CHAPTER 2
MICROBIOLOGICAL INVESTIGATIONS

by

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Introduction

The crew microbiology program was initiated in response to requirements made by the Interagency Committee on Back-Contamination* in recognition of the possibility of returning terrestrial contaminants in the lunar soil. In order to characterize contaminants as terrestrial, and not extraterrestrial, a catalog of the crew microflora was prepared prior to each Apollo mission. Since crewmen were the prime source for lunar soil contamination, this catalog provided an invaluable method to assist in establishing the terrestrial origin of a recovered contaminant.

Analysis of crew specimens was performed to satisfy three objectives in addition to lunar contaminant evaluation. The primary objective was to detect potentially pathogenic microorganisms so that associated medical problems could be identified early and preventive measures established. A second objective was to identify medically important microorganisms recovered from ill crewmen to aid in diagnosis and treatment. The third objective was to collect microbiological data that would aid in elucidating the response of the crew microbial autoflora to the space flight environment and in evaluating the resultant effect on the crewmember.

Microbiological sampling of selected sites in the Command Module (CM) was initiated in support of the quarantine program. These samples were also important from a medical standpoint because crewmen would be exposed to microorganisms in the closed spacecraft environment during space flight.

During lunar quarantine missions (Apollo 11 through 14), microbial screening was accomplished for all support personnel to be isolated with the returning crewmen.

*The Interagency Committee on Back-Contamination included members from the National Academy of Sciences and representatives from the U.S. Public Health Service, U.S. Department of Agriculture, and U.S. Department of Interior. See Section V, Chapter 1, *The Lunar Quarantine Program* for more detail.

Diagnostic microbiology was provided for all astronauts, their wives, and families; for personnel in the lunar (sample) processing area, and for personnel in the quarantine area. Microbiological support was also provided for the biological test systems used to screen the lunar materials for life forms and for maintenance of sterile Class III biological glove box systems.

Virology support for the Apollo Program consisted of characterization of the viral and mycoplasma flora of the crewmembers; performance of viral serology for crewmembers, crew contacts, and key mission personnel; and analysis of specimens obtained as a result of crew illnesses and from the conduct of the mission personnel surveillance program and the Flight Crew Health Stabilization Program. These programs were designed to ascertain the nature of illnesses in personnel who were either in contact with the crew or worked with lunar soil behind the biologic barrier. Serology studies were initiated with the Apollo 14 mission. The mission personnel surveillance program was in effect during the Apollo 11, 12, 13, and 14 missions, and the Flight Crew Health Stabilization Program was in effect during the Apollo 14, 15, 16, and 17 missions.

Procedures

Crew Microbiology

Each flight crewman and backup crewman assigned to Apollo missions 7 to 12, was sampled at four different time periods to provide the data needed to develop a catalog of microorganisms: 30 and 14 days before flight (F – 30 and F – 14, respectively), immediately before the flight (F – 0), and immediately upon recovery (R + 0). For the Apollo 13 to 17 missions, sampling times were varied according to mission constraints. Generally, an additional postflight sampling period was added at approximately two weeks following recovery.

Eleven samples were obtained from each crewmember on the morning of each preflight sampling date before initiation of personal hygiene activities, eating, or urination. Postflight samples were collected on board the recovery vessel immediately upon recovery and before other medical tests were performed. All specimens were analyzed by the microbiology laboratories at the NASA Lyndon B. Johnson Space Center. The body surface sites generally sampled were as follows.

1. A 13-cm^2 area of the scalp below the hairline at the base of the neck.
2. The auditory canals. (Two revolutions were made with each swab in each ear canal.)
3. The internal area of the umbilicus and a surrounding 13-cm^2 area. (Two revolutions were made with each swab.)
4. A 6.5-cm^2 area below the hairline of each axilla.
5. An area from front to rear of the left and right side of the groin.
6. An area between the first and large toe of each foot.
7. A 6.5-cm^2 area on each palm.

Both nostrils of each crewmember were sampled by making two revolutions with each swab in each nasal canal.

Each body surface site and the nasal passages were sampled separately with two sterile calcium alginate swabs moistened with a phosphate buffer. One swab from each sample area was placed in a screwcap tube containing 10.0 ml of sterile trypticase soy broth (TSB) for aerobic analysis. The second swab was placed in a tube of sterile veal infusion broth (VIB) for anaerobic analysis.

Each crewmember gargled with 60 ml of sterile phosphate buffer. The gargle was rinsed three times through the oral cavity to obtain a combination throat-mouth sample. The wash was then emptied into a sterile, widemouthed bottle.

A first-void, midstream urine specimen and a fecal specimen from each subject were collected in separate sterile containers on the morning of preflight sampling. Postflight specimens were collected as available.

The body surface samples, the nasal samples, the throat-mouth gargle, the urine, and the feces were maintained at 277°K (4°C) during transport to the laboratory. Approximately 12 hours elapsed between sampling and initial culture.

One-milliliter aliquots of the throat-mouth gargle and the urine were transferred to 9.0 ml each of TSB and VIB. Portions of fecal material weighing 0.1 gm each were transferred to TSB, VIB, and tetrathionate broth. In addition, a 0.1-gm portion of fecal material was heatshocked for five minutes at 353°K (80°C).

Dilution series from each TSB and VIB sample tube were prepared by aseptically transferring 1.0 ml aliquots to 9.0 ml of TSB or VIB. Body and nasal samples were diluted to 1×10^4; throat-mouth gargle samples, to 1×10^5; urine samples, to 1×10^2; and feces samples to 1×10^{10}. All tubes were maintained at 277°K (4°C) during the diluting process.

Aliquots of 0.1 ml from the initial sample tube and from the TSB and the VIB dilution series were transferred to agar media for quantitation and isolation of aerobic and anaerobic species (figures 1 and 2). Individual sterile glass rods were used to spread inoculum over the agar surface. Aerobic media were incubated for 48 hours at 308°K (35°C). Anaerobic media were incubated for 96 hours at 308°K (35°C) using an anaerobic Gas Pak (BBL, Division of Bio-Quest, Cockeysville, Md.) for generation of hydrogen gas. Colony counts were performed on the aerobic and anaerobic quantitation media. After 4.0 ml aliquots were transferred from each TSB sample tube to a labeled sterile screwcap tube for mycological analysis (figure 3), the TSB and VIB sample tubes were incubated for 24 hours at 308°K (35°C). Inocula from the sample tubes were then streaked on each isolation medium. The isolation streaks were prepared to culture organisms too few in number to be isolated on the quantitative agar media. Inoculated chocolate agar was incubated at 308°K (35°C) under an atmosphere of 8 to 10 percent carbon dioxide.

A portion of each colony type isolated on the quantitation and isolation media was transferred to a TSB or thioglycolate broth tube and incubated at 308°K (35°C) until turbid. The pure culture of each isolated organism was used to prepare stained slides and to inoculate biochemical media or to perform biochemical tests for identification.

State-of-the-art procedures that consisted of challenging tissue cultures, embryonated eggs, suckling mice, and mycoplasma media with specimens obtained at various times before and after flight were used in characterizing the viral and mycoplasma flora.

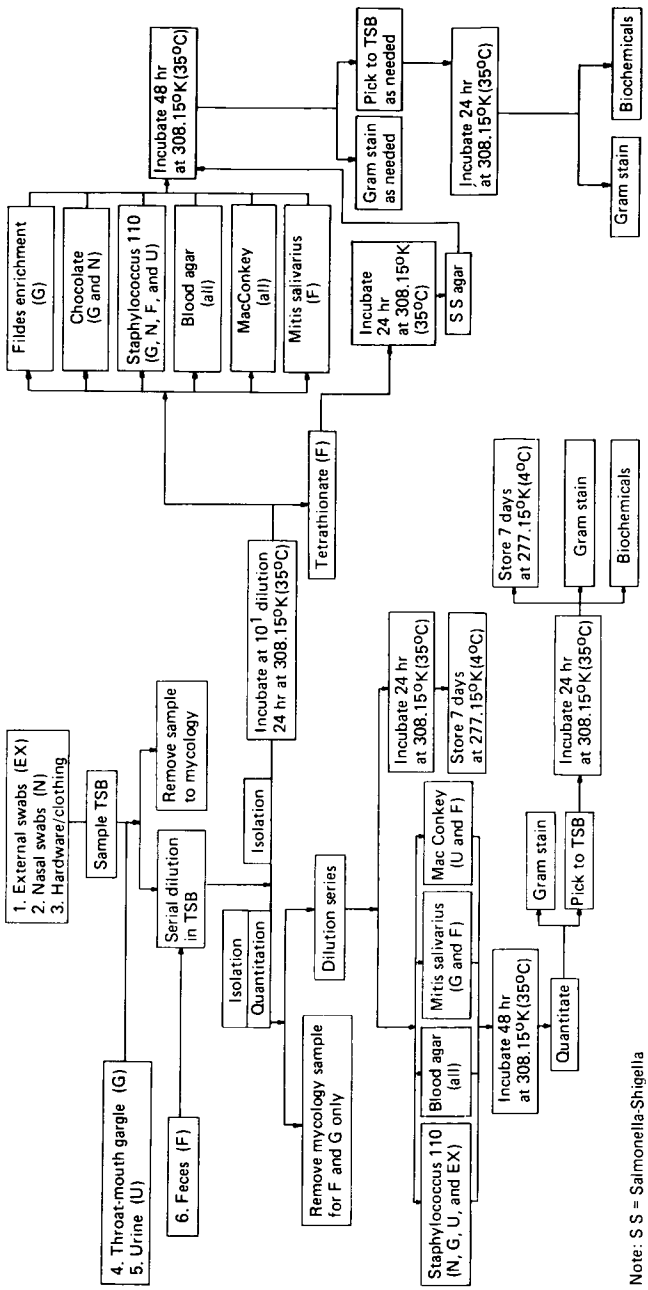

Figure 1. Crew bacteriology protocol for aerobic scheme.

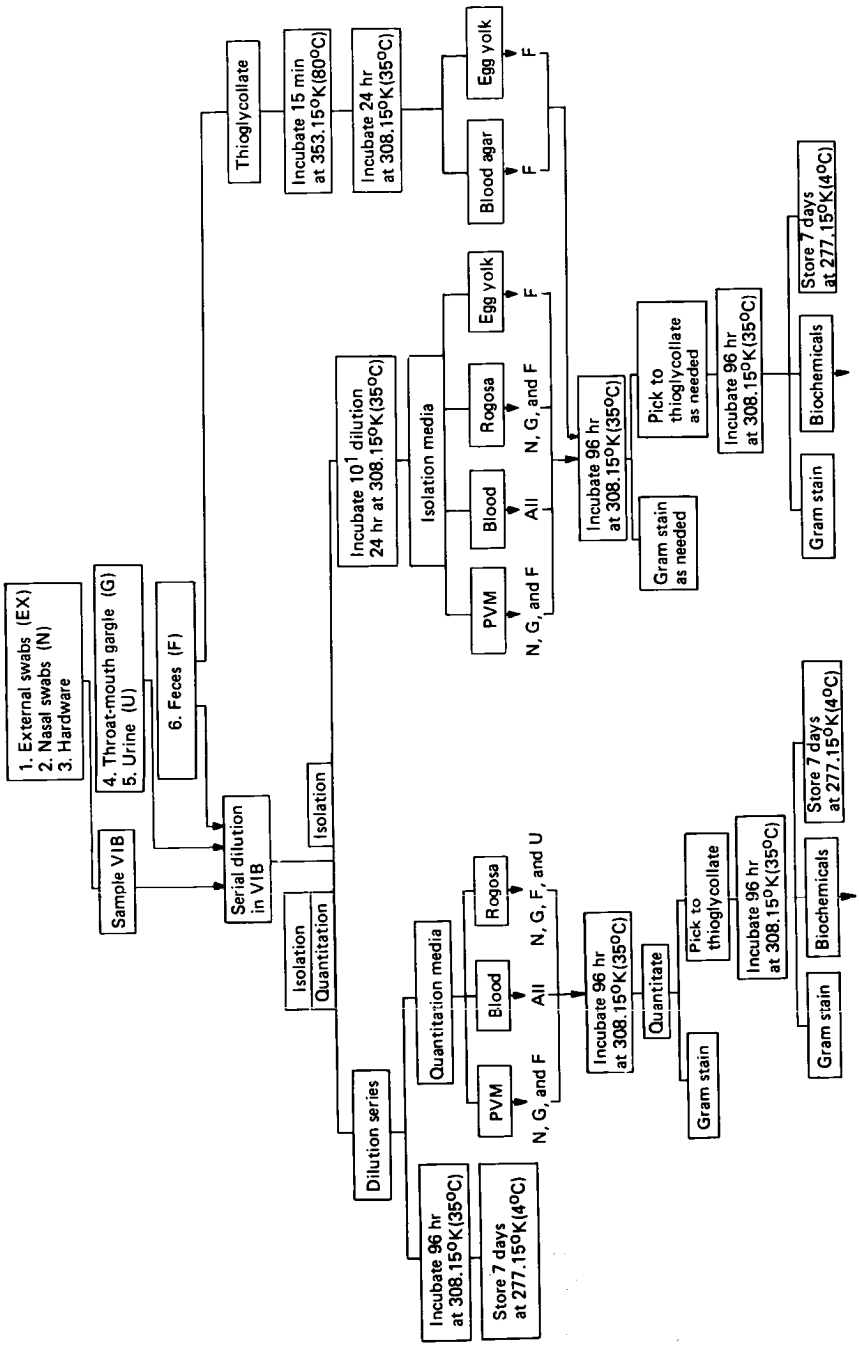

Figure 2. Crew bacteriology protocol for anaerobic scheme.

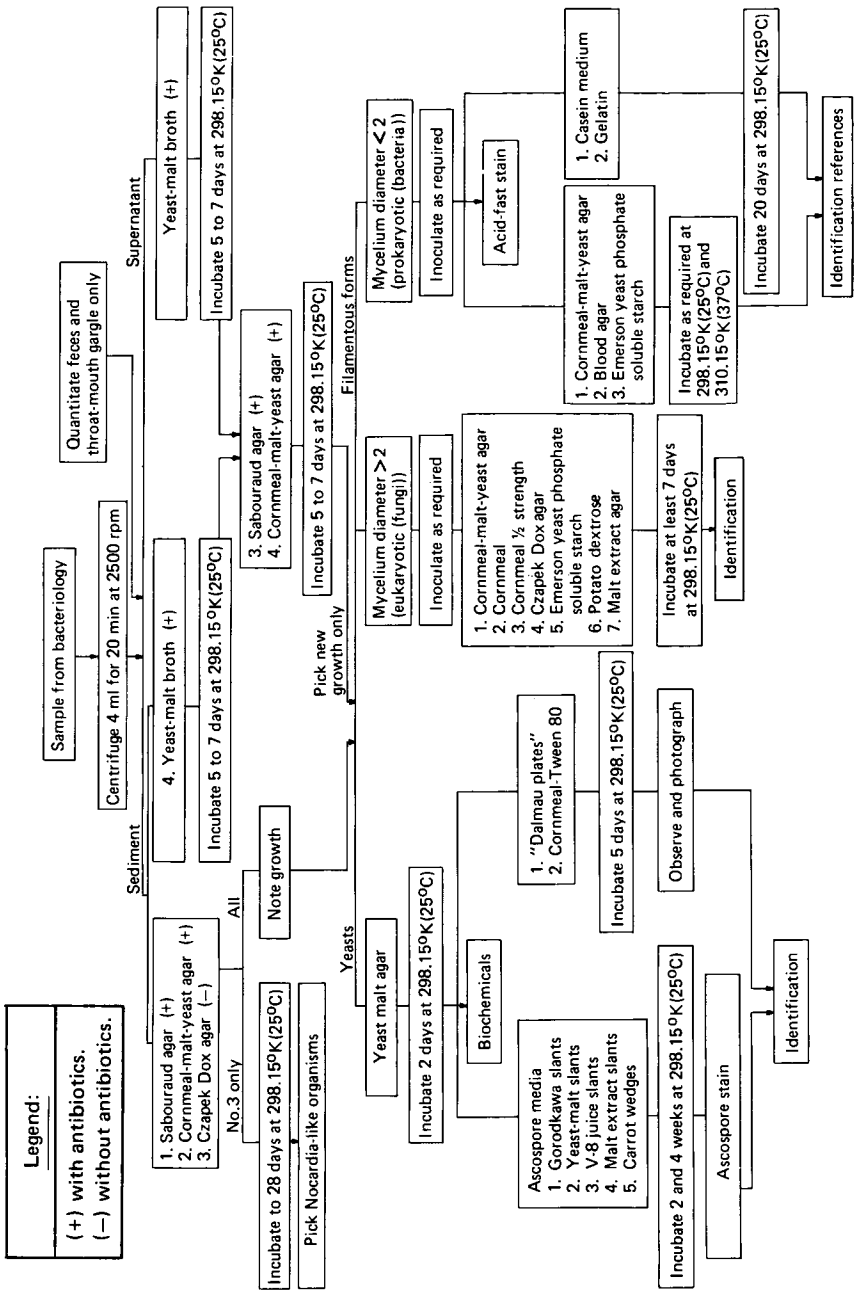

Figure 3. Crew mycology protocol.

Spacecraft Microbiology

Immediately before and after flight, swab samples were obtained from four selected sites inside the Command Module. Sterile calcium alginate swabs were moistened with 0.85 percent saline containing 0.0003 molar phosphate buffer. Two swabs were used to sample each of the following sites: (1) the total surface area of the mouthpiece of the drink gun; (2) a 13-cm^2 area of each pistol grip of the Command Module Pilot (CMP) maneuver controller; (3) a 13-m^2 area of each head strut; and (4) a 26-cm^2 area of the floor beneath the foot of the center couch. After the sampling, one swab from each site was placed in 5.0-ml TSB and another in 5.0-ml VIB. The tubes were maintained at 277°K (4°C) during transport to the laboratory. Each tube was vortexed, and the appropriate medium was used to serially dilute the contents. An aliquot of each TSB dilution was plated onto five percent sheep blood agar and incubated aerobically at 308°K (35°C) for 48 hours. An aliquot of each VIB dilution was plated onto sheep blood agar containing 10 mg/liter vitamin K and 5 mg/liter hemin. Gas Paks (Bio-Quest) were used to obtain anaerobic conditions. The plates were incubated at 308°K (35°C) for 96 hours.

Four milliliters of the undiluted TSB samples were used for mycological analysis. Each sample was centrifuged at 2500 rpm for 15 minutes. The supernatant from each was mixed with 10 ml of yeast-malt broth containing 33 000 units/liter penicillin G and 62 mg/liter streptomycin. The sediment was sampled with sterile calcium alginate swabs. The swabs were used to streak the surface of cornmeal-malt-yeast agar (containing antibiotics), Sabouraud dextrose agar (containing antibiotics), and Czapek Dox agar. The swabs were then placed into 10 ml of yeast-malt broth (containing antibiotics). All mycological media were incubated at 298°K (25°C) for 120 hours.

Following identification of all microorganisms, the laboratory data on each isolate were stored in a Univac 1108 computer. A computer program was developed to provide a "match test" of all stored data with the data that would be gathered from a lunar soil isolate. The program was designed to search the catalog of data on known terrestrial microorganisms and to select those microorganisms with the greatest number of like characteristics and test results.

Results and Discussion

Crew Microbiology for Quarantine

The final identification of microorganisms isolated at the various sampling sites resulted in approximately 150 to 175 identifications per sampling period. Often, the same microorganism was isolated at more than one site. At the time the first lunar material was returned by the Apollo 11 crewmen, the catalog contained laboratory identification data of approximately 4000 microbial isolations.

Throughout the Apollo missions, no microorganism was isolated from the lunar soil. This result attests to the successful operation of the Lunar Receiving Laboratory and the development of adequate aseptic techniques in the handling and processing of the lunar soil.

Crew Medical Microbiology

Increased Incidence and Transfer of Microorganisms Between Crewmen. An increased incidence of medically important gram-positive cocci was found for the Apollo 7 and 12 missions. On the Apollo 7 flight, 16 postflight isolations of *Staphylococcus aureus* were made at the various sampling sites of all three crewmen. During the period immediately before flight, five isolations were made from the samples of only two crewmen (table 1). The isolation sites on crewmember C were different at the two preflight periods, and the *S. aureus* was not isolated at any time from throat or nasal samples of this crewman. It is likely that the *S. aureus* was a transient microorganism on crewmembers C and B, and that crewmember A was a carrier.

Table 1

Occurrence and Distribution of *Staphylococcus Aureus* for the Apollo 7 Crewmembers

Sample Area	30-day Preflight			Immediate Preflight			Immediate Postflight		
	A[a]	B	C	A	B	C	A	B	C
Axilla	0[b]	0	0	0	0	0	0	0	0
Umbilicus	0	0	+[c]	0	0	0	+	0	+
Inguinal	0	0	0	0	0	+	+	0	+
Hands	+	0	0	+	0	+	+	+	0
Throat	0	+	0	+	0	0	+	+	0
Scalp	+	+	0	0	0	0	+	+	0
Nasal	+	+	0	+	0	0	+	+	0
Urine	0	0	+	0	0	0	0	+	0
Toes	0	0	0	0	0	0	+	0	+
Ears	0	0	+	0	0	0	+	0	0

[a]Crewmembers listed as A, B, and C.

[b]+ = isolation.

[c]0 = no isolation.

On the Apollo 7 flight, although no isolations of β-hemolytic streptococci were made from the throat samples of the crewmen at any preflight sampling period, β-hemolytic streptococci were present after the flight in the throat gargle sample of all three crewmen (table 2). Each sample contained 1×10^5 streptococcal cells per cubic centimeter of gargle. The presence and abundance of β-hemolytic streptococci and *S. aureus* on the Apollo 7 flight undoubtedly contributed to the nasal congestion and discomfort experienced by the crewmen.

Table 2

Occurrence and Distribution of Beta-hemolytic Streptococci for the Apollo 7 Crewmembers

Sample Area	30-day Preflight			Immediate Preflight			Immediate Postflight		
	A[a]	B	C	A	B	C	A	B	C
Axilla	+[b]	0[c]	0	0	0	0	0	0	0
Umbilicus	+	+	+	0	0	0	0	0	0
Inguinal	+	0	+	+	0	+	+	0	+
Hands	0	0	0	0	+	0	0	0	0
Feces	0	0	+	+	0	+	0	+	+
Urine	0	0	0	0	0	+	0	0	+
Throat	0	0	0	0	0	0	+	+	+

[a]Crewmembers listed as A, B, and C.

[b]+ = isolation.

[c]0 = no isolation.

A third microorganism, *Aspergillus fumigatus,* increased in number and apparently spread over the body surfaces of the Apollo 7 crewmen (table 3). With a single exception, all preflight isolations of *A. fumigatus* were made from the samples obtained from crewmember A. After flight, three or more isolations were made from the samples of each crewman. The organism was apparently transferred in flight from crewman A to crewmembers B and C. No significant increase of *A. fumigatus* or of any other fungus occurred on any mission through the Apollo 12 mission.

After the apparent transfer of microorganisms between crewmen during the Apollo 7 mission, strain-specific bacteriophage typing was developed in the laboratory and performed on all *S. aureus* recovered from later missions to better substantiate the suspected transfer.

An increased incidence of *S. aureus* did not reoccur until the Apollo 12 flight (table 4). Although only two isolations of *S. aureus* were made from one crewmember immediately before flight, seven of the twelve crewmember samples analyzed after flight were positive for *S. aureus.* Six additional isolations were made from the clothing samples and the internal Command Module samples. The organisms obtained immediately before and after flight were phage typed (table 5). Both isolates of *S. aureus* obtained from crewmember A immediately before flight were typed 3A. The microorganism was evidently transferred to crewmember B, to the urine collection device (UCD) of crewmember C, and to the couch support struts of the Command Module. The *S. aureus* phage type 187 was possibly a spacecraft contaminant. Although no inflight samples were obtained and the pustules on the crewmen's skin had dried at the postflight examination, *S. aureus* may have been the causative agent of the skin infections on the Apollo 12 flight.

Table 3

Occurrence and Distribution of *Aspergillus Fumigatus* for the Apollo 7 Crewmembers

Sample Area	30-day Preflight			Immediate Preflight			Immediate Postflight		
	A[a]	B	C	A	B	C	A	B	C
Scalp	+[b]	0[c]	0	0	0	0	+	0	0
Exterior auditory canal	0	0	0	0	0	0	+	+	+
Umbilicus	0	0	0	0	0	0	+	0	+
Hands	0	+	0	0	0	0	0	0	+
Inguinal	+	0	0	0	0	0	+	+	+
Toes	0	0	0	+	0	0	+	0	+
Gargle	+	0	0	+	0	0	+	0	0
Axilla	0	0	0	0	0	0	0	+	0

[a]Crewmembers listed as A, B, and C.

[b]+ = isolation.

[c]0 = no isolation.

Table 4

Occurrence and Distribution of *Staphylococcus Aureus* for the Apollo 12 Crewmembers

Sample Area	30-day Preflight			Immediate Preflight			Immediate Postflight		
	A[a]	B	C	A	B	C	A	B	C
Axilla	+[b]	0[c]	0	0	0	0	–[d]	–	–
Inguinal	+	0	0	0	0	0	–	–	–
Scalp	+	0	0	0	0	0	–	–	–
Toes	0	0	0	+	0	0	–	–	–
Hands	+	0	+	0	0	0	0	+	0
Nasal	+	0	0	+	0	0	+	+	+
Throat	0	0	0	0	0	0	0	+	+
Ears	+	0	0	0	0	0	0	+	0

[a]Crewmembers listed as A, B, and C.

[b]+ = isolation.

[c]0 = no isolation.

[d]– = no culture made.

Table 5

Distribution of Phage Typed *Staphylococcus Aureus* for the Apollo 12 Crewmembers and Command Module

Sample Area	Immediate Preflight			Immediate Postflight		
	Crewmembers			Crewmembers		
	A	B	C	A	B	C
Toes	3A	0[a]	0	_[b]	–	–
Hands	0	0	0	0	3A	0
Nasal	3A	0	0	3A	3A	187
Throat	0	0	0	0	3A	187
Ears	0	0	0	0	3A	0
Gloves	0	0	0	3A	3A	0
UCD	0	0	0	3A	0	3A
	Command Module			Command Module		
Maneuver Control	0			187		
Struts	0			3A		

[a] 0 = no isolation.

[b] _ = not cultured.

An increase in *S. aureus* did not occur on the Apollo 8, 9, 10, or 11 missions, even though, on two of these missions, at least one crewmember was carrying a nasal *S. aureus*. The microorganism did not increase in number as observed on the Apollo 7 and 12 missions, and was not exchanged between crewmen.

During the Apollo 13 flight, the transfer of *S. aureus* was again demonstrated. The Commander (CDR) and the Command Module Pilot (CMP) each carried *S. aureus* before flight, but the organisms were of different strains. Both strains were recovered after flight from the Lunar Module Pilot (LMP), who had not exhibited either strain before flight (table 6).

The Apollo 15 flight was an example of a more common occurrence, in which one crewmember, exhibiting multiple strains, probably acted as a reservoir to effect a transfer of one strain to another previously uncolonized crewmember during the flight. The transfer was from the CDR to the LMP, who spent more time with the CDR during the mission than did the CMP (table 7). The occurrence of intercrew transfer of microorganisms was demonstrated on many Apollo missions.

Specific Medical Microbiological Problems Associated with the Apollo 13 and 17 Flights. Urinalyses were performed on specimens from the Apollo 14 CMP several times during the 26 months preceding lift-off in response to a recurrent urethritis of possible microbial origin. However, no microorganisms were recovered until seven months before

C) 12

launch. Urine samples were evaluated periodically through the day of launch, and seven different medically important microorganisms were isolated (table 8). Of the microorganisms listed, the *Haemophilus* species was the most likely to cause a bacteria-mediated recurrent urethritis. Clinical symptoms were not expressed during the Apollo 14 space flight, although *Haemophilus* species was again isolated two weeks following recovery. As was usually the case, the presence of potentially pathogenic microorganisms *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Herellea vaginicola* in the postflight urine reflected the similar buildup observed in the urine collection device.

Table 6

Transfer of *Staphylococcus Aureus* Phage Types I and II from Two Apollo 13 Crewmembers to the Third Crewmember

Subject	Types of Bacteriophage Groups Present	
	Preflight (F-0)	Postflight (R + 0)
CDR	I	I
CMP	II	II
LMP	Absent	I and II

Table 7

Distribution of *Staphylococcus Aureus* Phage Types Among Apollo 15 Crewmembers

Subject	Sample Area	Sample Collection Period			
		F - 30	F - 5	F - O	R + O
CDR	Nasal	29/52	X[a]	X	X
	Throat	29	X	3A	29
	Gargle	X	N.T.[b]	29	29
LMP	Gargle	X	X	X	29/52
CMP	N.A.[c]	X	X	X	X

[a]Indicates no isolation of S. aureus.

[b]Nontypable S. aureus isolated.

[c]Not applicable.

An inflight malfunction of the Service Module, which caused early termination of the Apollo 13 mission, created a suboptimal environment and a stressful situation for the crew. Examination of the crew immediately after flight revealed that the LMP had a severe urinary tract infection from which *Pseudomonas aeruginosa* was isolated as the

causative agent. Antibiotic therapy was administered and closely monitored for 48 days following recovery (figure 4). Viable microbes had disappeared in the midstream urine samples within nine days following splashdown, although *P. aeruginosa* could still be recovered following prostatic massage after 16 days.

Table 8

Isolates from Urine of Apollo 14 CMP

Microorganisms	CMP Urinanalysis										
	Preflight by Month									Postflight by Month	
	26	13	7	4	3	2	1	0.5	0	0	0.5
Micrococcus species	–[a]	–	+[b]	–	–	–	–	–	–	–	–
Corynebacterium species	–	–	+	–	–	–	–	–	–	–	–
Haemophilus species	–	–	+	–	+	+	+	–	–	–	+
Staphylococcus epidermidis	–	–	–	+	+	+	–	–	–	–	–
Diphtheroid	–	–	–	+	–	–	–	–	–	–	–
Streptococcus species (β-hemolytic)	–	–	–	+	–	–	–	–	–	–	–
Klebsiella pneumoniae	–	–	–	–	–	–	+	–	+	+	–
Proteus mirabilus	–	–	–	–	–	–	–	–	–	+	–
Herellea vaginicola	–	–	–	–	–	–	–	–	–	+	–

[a] Indicates designated species not isolated.

[b] Indicates isolation of designated species.

The illness occurrence illustrates the types of infectious problems that can occur when the life support system is operating suboptimally for even a short period. Another example of the effect of unfavorable environmental conditions and poor hygiene was observed with the increased incidence of pathogenic microorganisms on the body surface. (figure 5). Whereas only three species (*Staphylococcus aureus, Escherichia coli,* and *Herellea vaginicola*) were recovered on the morning of launch, seven medically important species were recovered immediately after splashdown. In addition, the number of isolates of each species was generally higher after flight. Although there was generally a slight postflight increase in the incidence of pathogens in other crews, the Apollo 13 increase was significantly elevated. An average of 175 percent more medically important species was recovered from the seven Apollo 13 postflight skin swabs as compared with an average increase of only 33 percent for the same samples from the Apollo 14 flight.

It was not unusual to find at least one crewmember from each Apollo team harboring the pathogenic yeast *Candida albicans* in the mouth. The presence of this species generally does not pose a significant threat to healthy adults. However, the other fungi that normally exercise a controlling influence on *C. albicans* populations through

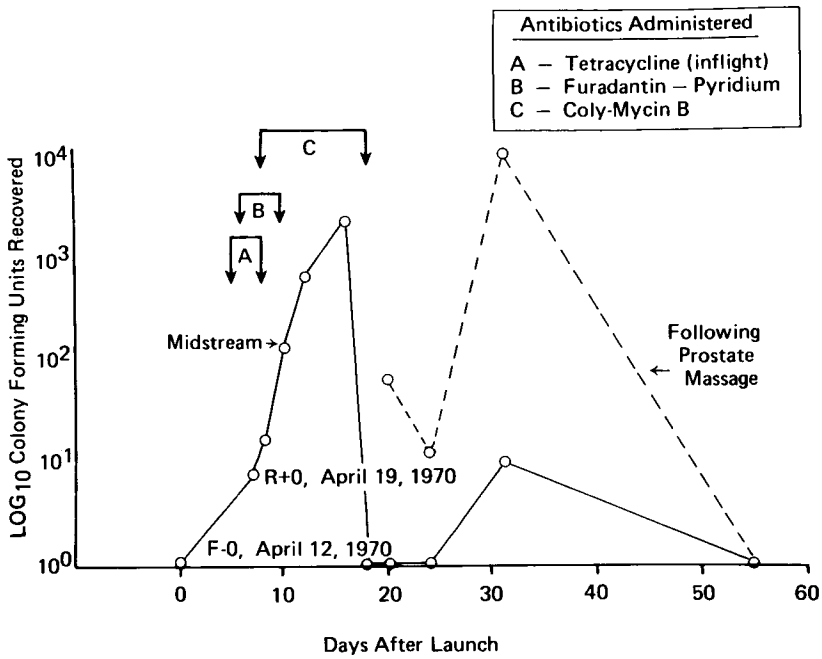

Figure 4. Presence of *Pseudomonas aeruginosa* in urine of Apollo 13 Lunar Module Pilot.

microbial competition have decreased dramatically during space flight. This population shift creates a situation in which the natural resistance to infection may be decreased at a time when clinical diagnosis and treatment are most difficult.

The presence of *C. albicans*, as well as other species of *Candida* that have similarly been implicated in a variety of pathogenic situations, was carefully monitored during each Apollo flight. No anomalies were noted among any of the Apollo crewmembers that could be traced to yeast infections. Whether this lack of microbial competition could mediate a disease state during missions of longer duration is a matter of conjecture, but the Apollo data demonstrate the existing possibility.

The Apollo 17 Command Module Pilot exhibited a chronic dermatitis on the skin of the groin and both feet before and after flight. The pathogenic fungus *Trichophyton rubrum* was isolated as the causative agent at each sampling period. A similar dermatitis was present on the skin of the Commander's toes, although the causative agent could not be cultured. The presence of active dermatophyte infections on two of the Apollo 17 crewmembers afforded the opportunity to study the response of this type of disease condition to short-term space flight. Analysis of the lesions after flight revealed no discernible change from the preflight condition. Likewise, there was no evidence of transfer of *T. rubrum* to other parts of the body.

A potential avenue of secondary infection was carefully monitored in the Apollo 17 crewmen. The opportunistic pathogen *Pseudomonas aeruginosa* was present on the toes

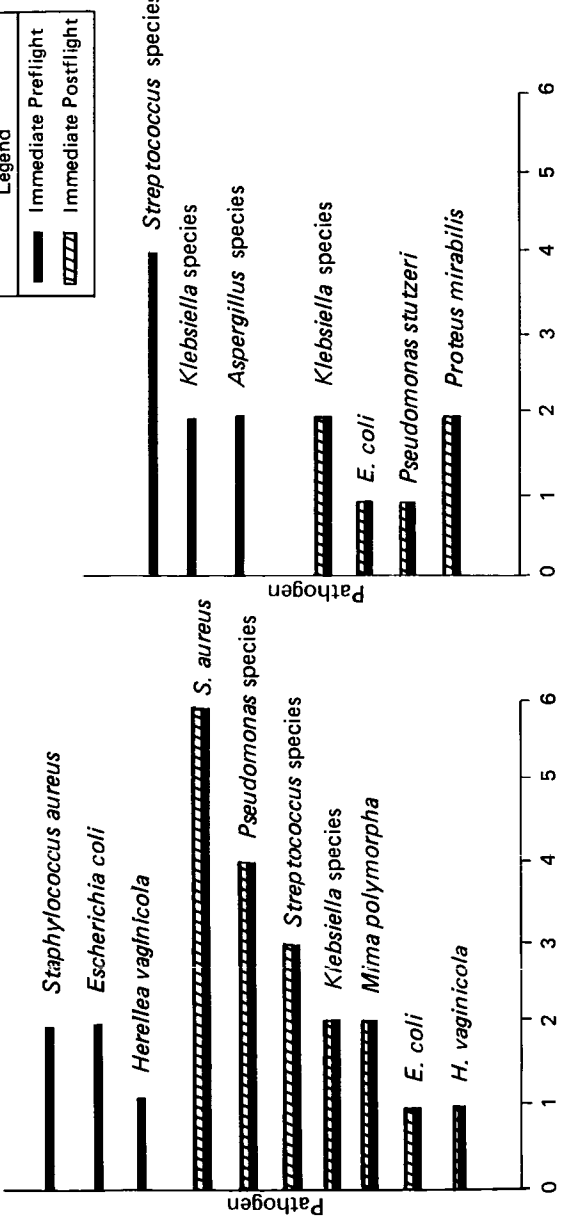

Figure 5. Incidence of pathogen isolation from Apollo 13 and 14 crew skin swabs.

of the CDR before flight and spread to the toes of the CMP and the LMP during flight. However, the presence of this species near the dermatophytic lesions did not result in a secondary, *P. aeruginosa* mediated infection.

Spacecraft and Clothing Microbiology

Spacecraft microbial samples from the Apollo 7 through 12 missions were evaluated. The microorganisms obtained from the four preflight and postflight Command Module samples were grouped according to morphological type (table 9). Although the sample population was small, a definite trend of increased numbers of potential pathogens was observed.

Table 9

Morphological Types Isolated From Four CM Sampling Sites: Apollo 7 through 12

Morphological Type	Total Isolations	
	Immediate Preflight	Immediate Postflight
Gram-positive cocci	38	47
Bacillus species	13	4
Diptheroids	8	13
Gram-negative rods	0	9
Filamentous fungi	14	9
Yeasts	6	3

A potentially pathogenic microorganism (*Staphylococcus aureus*) was isolated at the preflight sampling period only on the Apollo 10 spacecraft (table 10). The organism, recovered in each of the four preflight samples collected, was not recovered after flight from any sampled site. However, several medically important organisms were isolated at the postflight period of most of the Apollo 7 through 12 missions.

Of the 79 morphological types isolated before flight, only ten species were reisolated after the flight on the same mission. The reisolated types were primarily *Staphylococcus epidermidis* and *Micrococcus* species. These isolates are predominant in the human microflora and their reisolation from spacecraft samples is probably attributable to recontamination of the sample sites by the crewmen rather than to survival in the Command Module.

The transfer of microorganisms between the crewmen and the Command Module or the extravehicular activity clothing became more obvious in the analysis of subsequent missions. The data obtained from the Apollo 14 flight illustrate what generally happens to the microbial load of selected Command Module sites during a space flight (table 11). The various sites sampled harbored a variety of microbial species. Command Module habitation did not generally affect a significant change in the number of contaminating species. However, there was an obvious loss of the original contaminants on each site with a concurrent invasion of microbes of different species.

Table 10

Medically Important Microorganisms Isolated From the Apollo CM

Micro-organism	Areas of Isolation by Apollo Mission											
	7		8		9		10		11		12	
	Preflight	Postflight	Preflight	Postflight	Preflight	Postflight	Preflight	Postflight	Preflight	Postflight	Preflight	Postflight
Aspergillus fumigatus	–[1]	M.C.[2]	–	M.C.	–	F.[3]	–	–	–	–	–	–
Coliform	–	F.	–	–	–	–	–	–	–	–	–	–
Herellea species	–	F.	–	–	–	–	–	–	–	–	–	–
Klebsiella aerobacter	–	–	–	–	–	–	–	–	–	–	–	F.
Proteus mirabilis	–	–	–	–	–	–	–	–	–	–	–	F.
Pseudomonas maltophilia	–	S.A.[4]	–	–	–	–	–	–	–	–	–	–
Pseudomonas pseudomallei	–	–	–	–	–	–	–	F.	–	–	–	–
Pseudomonas species	–	–	–	D.G.[5]	–	–	–	–	–	–	–	–
Staphylococcus aureus	–	D.G.	–	–	–	F.	M.C. D.G. F. S.[6]	–	–	–	–	S. M.C.

[1] Indicates no pathogenic organism found.
[2] M. C. = Maneuver Controller
[3] F. = Floor
[4] S. A. = Shock Absorbers
[5] D. G. = Drink Gun
[6] S. = Strut

Table 11

Analyses of Aerobic Species Recovered From Apollo 14
CM Hardware Sites

Sample Site	Microorganisms Recovered	
	Preflight (F−0)	Postflight (R+0)
Floor	*Micrococcus* species 3	–[a]
	Micrococcus species 5	–
	Micrococcus species 14	–
	Pseudomonas maltophilia[b]	–
	Staphylococcus epidermidis	–
	–	*Herellea vaginicola*[b]
	–	*Klebsiella pneumoniae*[b]
	–	*Proteus mirabilis*[b]
	–	*Streptococcus faecalis*[b]
Head strut	*Gaffkya tetragena*	–
	–	*Staphylococcus epidermidis*
	–	*Bacillus* species
Rotational hand controller	*Micrococcus* species 10	–
	Micrococcus species 19	-
	–	*Staphylococcus epidermidis*
	Corynebacterium bovis	–
	Gaffkya species	–
	Micrococcus species 4	*Micrococcus* species 4
	Micrococcus species 29	–
	–	*Gaffkya tetragena*
	–	*Staphylococcus epidermidis*

[a] = Indicates designated species not recovered during this sampling period.
[b] = Medically significant species.

The Apollo 14 data also illustrate the general phenomenon of buildup of medically important species during the space flight. Only one potentially pathogenic species (*Pseudomonas maltophilia*) was recovered from the Command Module sites before lift-off, whereas four different potential pathogens, *Herellea vaginicola*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Streptococcus faecalis*, were recovered after flight. This same pattern was generally noted in each of the flights for which the appropriate samples were collected.

The increased incidence of medically important microorganisms is even more obvious from urine collection device analysis. Table 12 illustrates a common pattern with the UCD samples. UCDs were first sampled in the clean room at the NASA John F. Kennedy Space Center the morning of launch, and were generally free of microbes. However, samples collected in the Command Module immediately upon recovery contained a variety of contaminants. All but one species (*Bacillus*) of the microbes recovered after

flight from the Apollo 14 devices were potential pathogens. The buildup of *Proteus mirabilis* on the UCD reoccurred throughout most of the Apollo missions. Close contact of susceptible parts of the body with a contaminated UCD presented a significant medical hazard.

Table 12

Number of Aerobic Specimens Recovered From Samples of Three Apollo 14 UCSs

Organism	Preflight	Postflight
Bacillus species	–[a]	1
Klebsiella pneumoniae[b]	–	3
Proteus mirabilis[b]	–	1
Pseudomonas maltophilia[b]	–	2
Staphylococcus epidermidis	2	–

[a]Indicates designated species not isolated during this sampling period.
[b]Medically important species.

Statistical Analysis of Crew Microflora

Paired t-tests were performed on the crew bacterial flora of the Apollo 7 to 11 missions to identify significant changes in the number or occurrence of microorganisms in the postflight period as compared with the preflight period. Comparisons were made by testing both the sum of actual bacterial counts within a genus and the sum of occurrence of a particular genus at each sampling site. Times selected for comparison of the paired genera in the identified groups were F-30 and F-0; F-30 and R+0; and F-0 and R+0. The tests were performed on the microflora of the stool, urine, throat-mouth gargle, and inguinal region samples. All body surface samples, which included the inguinal region samples, were tested as a single group. The microflora of each sample area were further divided into groups of aerobic gram positives, anaerobic gram positives, aerobic gram negatives, and anaerobic gram negatives.

Significant alteration at the 0.05 level in the count, or occurrence, of microorganisms during these missions was indicated only in the inguinal region by this test method. Alteration of the microflora in this sample area was expected because of the poor personal hygiene measures available to the Apollo crewmen following defecation. In general, a high degree of variation was observed in the microflora between sampling periods, between crewmen, and between missions. No other consistent alteration to the microflora was observed by this test method.

Apollo Crew Virology

Serological titers were determined preflight on crewmen, crew contacts, and key mission personnel to ascertain immune status to mumps, rubella, and rubeola. The immune status of all astronauts to poliomyelitis virus types 1, 2, and 3 was also determined. In addition, complement-fixation antibody titers to influenza A, influenza B, ECHO virus (group), adenovirus (group), parainfluenza, herpes simplex, *Mycoplasma pneumoniae*, cytomegalovirus, and respiratory syncytial virus were determined for the crewmembers.

Poliomyelitis virus was isolated from the preflight stools of the Apollo 11 crewmen after the crewmen had been given poliomyelitis boosters. Herpes simplex virus was isolated from the throat specimen collected immediately before flight from one Apollo 15 crewmember. This virus was not isolated from postflight specimens.

An investigation of the postflight illness of Apollo 7 crewmen established A_2 Hong Kong influenza as the causative agent by serological confirmation. Postflight illnesses in two Apollo 9 crewmembers were confirmed as influenza B virus by virus isolation and identification.

A study of the rubella virus exposure of the Apollo 13 crewmembers definitely established that a backup crewmember was infected with rubella virus. The source of the backup crewmember's exposure was also identified. After the immune status of the Apollo 13 crew was determined, one crew reassignment was made and the scheduled initiation of the flight was permitted. The following viruses were isolated from personnel who either worked behind the biological barrier or were contacts of the crew: rhinovirus; herpes simplex; adenovirus type 2 and type 5; Coxsackie A6, A24, B1, and B3; and enteric cytopathogenic human orphan (ECHO) virus type 1. The crewmen remained free from manifestations of similar illnesses.

Mycoplasma species were routinely isolated from preflight and postflight specimens from all Apollo crewmen. Throat specimens frequently yielded *Mycoplasma salivarium* and *Mycoplasma orale I*, and *Mycoplasma hominis* was isolated from the urine. *Mycoplasma laidlawii A* was isolated from throat and urine specimens of Apollo 12 crewmen. Some evidence of cross infection was noted. Usually, *Mycoplasma* species were isolated from one or two crewmembers, and the same species were isolated before and after flight. The largest number of isolations was obtained from the Apollo 12 and 13 crewmen. *Mycoplasma* species were isolated from preflight and postflight specimens obtained from all crewmembers of these missions.

Summary and Conclusions

The return of sterile lunar soil indicated the success of measures developed to prevent lunar soil contamination. The likelihood of returning a lunar microorganism was recognized as being very small. However, the possibility of lunar soil contamination with terrestrial organisms was considerably greater. Had the soil become contaminated, the catalog developed before flight of microorganisms carried to the moon would have been extremely useful in identifying a terrestrial contaminant. The need for a premission microbial catalog will exist for future manned missions to other planets unless substantial advances can be made in the collection and transportation procedures of foreign soil, thus ensuring the return of the soil in its original state.

Considerable variation in the microfloral response was observed on the Apollo missions. The variables of host susceptibility, external environmental factors, and ecological relationships among competing species of microorganisms were undoubtedly responsible for the observed response of the microflora.

An increased incidence and spread of potentially pathogenic microorganisms between crewmen was demonstrated on several missions. In all cases, the organisms carried by each

crewman were carefully monitored throughout the preflight and postflight phases in an effort to prevent, or control, infectious disease events. A major consideration for future missions of longer duration should be to develop improved preventive measures and inflight monitoring and diagnostic systems. Such systems will provide coverage for inflight illness events and will provide additional understanding of the microfloral response and its relationship to illness events.

Preflight and postflight microbial analysis of samples obtained from the Command Module showed a loss of the preflight microorganisms occurs during the mission. Microflora isolated at sampling sites before flight were replaced by microorganisms from the crew.

No observations made suggest the spacecraft environment predisposes the crewmen to viral or mycoplasma-induced illness.