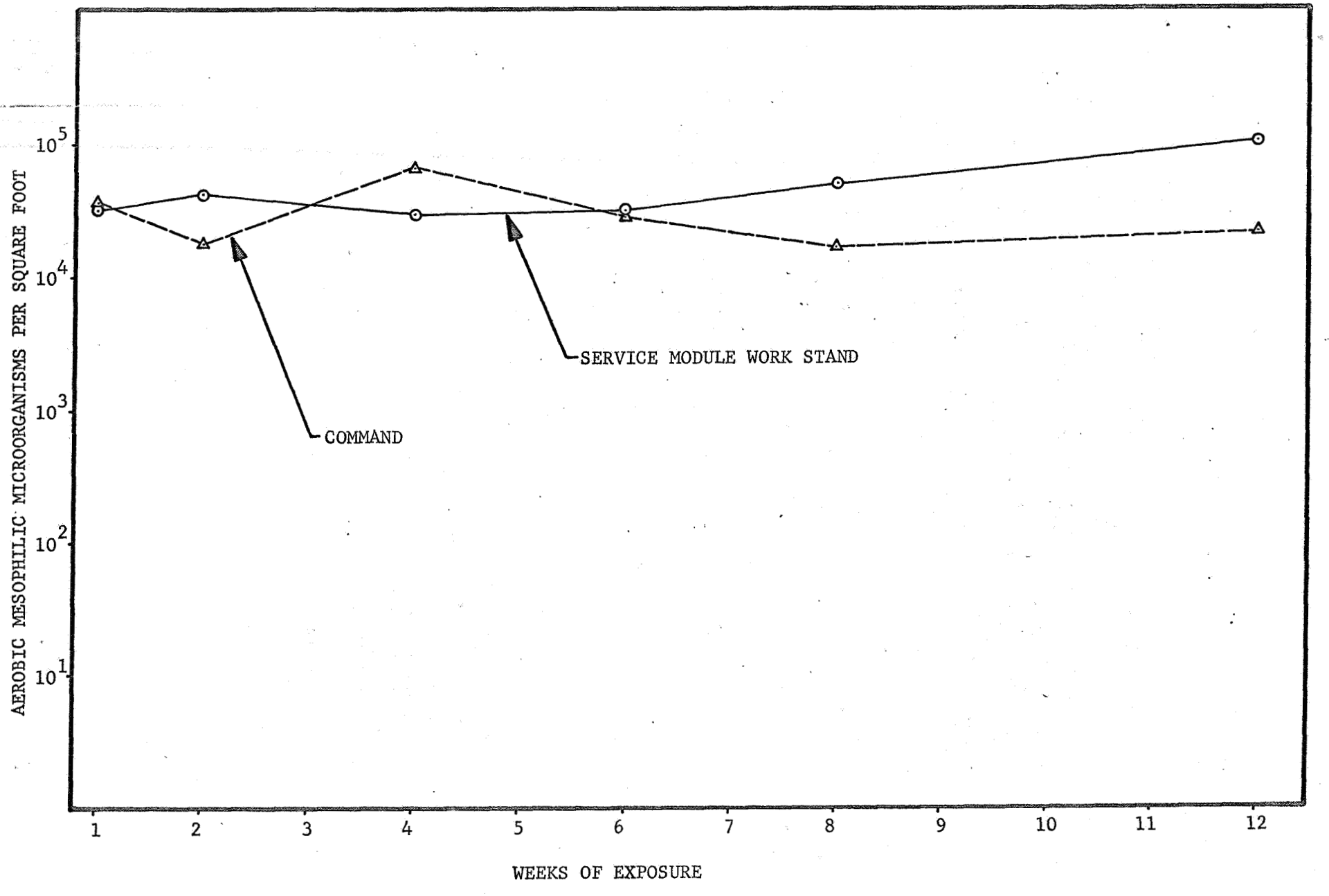

FIG. 2. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL SURFACES EXPOSED TO THE INTRAMURAL AIR OF THE COMMAND AND SERVICE MODULE WORK STANDS.

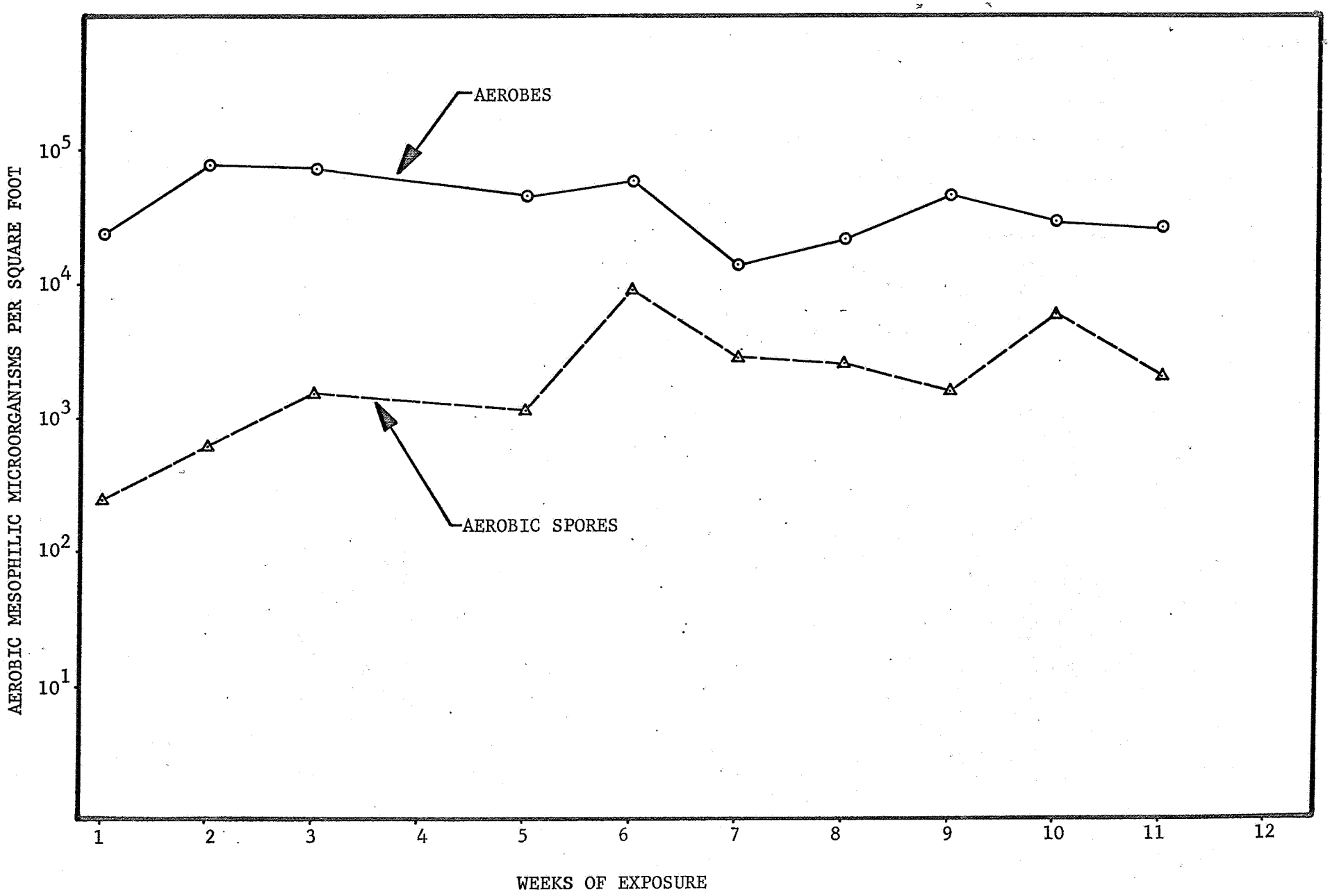

FIG. 3. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL SURFACES EXPOSED TO THE INTRAMURAL AIR OF THE INTEGRATED WORK STAND NO. 2.

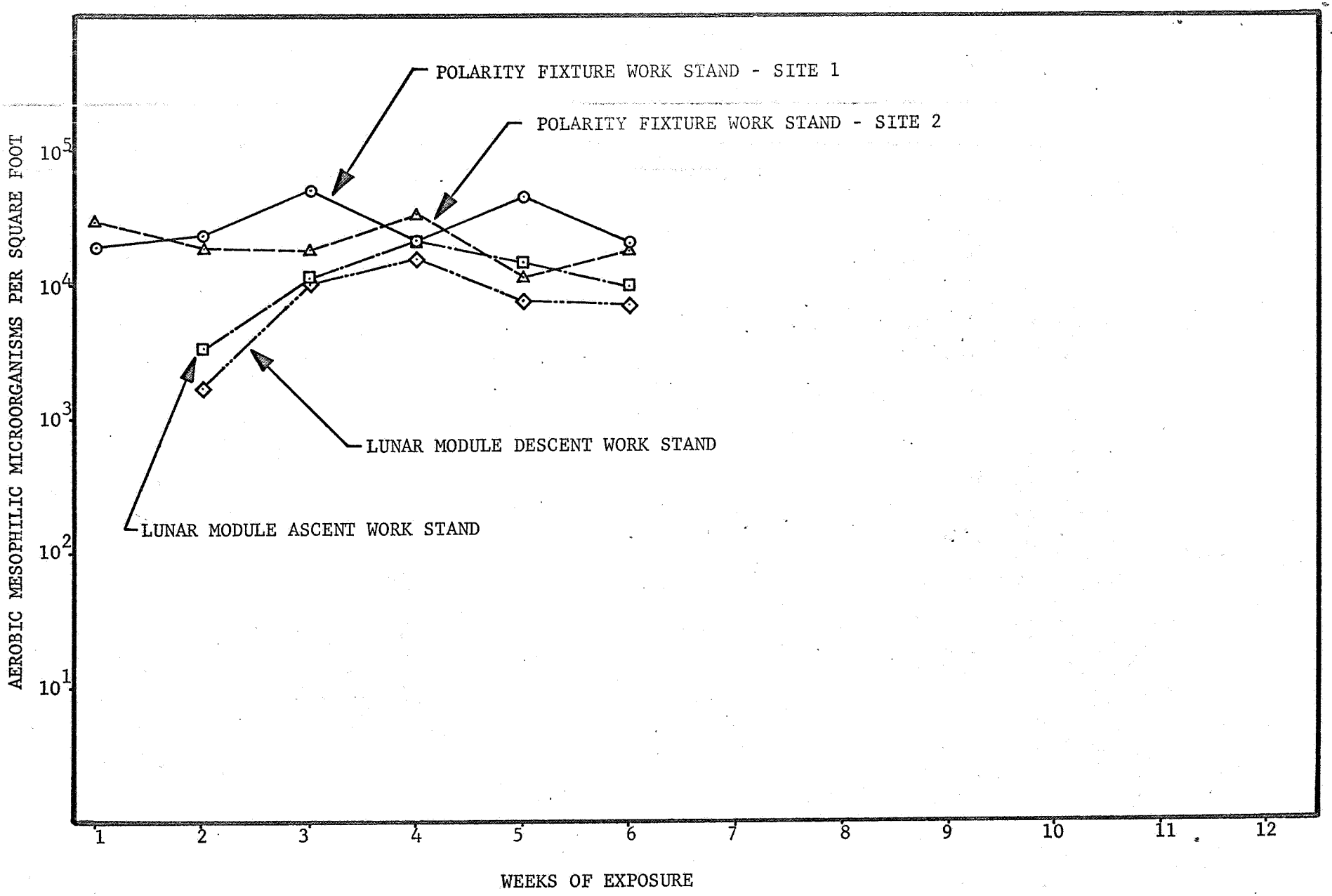

FIG. 4. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL SURFACES EXPOSED TO THE INTRAMURAL AIR OF SEVERAL WORK STANDS.

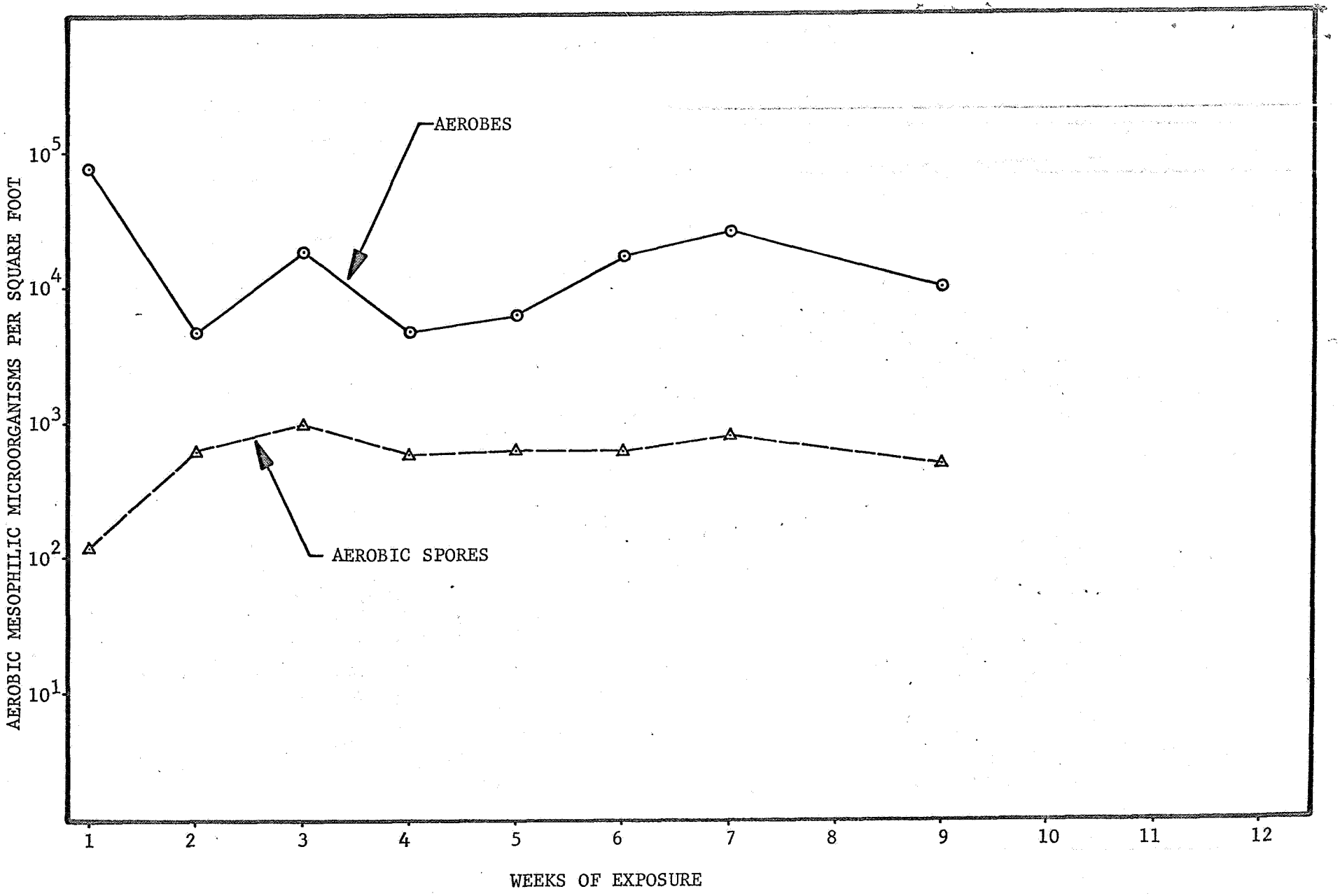

FIG. 5. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL SURFACES EXPOSED TO THE INTRAMURAL AIR OF THE SPACESUIT CLEAN ROOM.

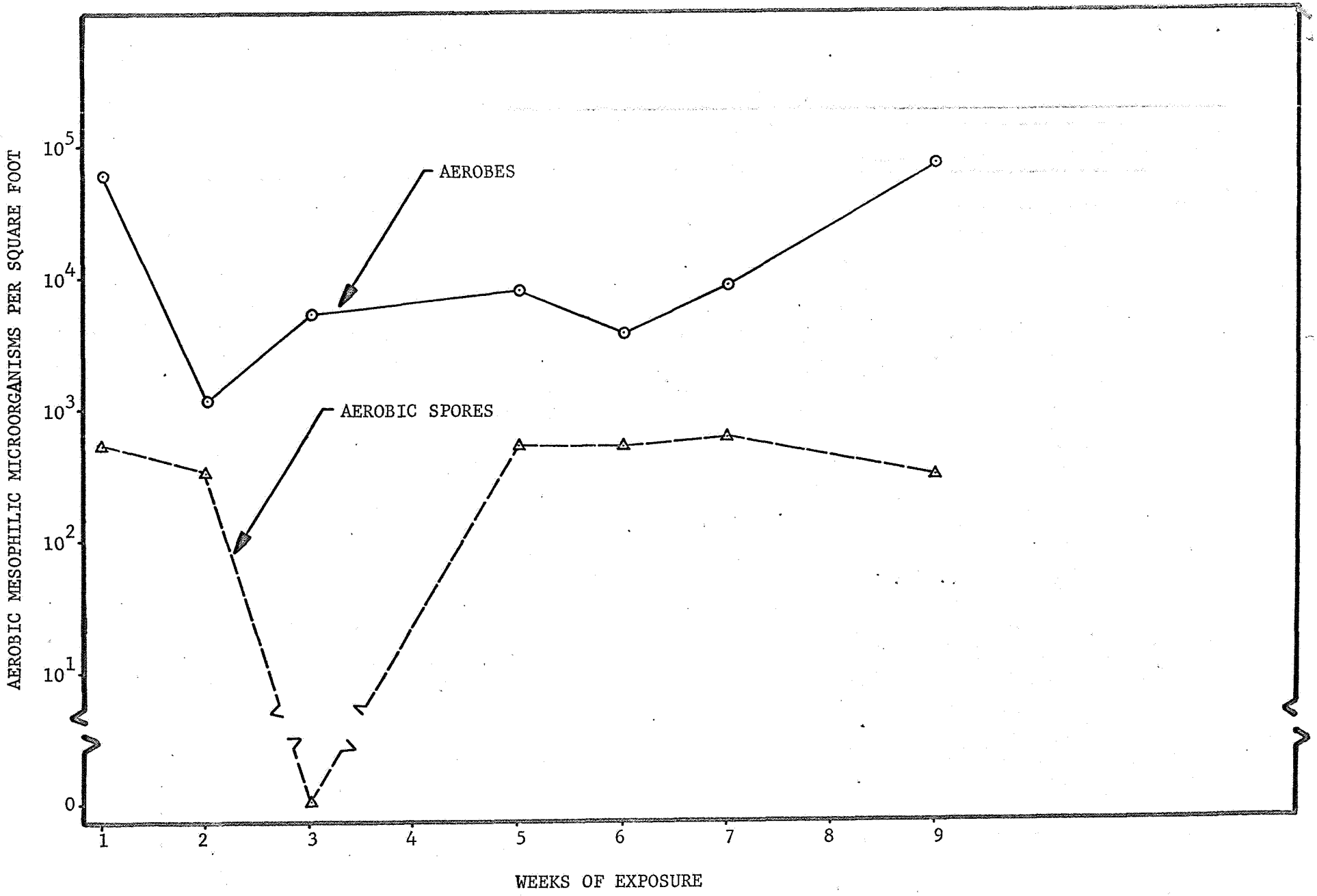

FIG. 6. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL SURFACES EXPOSED TO THE INTRAMURAL AIR OF THE BACK PACK LIFE SUPPORT CLEAN ROOM.