NASA CR - 119313

Services Provided in Support of the Planetary Quarantine Requirements
of the
National Aeronautics and Space Administration
under Contract W-13,062

Report No. 34
April - June 1971

Applied Microbiology and Planetary Quarantine Section
Phoenix Laboratories
Ecological Investigations Program
Center for Disease Control
Public Health Service
U.S. Department of Health, Education, and Welfare
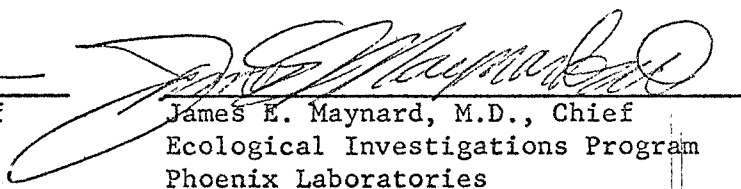Phoenix, Arizona

Contributors:

Biophysics Unit	Experimental Microbiology Unit	Spacecraft Bioassay Unit
N. Petersen	W. Bond	J. Puleo
J. Marshall	L. Carson	G. Oxborrow
D. Collins	M. Korber	N. Fields
		C. Herring

Report submitted by: Report reviewed and forwarded by:

Martin S. Favero, Ph.D., Chief   James E. Maynard, M.D., Chief
Applied Microbiology and   Ecological Investigations Program
Planetary Quarantine Section   Phoenix Laboratories

CASE FILE
COPY

1. The evaluation of certain aspects of the swab-rinse technique was continued by investigating the effect of training and experience on the subsequent performance of inexperienced individuals. A second individual (Trainee #2) who achieved very poor removal (70%) in the initial test was selected for study. The subject was trained and his performance was measured as described for Trainee #1 (Q.R. #33). The results achieved by Trainee #2 were compared with the results achieved by the instructor (Table 1). The mean removal value for the instructor was significantly higher than the value for the trainee. However, in each of the five tests the removal value for the trainee was markedly higher than the value he achieved in his initial test as an inexperienced individual. Also, the trainee's mean recovery value was not significantly different from that of the instructor.

   As a result of the tests conducted with inexperienced individuals and trainees, it was concluded that removal of surface contamination with a swab is not the major source of variation in results achieved using the swab-rinse technique. These experiments demonstrated that with a minimum of training, inexperienced individuals remove as much or nearly as much surface contamination with a swab as do experienced individuals. Furthermore, recovery of microorganisms from samples collected by inexperienced individuals always equaled or exceeded that from samples collected by experienced individuals. Since the recovery value most directly influences the results of surface sampling using the swab-rinse technique, it was concluded that the experience and training of the individual collecting the sample was not a significant variable.

   An effort was made to determine the major source of variation in recovery values achieved using the swab-rinse technique. Twenty-four swab samples were collected from 2 x 2" stainless steel strips by a single experienced individual and subjected to the standard assay procedure of vortexing, insonation, plating of rinse fluid, addition of fresh rinse fluid, vortexing and plating of second portion of rinse fluid. Following this procedure, 5 ml of rinse fluid were added to the tubes containing the swabs and the entire standard procedure was repeated. Plate counts from both procedures were totaled for each swab. The plate count from the first procedure was then expressed as a percentage of the total plate count for each swab to determine the efficiency of the first procedure. The standard deviation of this mean was only 2%, indicating that the procedure was quite consistent. At this point the major source of observed variation in the recovery of microorganisms from swabs has not been defined. However, these data suggest that for purposes of estimating the level of microbial contamination on surfaces, the efficiency of the swab-rinse technique can be approximated at 50%.

2. Studies were initiated using the biodetection grinder to detect and quantitate buried microbial contamination in a methyl methacrylate model system. The initial objective was to relate the efficiency of recovering microorganisms to particle size distribution of the ground material. It was anticipated that the design of the biodetection grinder would permit grinding seeded methyl methacrylate to achieve distinctly different particle size distributions. However, experimentation revealed that

in spite of varying the parameters of the biodetection grinder within maximum limits, ground methyl methacrylate consistently produced similar particle size distributions. As can be seen in Table 2, this was not in agreement with the findings reported by the developers of the bio-detection grinder at MSFC. The observed differences were probably due to different techniques used in collecting the particles for sizing. At MSFC particles were collected and sized in a dry condition, while at this laboratory particles were collected in standard rinse solution and dispersed for sizing on a dark membrane filter. It was observed that large particles produced by grinding invariably broke-up into many small particles upon contacting the rinse solution. Since microbiologic assay of the particles is performed in liquids, it was felt that the particle size distribution of the fines in liquid was most meaningful. Since the particle size distribution could not be varied in our laboratory, the plan to relate recovery to particle size distribution could not be implemented.

Experiments were conducted to determine the fraction of Bacillus subtilis var. niger spores buried in methyl methacrylate that survive grinding in the biodetection grinder as well as the fraction that can be detected by direct plating as a result of being released. Eighteen samples of seeded methyl methacrylate prepared in this laboratory and six samples prepared by Boeing were subjected to the assay procedure. The surface of each sample was decontaminated using the NASA standard peracetic acid procedure. A measured volume of each sample was aseptically ground using the bio-detection grinder and the fines were collected in 10 ml of sterile rinse solution to produce dispersion of the particles. The dispersed particles were plated in TSA and incubated at 32 C. The plates were inspected each day for 10 days and B. subtilis var. niger colonies were counted. A measured volume from each of six samples prepared in this laboratory and the six samples prepared at Boeing was ground, dissolved in acetone and the concentration of surviving spores was determined using a membrane filter assay technique.

The results of these assays, presented in Table 3, indicated that only a very small percent of spores buried in methyl methacrylate survive the grinding procedure and the percentage of buried spores that is re-leased for detection by direct plating is even smaller. In fact, none of the 7.8×10^3 spores in the 12 ground samples from the second set prepared at this laboratory was detected. Six samples of seeded Ecco-bond prepared at Boeing also were assayed using the biodetection grinder. Although Eccobbond cannot be dissolved to determine the actual concentra-tion of buried spores, the best estimates made at Boeing placed the concentration at very near that in the Boeing methyl methacrylate (2×10^4 spores per cm^3). However, the grinding procedure detected 40 times as many spores in the Eccobond as in the methyl methacrylate. Even at this, assuming the Boeing concentration estimate to be correct, the procedure detected only 1% of the buried spores in the ground Eccobond. The particle size distribution for ground Eccobond and methyl methacryl-ate in the samples tested is presented in Table 4.

The disparity between recovery results with Eccobond and those with methyl methacrylate indicate a need for additional studies with model systems to better define the variables influencing the survival and detection of buried spores in ground solids. Only in this way can a meaningful value be selected for use in extrapolating results from bio-detection grinder assays of spacecraft piece-parts to the actual concentration of buried microbial contamination in the spacecraft.

Two types of resistors and three types of printed circuit boards, representative of piece-parts to be used in planet-bound spacecraft, were acquired from the Martin-Denver Company and were assayed for buried microbial contamination using the biodetection grinder. Wire leads were trimmed from the resistors and circuit boards were cut into pieces small enough to fit the grinder chuck. All pieces were surface decontaminated and surface sterility tests were performed on appropriate samples with negative results. Each piece was assayed aseptically using the biodetection grinder. All procedures were performed in a vertical laminar flow clean room or in cross flow benches in the clean room. A measured volume of each piece was ground and the fines collected in 10 ml of sterile rinse solution. The fines were then plated in TSA containing neutralizer, incubated at 32 C, and examined for the appearance of colonies each day for 10 days. Colonies that appeared were counted and subcultured for subsequent identification. A second measured volume was ground and the fines placed in tubes containing TSB with neutralizer. Tubes were incubated at 32 C and observed for the presence of growth for 10 days. Growth appearing in tubes was subcultured for subsequent identification. After 10 days of incubation, five negative tubes containing each of the five ground materials were inoculated with 1×10^2 spores of B. subtilis var. niger and again incubated at 32 C to determine whether inhibitory materials were present. No inhibition was detected in any of the tubes.

The quantitative results of the grinder assays are presented in Tables 5, 6, 7, 8, and 9. Most noteworthy was the frequency with which microorganisms were detected in hot molded resistors.

Forty-two percent of samples ground from hot molded resistors demonstrated the presence of viable microorganisms compared to only 2 percent of the samples ground from all other pieces. The types of microorganisms isolated from ground samples are listed in Table 10. As would be expected, Bacillus spp. predominated. An unexpected finding was the number of Actinomycetes and Micrococcus spp. that were recovered. The presence of these organisms, as well as the two Gram negative isolates could be interpreted as contaminants introduced in the assay procedure. However, the effort made to prevent such contamination leads us to give equal weight to the possibility that the organisms were in fact buried in the piece-parts. It may be significant that both Gram negative organisms were isolated from mixed cultures, both including Bacillus spp.

3. The sieve processed vacuum cleaner dust from Hangar AO (KSC) reported last quarter was subjected to heat resistance testing. One gram of the

dust that passed the 43 micron sieve was suspended in 10 ml of 95%
ethanol, and 0.1 ml was applied to standard 1/2 x 1/2" stainless steel
strips. Four inoculated strips and one uninoculated control were sus-
pended in a forced air oven at 125 C at each interval and were assayed
for survivors in the usual manner (Q.R. 31) with supplemented TSA. All
plates were overlayed with the TSA to retard spreading growth and were
incubated for one week at 35 C. All control strips were negative, and
Figure 1 shows the results. The D_{125C} value obtained from the best fit
line ignoring N_0 was 94 min. The vacuum cleaner dust contained approx-
imately 10^5 spores per gm after sieving which was the highest titer so
far in a sample such as this. As reported previously (Q.R. 31) there
were about 10^7 spores per gm in the sieved Cape Kennedy soil. Figure 1
shows that the viable survivors per strip at the 120 min interval ranged
from 4 to 11, which are obviously not desirable numbers from a statistical
standpoint. It has been our experience in the past that the more heat
resistant organisms in a naturally occurring population are usually found
in relatively low numbers, i.e., 1-2 logs below the starting concentra-
tion. Work is currently underway to develop methods whereby naturally
occurring spores may be physically separated and concentrated from soils
and dusts. In lieu of these methods at present, 0.1 gm portions of the
AO Hangar vacuum cleaner dust are being heat tested in order to raise
the concentration of the resistant spores to a more accurately measurable
range. Also extended incubation of platings will be attempted employing
agar underlay and double overlays to inhibit spreading growth.

4. Bacillus sp. 125-48, the organism reported last quarter that formed
   spherical, rough surfaced spores, was identified biochemically (Identi-
   fication Scheme for Microorganisms Isolated from Apollo Spacecraft) as
   B. lentus. According to Smith, et.al., (Smith, N. R., R. E. Gordon, and
   F. E. Clark, 1952, Aerobic Sporeforming Bacteria, Agriculture Monograph
   No. 16, USDA), B. lentus forms oval, thin-walled spores with the sporangium
   only slightly swollen, if at all. Colonial characteristics, such as a
   slimy or mucoid consistency, were not indicated for B. lentus. A colony
   of Bacillus sp. 125-48 on TSA is initially small and slow-growing, in-
   creasing in size and mucoid consistency after 1 week of incubation,
   resembling the description of B. polymyxa growth. B. polymyxa spores
   are oval rather than spherical, however they exhibit much the same sur-
   face roughness and pattern as Bacillus sp. 125-48 spores. Considering only
   spore morphology, Smith, Gordon, and Clark would identify Bacillus sp.
   125-48 as B. sphaericus: "0.7-1.2 μ in diameter; round --- spore wall
   usually thick, stainable; --- the surface rough and spiny; --- sporu-
   lation variable --- ". B. sphaericus is described as inactive physio-
   logically, its morphology and unreactive character determining its
   identity for the most part. It is likely that Bacillus sp. 125-48 is
   a variant of B. sphaericus. It will be referred to by its numerical
   designation until a more certain identification can be made.

Two spore suspensions of Bacillus sp. 125-48 were prepared using TAM
and AK#2 sporulation agar. The media were inoculated with heat shocked
(80 C, 15 min) growth from TSA and were incubated at 25 C for 30 days.

The growth from two plates of each medium, all having a considerable amount of slime material, were harvested in cold sterile distilled water. The resultant suspensions were used as inocula for initial heat resistance testing. One-tenth ml amounts of these suspensions on stainless steel strips gave N_0 values of approximately 5×10^2 spores/strip. Strips were heated in the usual manner (Q.R. 31) at 125 C using 8 hr intervals to a maximum time of 48 hr. Insufficient data for presentation of survivor curves have been gathered at this time, however, D_{125C} values appear to be in the range of 50 hr. More complete data will be presented next quarter. Larger crops of Bacillus sp. 125-48 are being prepared using TAM and AK. Repeated attempts to culture the organism in SSM-10 liquid sporulation medium (Q.R. 25) have not been successful.

5. Work has begun to prepare additional subcultured spore crops for heat resistance testing. Pertinent isolate sources include Bacillus firmus, B. lentus, and B. sphaericus cultures from recent Apollo spacecraft samplings, heat resistant isolates from the AO Hangar vacuum cleaner dust and the Cape Kennedy soil suspension, and spore formers recovered from spacecraft piece-parts using the biodetection grinder.

6. A total of 1123 microorganisms were isolated and identified from the Apollo 14 spacecraft. The number and types of microorganisms are shown in Table 11. Thirty two different species or groups were detected. Table 12 shows the types of aerobic mesophilic microorganisms isolated from each of the different component parts of the spacecraft. The types of microorganisms detected on components of Apollo 14 were similar to those found on previous Apollo spacecraft (Q.R. 27, 29, and 30).

The vast majority of microorganisms detected on the Command Module (CMI), Lunar Module (LAI), and Spacecraft Lunar Module Adapter (SLA) interior surfaces were those considered to be indigenous to human hair, skin, and respiratory tract. The occurrence of microorganisms associated with soil and dust in the environment were found in higher levels on the other component parts of the spacecraft (Table 13). A comparison of the microorganisms indigenous to humans detected on five Apollo spacecraft is shown in Table 14. The data show that the Apollo 14 spacecraft had a decrease of these types when compared to previous Apollo spacecraft.

The microorganisms isolated and identified from the Apollo 14 spacecraft were lyophilized and stored for future reference. In addition, all data pertaining to enumeration and identification of microorganisms from the Apollo spacecraft were treated and stored in a CDC 3100 computer at Cape Kennedy for rapid retrieval. Computer printouts were compliled and sent to the Planetary Quarantine Officer.

Microbiological assays of the two Mariner-Mars 1971 spacecraft were accomplished at Cape Kennedy as described by the "Mariner-Mars 1971 Microbiological Assay and Monitoring Plan". Independent verification of the Jet Propulsion Laboratory's microbiological assays was conducted by PHS personnel who assayed half of all swab samples taken from the two

flight spacecraft. A total of 463 microorganisms were isolated from the two Mariner-Mars 1971 spacecraft and these isolates are in the process of being identified.

The microorganisms isolated from the Apollo 14 spacecraft were identified both manually and by a CDC 3100 computer using the Qualitative Identification Program as developed by Sandia Corporation. There were a total of 1123 bacterial cultures identified from Apollo 14. Of this total, 224 differences existed between the computer and manual identification methods. An analysis of these differences revealed that in 87 cases both identification methods were correct. This can occur when there are two possible identifications for a microorganism, each differing by only one test reaction. The computer is programmed to pick the first one of the two in the sequence. Subtracting the cases with equal identification from the total differences leaves 137 cases in which the manual identification was found to be incorrect and the computer made the correct identification.

An analysis of the above data shows that 20% of the microorganisms identified differed between the two identification methods. Of this difference, 7.8% were due to cases where either identification would be correct and 12.2% were due to errors made by the manual identification. These results indicate that identification by the computer system is superior to manual identification of microorganisms. Similar results were obtained by the Sandia Corporation who compared both identification methods with the Apollo 10, 11, and 12 bacterial isolates. They showed that the computer system agrees with the manually determined identification more than 90% of the time. The human errors present when handling a large number of similar material are eliminated by use of the computer. Because of the results and reasons stated above, all bacterial cultures from Apollo 14 were identified by the computer system, as will all future isolates.

7. The study for the evaluation of a terminal sterilization process for unmanned lander spacecraft was continued. The preliminary results are described in the "Ecology and Thermal Inactivation of Microbes In and On Interplanetary Space Vehicle Components", (Q.R. 24) FDA, Cincinnati, Ohio. In this quarter, experiments have been conducted to insure that laboratory techniques are adequate and that the sterile cups can be handled aseptically. (Probability of background contamination, if any, must be less than 1×10^{-3}.) The oven was filled with 2100 empty cups, sealed, and the temperature raised to 150 C for a minimum of 6 hours and allowed to cool to room temperature. The sample cups were then removed, nutrient media were added to each cup, and allowed to incubate for 7 days. The cups were then inspected and scored for growth. Three replicate experiments were conducted with no positives observed. These experiments fulfill the aseptic technique constraint. In addition, two tests for independence of cups have been done. These results have been forwarded to Dr. Campbell, FDA, Cincinnati, Ohio, for statistical analysis.

TABLE 1.   COMPARISON OF SWAB-RINSE RESULTS ACHIEVED BY INSTRUCTOR AND TRAINEE # 2.

Individual	No. of Samples	Removal		Recovery	
		Mean %	Coefficient of Variation %	Mean %	Coefficient of Variation %
Instructor	30	91	4	68	12
Trainee # 2	30	87	5	71	18

TABLE 2. EFFECT OF GRIT NO. ON PARTICLE SIZE DISTRIBUTION OF METHYL
METHACRYLATE OBTAINED WITH BIODETECTION GRINDER.

Lab	Cutter Grit No.	% Particle Size - Microns		
		2 - 4	5 - 8	> 8
MSFC	60	21	22	57
PHS	60	43	30	27
MSFC	240	63	20	17
PHS	240	41	39	20

TABLE 3. RESULTS OF BIODETECTION GRINDER ASSAYS OF SEEDED METHYL METHACRYLATE.

Source	PHS-1	PHS-2	Boeing
No. of Samples	6	12	6
Control Concentration (spores/cm^3)	3.5×10^4	2.5×10^4	2.1×10^4
Mean Size of Sample (cm^3)	5.4×10^{-2}	2.6×10^{-2}	1.3×10^{-1}
Dissolved Fines Concentration (spores/cm^3)	810	-	17.
Plated Fines Concentration (spores/cm)	58	0	7
% Surviving Grinding	2.50	-	.08
% Recovery of Survivors	10.7	-	41.1
% Recovery of Control	.27	0	.03

TABLE 4.   EFFECT OF SAMPLE MATERIAL ON PARTICLE SIZE DISTRIBUTION

OBTAINED WITH BIODETECTION GRINDER.

| Sample Material | % Particle Size - Microns | | | |
	< 5	5 - 15	15 - 45	> 45
Methyl Methacrylate	34	38	18	10
Eccobond	22	58	17	3

TABLE 5. RESULTS OF BIODETECTION GRINDER ASSAYS OF HOT MOLDED RESISTORS.

Pieces Sampled	24
Mean Vol. per Piece (cm^3)	9.7×10^{-2}
Mean Vol. per Sample Plated in TSA (cm^3)	2.4×10^{-2}
No. Plates With Positive Samples	13
Mean Concentration in Positive Samples (Microorganisms/cm^3)	77
Mean Concentration in All Samples (Microorganisms/cm^3)	42
Mean Vol. per Sample Placed in TSB (cm^3)	2.4×10^{-2}
No. TSB Tubes With Positive Samples	7

TABLE 6.   RESULTS OF BIODETECTION GRINDER ASSAYS OF METAL FILM RESISTORS.

Pieces Sampled	8
Mean Vol. per Piece (cm^3)	1.7×10^{-1}
Mean Vol. per Sample Plated in TSA (cm^3)	3.1×10^{-2}
No. Plates With Positive Samples	.1
Mean Concentration in Positive Samples (Microorganisms/cm^3)	65
Mean Concentration in All Samples (Microorganisms/cm^3)	8
Mean Vol. per Sample Placed in TSB (cm^3)	3.1×10^{-2}
No. TSB Tubes With Positive Samples	0

TABLE 7. RESULTS OF BIODETECTION GRINDER ASSAYS OF PRINTED CIRCUIT BOARD # 1.

Pieces Sampled	30
Mean Vol. per Piece (cm^3)	6.4×10^{-1}
Mean Vol. per Sample Plated in TSA	6.7×10^{-2}
No. Plates With Positive Samples	1
Mean Concentration in Positive Samples (Microorganisms/cm^3)	15
Mean Concentration in All Samples (Microorganisms/cm^3)	0.5
Mean Vol. per Sample Placed in TSB (cm^3)	6.7×10^{-2}
No. TSB Tubes With Positive Samples	0

TABLE 8. RESULTS OF BIODETECTION GRINDER ASSAYS OF PRINTED CIRCUIT
BOARD # 2.

Pieces Sampled	12
Mean Vol. per Piece (cm^3)	8.7×10^{-1}
Mean Vol. per Sample Plated in TSA (cm^3)	1.6×10^{-1}
No. Plates with Positive Samples	0
Mean Concentration in Positive Samples (Microorganisms/cm^3)	0
Mean Concentration in All Samples (Microorganisms/cm^3)	0
Mean Vol. per Sample Placed in TSB (cm^3)	8.0×10^{-2}
No. TSB Tubes With Positive Samples	1

TABLE 9. RESULTS OF BIODETECTION GRINDER ASSAYS OF PRINTED CIRCUIT

BOARD # 3.

Pieces Sampled	20
Mean Vol. per Piece (cm^3)	8.1×10^{-1}
Mean Vol. per Sample Plated in TSA (cm^3)	8.1×10^{-2}
No. Plates with Positive Samples	0
Mean Concentration in Positive Samples (Microorganisms/cm^3)	0
Mean Concentration in All Samples (Microorganisms/cm^3)	0
Mean Vol. per Sample Placed in TSB (cm^3)	8.1×10^{-2}
No. TSB Tubes With Positive Samples	0

TABLE 10. TYPES OF MICROORGANISMS ISOLATED FROM GROUND SAMPLES OF PIECE-PARTS.

Part	Colonies from TSA Plates		Growth from TSB Tubes	
	No.	Type	No.	Type
Hot Molded Resistor	12	Bacillus spp.	8	Bacillus spp.
	8	Actinomycete	1	Micrococcus sp.
	2	Micrococcus sp.	1	Gram Negative Rod
	1	Gram Negative Rod		
Metal Film Resistors	1	Bacillus spp.	0	
	1	Actinomycete		
Circuit Board # 1	1	Bacillus spp.	0	
Circuit Board # 2	0		1	Micrococcus sp.
Circuit Board # 3	0			

TABLE 11. COMPARISON OF THE NUMBERS AND TYPES OF MICROORGANISMS DETECTED ON APOLLO 14 SPACECRAFT.

	Number	Percent
Staphylococcus spp.		
Subgroup I	11	1.0
Subgroup II	161	14.3
Subgroup III	105	9.4
Subgroup IV	85	7.6
Subgroup V	110	9.8
Subgroup VI	118	10.5
Micrococcus spp.		
Subgroup 1	28	2.5
Subgroup 2	2	0.2
Subgroup 3	11	1.0
Subgroup 4		
Subgroup 5	7	0.6
Subgroup 6		
Subgroup 7	121	10.8
Subgroup 8	1	0.1
Bacillus spp.		
B. alvei	1	0.1
B. badius	1	0.1
B. brevis		
B. cereus	4	0.4
B. circulans	15	1.3

	Number	Percent
Bacillus spp.		
B. coagulans	20	1.8
B. firmus	5	0.4
B. laterosporus		
B. lentus	4	0.4
B. licheniformis	2	0.2
B. macerans	2	0.2
B. megaterium		
B. pantothenticus		
B. polymyxa	10	0.9
B. pulvifaciens		
B. pumilus	3	0.3
B. sphaericus	13	1.2
B. subtilis	3	0.3
Corynebacterium-Brevibacterium Group	158	14.1
Actinomycetes	7	0.6
Streptomycetes	4	0.4
Yeasts	2	0.2
Molds	31	2.8
Atypical Micrococcus spp.	36	3.2
Atypical Bacillus spp.	30	2.7
No growth on subculture	12	1.1
Number Isolated	1123	

TABLE 12. TYPES OF MICROORGANISMS DETECTED ON APOLLO 14 SPACECRAFT USING TRYPTICASE SOY AGAR.

Type	CSM-110 %	LAI-S %	LAE-S %	LDE-S %	SLA-14 %	IU-14 %	S-4B-14 %	All Components of Spacecraft %
Staphylococcus spp.								
Subgroup I		1.8		1.5			1.1	1.0
Subgroup II	21.6	17.5	14.3	9.8	5.9	3.1	4.5	14.3
Subgroup III	10.3	13.8	7.5	7.6	2.9	2.1	1.1	9.4
Subgroup IV	11.8	8.3	6.0	5.3	8.8	3.1	4.5	7.6
Subgroup V	12.2	13.1	4.5	7.6	2.9	7.2	4.5	9.8
Subgroup VI	18.6	9.2	7.5	9.1	5.9	13.4	5.6	10.5
Micrococcus spp.								
Subgroup 1	1.5	3.9	0.8	0.8		2.1	4.5	2.5
Subgroup 2	0.5	0.2						0.2
Subgroup 3		2.1		0.8		1.0		1.0
Subgroup 4								
Subgroup 5	0.5	0.7	1.5				1.1	0.6
Subgroup 6								
Subgroup 7	3.9	8.8	24.1	15.2	5.9	12.4	10.1	10.8
Subgroup 8			0.8					0.1

Type	CSM-110 %	LAI-S %	LAE-S %	LDE-S %	SLA-14 %	IU-14 %	S-4B-14 %	All components of Spacecraft %
Bacillus spp.								
B. alvei						1.0		0.1
B. badius							1.1	0.1
B. brevis								
B. cereus						2.1	2.2	0.4
B. circulans	0.5	0.2	0.8		5.9	3.1	7.9	1.3
B. coagulans	1.0	1.6	0.8	3.0		4.1	2.2	0.4
B. licheniformis		0.2					1.1	0.2
B. macerans	0.5			0.8				0.2
B. polymyxa	1.5	0.2				3.1	3.4	0.9
B. pumilus	0.5		0.8			1.0		0.3
B. sphaericus			0.8	0.8		4.1	7.9	1.2
B. subtilis				0.8			2.2	0.3

TABLE 12. TYPES OF MICROORGANISMS DETECTED ON APOLLO 14 SPACECRAFT USING TRYPTICASE SOY AGAR. (Continued)

Type	CSM-110 %	LAI-S %	LAE-S %	LDE-S %	SLA-14 %	IU-14 %	S-4B-14 %	All Components of Spacecraft %
Corynebacterium-Brevi-bacterium group	7.8	9.7	18.0	21.2	67.6	16.5	10.1	14.1
Actinomycetes	0.2			4.5				0.6
Streptomycetes	0.5					1.0	1.1	0.4
Yeasts	0.5							0.2
Molds	0.5	3.8	3.8	3.8		11.3	9.0	2.8
Atypical Micro-coccus spp.	2.9	3.7	3.0	3.8		2.1	3.4	3.2
Atypical Bacillus spp.	2.0	1.8	3.0	1.5		4.1	9.0	2.7
No growth on sub-culture	2.0	1.2	0.8	0.8		1.0		1.1
Number isolated	204	434	133	132	34	97	89	1123

TABLE 13. TYPES OF MICROORGANISMS ASSOCIATED WITH THE COMPONENT PARTS OF APOLLO 14 SPACECRAFT.

Source	Human Types Percent	Environmental Types Percent
CMI	94	6
LAI	94	6
LAE	89	11
LDE	83	17
SLA	94	6
I.U.	64	36
S-4B	51	49
Total Spacecraft	86	14

TABLE 14. MICROORGANISMS INDIGENOUS TO HUMANS ON APOLLO 10, 11, 12, 13, AND 14.

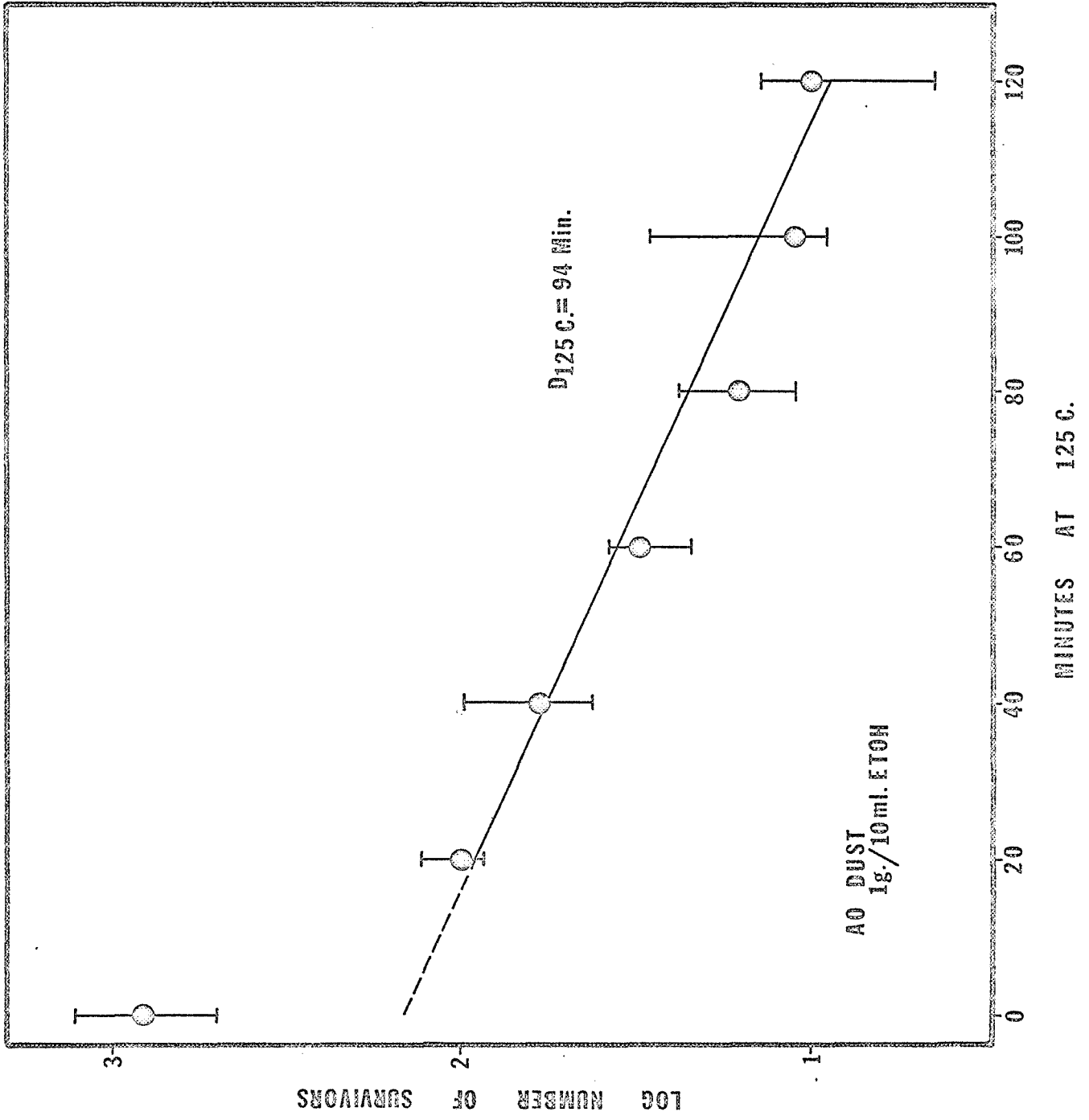| Apollo	CMI %	LAI %	LAE %	LDE %	SLA %	IU %	S-4B %
10	98	99	91	97	82	89	87
11	99	98	93	92	25	84	76
12	98	97	89	94	100	79	80
13	99	97	98	96	100	77	83
14	94	94	89	83	94	64	51

FIGURE 1. AO HANGAR VACUUM CLEANER DUST; SIEVE PROCESS DRY.