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JSC-11021

NASA TM X-58180
April 1976



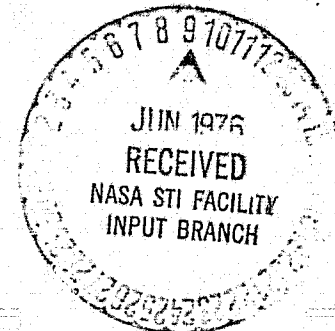
MEDICAL MICROBIOLOGICAL ANALYSIS
OF APOLLO-SOYUZ TEST PROJECT CREWMEMBERS

(NASA-TM-X-58180) MEDICAL MICROBIOLOGICAL
ANALYSIS OF APOLLO-SOYUZ TEST PROJECT
CREWMEMBERS (NASA) 26 p HC \$4.00 CSCL 06E

N76-24878

Unclas
G3/52 28242

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
LYNDON B. JOHNSON SPACE CENTER
HOUSTON, TEXAS 77058



1. Report No. NASA TM X-58180	2. Government Accession No.	3. Recipient's Catalog No.	
4. Title and Subtitle MEDICAL MICROBIOLOGICAL ANALYSIS OF APOLLO-SOYUZ TEST PROJECT CREWMEMBERS		5. Report Date	
		6. Performing Organization Code JSC-11021	
7. Author(s) Gerald R. Taylor, JSC, and S. N. Zaloguev, U.S.S.R. Ministry of Health		8. Performing Organization Report No.	
		10. Work Unit No. 953-36-00-00-72	
9. Performing Organization Name and Address Lyndon B. Johnson Space Center Houston, Texas 77058		11. Contract or Grant No.	
		13. Type of Report and Period Covered Technical Memorandum	
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration Washington, D.C. 20546		14. Sponsoring Agency Code	
		15. Supplementary Notes	
16. Abstract <p>The procedures and results of the Microbial Exchange Experiment (AR-002) of the Apollo-Soyuz Test Project are described. Included in the discussion of procedural aspects are methods and materials, in-flight microbial specimen collection, and preliminary analysis of microbial specimens. Medically important microorganisms recovered from both Apollo and Soyuz crewmen are evaluated.</p>			
17. Key Words (Suggested by Author(s))		18. Distribution Statement	
Microbiology Bacteriology Microorganisms Microbe	Infectious diseases Immunity Infection Aerospace medicine Bioastronautics	STAR Subject Category: 62 (Aerospace Medicine) <i>52</i>	
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 27	22. Price* \$4.00

*For sale by the National Technical Information Service, Springfield, Virginia 22161

NASA TM X-58180

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OF APOLLO-SOYUZ TEST PROJECT CREWMEMBERS**

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**MEDICAL MICROBIOLOGICAL ANALYSIS
OF APOLLO-SOYUZ TEST PROJECT CREWMEMBERS**

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Lyndon B. Johnson Space Center

SUMMARY

The purpose of the Microbial Exchange Experiment (AR-002) of the Apollo-Soyuz Test Project was to evaluate components of the infectious disease process in space flight by measuring alterations in three factors: (1) the composition of the microbial populations inhabiting the crewmembers and spacecraft, (2) the ability of each crewmember's defense mechanism to resist infection, and (3) the ability of certain microorganisms to originate infections. The impetus for performing the experiment arose from the variety of in-flight and ground-based microbial studies in the United States and the U.S.S.R., which have indicated that the conditions of space flight alter man and microorganisms in such a way that the normal fine balance between them may be adversely affected. The monitoring of two separate crews, which differed microbiologically and immunologically, provided an opportunity to study in-flight cross-contamination patterns. Because the Apollo-Soyuz Test Project crewmembers came from widely different geographical and ecological areas, it was possible to identify specific, naturally occurring, marker microorganisms for detailed analysis.

This report evaluates the operational aspects associated with the experiment and the activities of medically important microorganisms recovered from the Apollo and Soyuz crewmen. The majority of activities were performed as planned, and a large percentage of the anticipated data will be forthcoming. A variety of potential pathogens was recovered from prime and backup crewmembers before and after flight. However, no disease events were reported. Candida albicans and Staphylococcus aureus (type 52, 52A, 80, 81) were shown to be transferred from one crewmember to another during the flight. No other medically significant changes in the microbial population were observed.

INTRODUCTION

On early Apollo missions, before strict protective measures were instituted, in-flight infections were not unusual (ref. 1). In-flight illness of microbial origin was, however, completely absent from the Apollo 14 through 17 missions (ref. 2).

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There is no doubt that the implementation of extensive preventive measures (ref. 3) following the clinically significant Apollo 13 mission was a contributing factor. Preflight monitoring of pathogenic and potentially pathogenic species identified certain potential problems so that appropriate prophylaxis or treatment could be administered before the flight or could be provided during the flight. This procedure was highly effective and was recommended for all future U.S. manned space flights (ref. 2). Similar analyses conducted during the three flights to the U.S. Skylab demonstrated that, whereas there were several in-flight disease events and gross contamination of the orbital workshop did occur, such events were not shown to be limiting hazards for long-term space flight (ref. 4).

The results of microbiological studies conducted as part of ground-based experiments in the U.S.S.R. and during the flights of Soyuz spacecraft (ref. 5) indicate that the state of human beings in hermetically sealed cabins can be associated with certain shifts in the composition of their automicroflora. It has been reported (refs. 6 and 7) that these changes were of a dysbacteriological character and were expressed as a marked increase in the size of the microbial population in different areas of the integument. Such increases may be due to a predominance of individual representatives of the autoflora, such as staphylococci, streptococci, or Gram-negative rods of the genus Escherichia or Klebsiella. This predominance could be an important factor in the development of the symptomatology of infections. Increased intensity of shedding of microorganisms from the skin of crewmembers into the cabin environment (refs. 6 and 7) favors the creation of conditions suitable for the exchange of microorganisms between individuals.

The Apollo-Soyuz Test Project (ASTP), a unique space flight in which two teams of crewmembers from different geographical areas joined in space with two different spacecraft, presented an unusual opportunity for cross-contamination. Accordingly, it was necessary to identify and trace the presence of all microorganisms of potential medical importance. This report covers such an analysis of the three prime and three backup Apollo astronauts and the two flight and two backup Soyuz cosmonauts.

As an aid to the reader, where necessary the original units of measure have been converted to the equivalent value in the Système International d'Unités (SI). The SI units are written first, and the original units are written parenthetically thereafter.

METHODS AND MATERIALS

Routine Method

Nine sets of microbiological specimens were collected from the three prime Apollo crewmembers (43, 29, 18, 5, and 0 days before launch; once during flight; and 0, 18, and 29 days after recovery). Additionally, six sets of control specimens were collected from the three backup Apollo crewmembers (45, 32, 18, 5, and 0 days before the flight and immediately after the flight). Nine sets of microbiological specimens were taken from the two prime Soyuz crewmembers (48, 33,

15, 7, and 0 days before launch; once during flight; and 0, 7, and 15 days after recovery). In addition, four sets of microbiological samples were collected from the two backup Soyuz crewmembers (45, 29, 14, and 6 days before the flight).

During each preflight and postflight sample period, 10 microbial specimens were collected from each crewmember as outlined in table I. Calcium alginate swabs, wetted in 0.3-millimolar phosphate buffer, were used to sample each of the seven body surface areas. Although it is recognized that subsurface microbes may be overlooked by this method, procedures for sampling subsurface areas were not compatible with the flight program (refs. 2 and 8). Dry calcium alginate swabs were used to sample the surfaces of the tonsils and the posterior pharyngeal vault before collection of the gargle specimen. For this latter sample, the subject gargled with 0.3-millimolar phosphate buffer followed by a repeated rinse of the teeth with the same solution. Swabs were placed in 5 cubic centimeters of 0.3-millimolar phosphate buffer for transport to the laboratory. Analysis of all samples was initiated within 1 hour of specimen collection.

In-Flight Microbial Specimen Collection

In addition to the baseline samples previously described, in-flight samples were obtained from all five prime crewmembers and both spacecraft between 77:40 and 78:30 Soyuz ground elapsed time. In-flight samples consisted of the first six areas outlined in table I. For this set of samples, a specially developed sample collection device was employed (fig. 1). This device consisted of a cotton-tipped Teflon swab on a capillary tube that contained conservation fluid to keep the microorganisms alive. Each swab was housed within an airtight case to prevent desiccation. Groups of swabs were organized in Beta-cloth retaining bags (fig. 2).

Preliminary Analysis of Microbial Specimens

The contents of each swab and gargle sample were serially diluted under aseptic conditions and subsequently inoculated onto the surface of the nutrient media (table II). The variety of media, number of plates inoculated, and dilution range were selected on the basis of what was required to isolate and quantitate the autoflora components present in each sampled area (ref. 9). Sabouraud's dextrose agar (SAB) plates were incubated at 303 K (30° C) for 5 days. Cornmeal, malt-extract, yeast-extract agar (CMMY) plates were incubated at 298 K (25° C) for 7 days, and all others were incubated at 310 K (37° C) for 48 hours.

Following incubation under the appropriate conditions, all resulting colonies on every culture plate were categorized and counted. Subsequently, one sample of each morphologically different colony type was transferred from each dilution series to the appropriate nutrient media and was stained according to the method of Gram. Species were identified as previously outlined (refs. 9 and 10).

RESULTS AND DISCUSSION

Medical microbiological analyses of Apollo crewmembers were conducted at the NASA Lyndon B. Johnson Space Center laboratories in Houston, Texas, and evaluations of specimens from Soyuz crewmembers were conducted at the Institute of Biomedical Problems of the U.S.S.R. Ministry of Health in Moscow. Although these studies were conducted individually, a common methodology was used to ensure comparability of data. In addition, nutrient and selective culture media were exchanged so that these variables could be reduced. The following represents the analyses of medically important microorganisms contributed by each side.

Gram-Negative Rods Isolated From Apollo Astronauts

Enteric microorganisms. - A number of different microbes that normally occur in the intestinal tract, or are associated with intestinal infection, are placed in the enteric group of bacilli. The normally occurring members of this group are generally considered to be of potential medical importance when they are recovered repeatedly, or in large numbers, from sites other than the lower digestive tract. The following members of this group were recovered from areas on the ASTP crewmembers other than the lower digestive tract. An outline of the total recovery pattern for the Apollo crewmembers is presented in tables III to VI.

Escherichia coli: Escherichia coli is generally accepted as the most reliable evidence of fecal contamination. Outside the intestinal tract, under certain conditions, it often produces such diseases as urinary tract infections (cystitis and pyelitis), peritonitis, gallbladder infection, wound infection, septicemia, and enteritis. This species was repeatedly recovered from the groin of the prime Apollo command module pilot (CMP) and occasionally from the upper respiratory tract of the other two prime Apollo crewmembers. In all cases, neither the recovery pattern nor the quantitation indicated medical significance.

Enterobacter aerogenes: Enterobacter aerogenes often occurs in the large intestine of humans, although the number present is considerably smaller than that of E. coli. The pathogenic significance of E. aerogenes is similar to that of E. coli. As indicated in tables III and IV, this species was always carried in the nose and mouth of the prime Apollo CMP and docking module pilot (DMP) and was frequently isolated from the backup Apollo commander (CDR) and DMP. Although this is not a common occurrence, it should be noted that this species was not shown to spread to the prime Apollo CDR or to more sites on the carriers during the flight. Also, there was no postflight increase in quantitation. This occurrence is an excellent example of a potential pathogen, carried by two flight crewmembers, apparently being unaffected by the conditions of space flight.

Proteus mirabilis: Members of the genus Proteus may cause infections of the urinary tract and abscesses. Additionally, these microbes have been associated with outbreaks of enteric infection, particularly gastroenteritis. More often, they are secondary invaders of infections of the middle ear, mastoid process, meninges, wounds, and urinary tract. P. mirabilis is the most frequent species of this genus found in human clinical material. This species was carried, in low numbers, in

the nasal passage of the prime Apollo CDR throughout the monitoring period. As with E. aerogenes previously described, this unusual event provides a good model system for analyzing the response of Gram-negative rods to space-flight conditions. The ASTP mission had no detectable effect on the qualitative or quantitative presence of this microorganism.

Other Gram-negative rods. - Nonenteric Gram-negative rods discussed in this section include members of the genus Moraxella, Acinetobacter calcoaceticus, and four species of Haemophilus.

Moraxella species: Members of the genus Moraxella are parasites of the mucous membranes and are frequently involved in pathogenic activity such as conjunctivitis. Although carried in the oral cavity of the prime Apollo DMP for 45 days before the flight, no discernible alteration was demonstrated following the mission.

Acinetobacter calcoaceticus: Acinetobacter calcoaceticus (synonym: Mima polymorpha) is a species of minor potential medical importance. Isolated infrequently from multiple sites on the prime Apollo CMP, it did not contribute significantly to the load of Gram-negative rods recovered from ASTP prime crewmembers.

Haemophilus: Four species of Haemophilus were isolated from the oral cavity of each ASTP Apollo astronaut as outlined in tables V and VI. Although each of these is to some degree a common inhabitant of the human mouth, each is a strict parasite requiring certain growth factors present in blood. A discussion of the potential medical importance of each follows.

H. influenzae is found in lesions and in the upper respiratory tract of carriers. It may be either a primary or a secondary invader. As a primary incitant of disease, it is responsible for meningitis, septicemia, conjunctivitis, and upper respiratory tract infections. It is commonly a secondary invader in cases of influenza and pertussis. This species was isolated from each of the Apollo crewmembers before flight in quantities ranging from 10 000 to 600 000 viable cells/cm³ of gargle. The postflight loss from two Apollo crewmembers and the quantitative reduction in contamination of the Apollo CDR is contraindicative of a space-flight-mediated increase in infective potential.

H. haemolyticus and H. parahaemolyticus are often associated with acute pharyngitis when present in high numbers. As with the H. influenzae, the overall quantitation and incidence decreased following the ASTP flight.

H. parainfluenzae is the most benign of the four recovered species and is used largely as a marker organism because of its almost ubiquitous appearance.

Gram-Negative Rods Isolated From Soyuz Cosmonauts

The results of the studies of the content of Gram-negative rods on the skin of the cosmonauts of the prime Soyuz crew (table VII) revealed a postflight increase in the quantity of Acinetobacter calcoaceticus and an increase in the frequency of occurrence of microorganisms of the family Enterobacteriaceae. Data for the recovery of Gram-negative rods from the Soyuz backup crewmembers are given in

table VIII. Considering the medical importance of the bacteria of this family, the increase in the number of skin areas from which the mentioned microbes were isolated should be considered as unfavorable.

Candida Albicans

Candida albicans is a well-recognized component of the indigenous autoflora of humans (ref. 11) and has been recovered from astronaut specimens collected in association with each Apollo and Skylab mission (refs. 2, 4, and 8). Because C. albicans has been identified as the causative agent for serious oral cavity diseases (refs. 11 and 12), the presence of this microorganism in the mouths of ASTP crewmembers was carefully monitored. As previously reported (ref. 10), C. albicans was recovered from crewmembers following the Apollo 14 and 15 missions, whereas other fungal species, present before flight, were absent from samples obtained immediately after recovery. Analysis of the ASTP Apollo astronaut data presented in table IX indicates an in-flight transfer of this species to the Apollo CDR. No such transfer occurred with the Apollo backup crew, although C. albicans was carried in the mouth of the backup CMP. These events demonstrate the importance of this species in space flight. In-flight use of antibiotics could provide the opportunity for a loss of competing bacterial species and eventual overgrowth with C. albicans, thereby increasing the importance of this yeast (refs. 12 to 14).

Among the Soyuz cosmonauts, C. albicans was recovered only from the prime flight engineer (FE) 33 and 15 days before launch. Therefore, this microorganism was not considered to be of medical importance for the Soyuz crewmembers.

Staphylococcus Aureus

Although Staphylococcus aureus is not an uncommon skin and nasal contaminant, it is an important potential pathogen. It has been shown to be the causative agent of a wide range of infection and intoxications including boils, abscesses, meningitis, furunculosis, pyemia, osteomyelitis, suppuration of wounds, and food poisoning (ref. 15). Several space-flight-simulation studies (refs. 6, 7, and 16) have indicated increases in the toxigenic activity, virulence, or pathogenicity of this species with stressful confinement of the human host. If these events were to be duplicated during space flight, the resulting lesions could be especially important because of their interference with close-contact surfaces, such as the tight-fitting and abrasive pressure suits, oxygen masks, and other components of the life support system. Accordingly, the presence and activity of this species were monitored before and after each of the recent U.S. space flights. Data from the Apollo 13, 14, and 15 missions (ref. 2) and from the Skylab missions (ref. 4) confirm in-flight cross-contamination with S. aureus.

The recovery of this species from ASTP Apollo crewmembers in connection with previous space flights is presented in table X. The ASTP prime DMP had not previously been assigned to a space flight, rendering such data nonapplicable. Strains of S. aureus were recovered from all the other five ASTP astronauts in connection with at least one previous space flight.

The recovery of strains of S. aureus from the Apollo astronauts during the ASTP monitoring period is presented in table XI. These strains are expressed as numbered bacteriophage types. The data show that each Apollo prime and backup crewmember carried a different strain of S. aureus. Type 52, 52A, 80, 81 was carried by the prime DMP and was transferred to the prime Apollo CDR during flight. Apparently, colonization did not ensue because this strain was not recovered again from the prime Apollo CDR.

A postflight increase in the incidence of S. aureus isolation has previously been reported (refs. 2 and 4). This increase did not occur among the astronauts after the ASTP mission. Likewise, and contrary to some previous missions (ref. 4), no disease events resulted from the ubiquitous presence of S. aureus before and during the flight.

Total Load of Potential Pathogens

Several investigators have suggested that returning space travelers may experience a "microbial shock" and respond negatively to renewed contact with potentially pathogenic microorganisms that are absent in the space-flight environment (refs. 1, 17, 18, and 19). These warnings were based on the assumption that contact with potential pathogens during space flight would be very limited, resulting in a reduction of immunocompetence. However, there was no demonstrable decrease in the incidence of medically important microorganisms recovered from the ASTP crewmembers on recovery day. This finding supports results reported earlier (refs. 2, 5, and 9). Therefore, if a reduction in total immunocompetence were to occur during this mission, it could not be in response to decreased contact with medically important components of the resident microflora.

CONCLUDING REMARKS

This medical/microbiological analysis of the Apollo-Soyuz Test Project crewmembers was conducted as part of the Microbial Exchange Experiment (AR-002). Although a variety of potential pathogens was recovered from each of the prime and backup crewmembers before and after flight, no disease events were reported. Candida albicans and Staphylococcus aureus (type 52, 52A, 80, 81) were shown to be transferred from one crewmember to another during flight. No other medically significant changes in the microbial population were observed.

Lyndon B. Johnson Space Center
National Aeronautics and Space Administration
Houston, Texas, April 8, 1976
953-36-00-00-72

REFERENCES

1. Berry, Charles A.: Summary of Medical Experiences in the Apollo 7 Through 11 Manned Spaceflights. *Aerospace Med.*, vol. 41, no. 5, May 1970, pp. 500-519.
2. Taylor, Gerald R.: Recovery of Medically Important Microorganisms From Apollo Astronauts. *Aerospace Med.*, vol. 45, no. 8, Aug. 1974, pp. 824-828.
3. Wooley, Bennie C.: Apollo Experience Report - Protection of Life and Health. NASA TN D-6856, 1972.
4. Brockett, R. M.; Ferguson, J. K.; et al.: Skylab Environments and Crew Microbiology Studies. Biomedical Results From Skylab. NASA SP-377, 1976 (To be published).
5. Zaloguyev, S. N.; Shinkareva, N. M.; and Utkina, T. G.: State of the Auto-microflora of Skin Tissues and Certain Natural Immunity Indices in the Astronauts A. G. Nikolaev and V. I. Sevast'ianov Before and After Flight. *Kosmicheskaya Biologiya i Meditsina*, vol. 4, no. 6, 1970, pp. 54-59. (Translation available from Joint Publications Research Service, Washington, D.C.)
6. Nefedov, Yu. G.; Shilov, V. M.; Konstantinova, I. V.; and Zaloguyev, S. N.: Microbiological and Immunological Aspects of Extended Manned Space Flights. Life Sciences and Space Research, IX, COSPAR, Akademie-Verlag (Berlin), May 1971, pp. 11-16.
7. Shilov, V. M.; Lizko, N. N.; Borisova, O. K.; and Prokhorov, V. Ya.: Changes in the Microflora of Man During Long-Term Confinement. Life Sciences and Space Research, IX, COSPAR, Akademie-Verlag (Berlin), May 1971, pp. 43-49.
8. Taylor, G. R.: Space Microbiology. *Ann. Rev. Microbiol.*, vol. 401, 1974, pp. 23-40.
9. Taylor, Gerald R.: Apollo 14 Microbial Analysis. NASA TM X-58094, 1972.
10. Taylor, Gerald R.; Henney, Mary R.; and Ellis, Walter L.: Changes in the Fungal Autoflora of Apollo Astronauts. *Appl. Microbiol.*, vol. 26, no. 5, Nov. 1973, pp. 804-813.
11. Rosebury, T.: Microorganisms Indigenous to Man. Blackiston Division, McGraw-Hill, 1962.
12. Marwin, R. M.: Relative Incidence of Candida albicans on the Skin of Persons With and Without Skin Disease. *J. Investigative Dermatology*, vol. 1, 1949, pp. 229-241.

13. Ginsberg, H. S.: Immune States in Long-Term Space Flights. Life Sciences and Space Research, IX, COSPAR, Akademie-Verlag (Berlin), May 1971, pp. 1-9.
14. Huppert, M.; Cazin, J., Jr.; and Smith, H., Jr.: Pathogenesis of Candida albicans Infections Following Antibiotic Therapy. J. Bacteriol., vol. 70, no. 1, July 1955, pp. 440-447.
15. Buchanan, Robert E.; and Gibbons, Norman E.: Bergey's Manual of Determinative Bacteriology. Eighth ed. Williams & Wilkins Co. (Baltimore), 1974.
16. Chukhlovin, B. A.; Ostrovmov, P. B.; and Ivanova, S. P.: Development of Staphylococcal Infections in Human Subjects Under the Influence of Some Spaceflight Factors. Space Biol. & Med., vol. 5, no. 6, 1971, pp. 91-98. (Primary source - Kosmicheskaya Biologiya i Meditsina, vol. 5, 1971, pp. 61-65. (In Russian.))
17. Luckey, T. D.: Potential Microbic Shock in Manned Aerospace Systems. Aerospace Med., vol. 37, no. 12, Dec. 1966, pp. 1223-1228.
18. Spizizen, J.: Microbiological Problems of Manned Space Flight. Life Sciences and Space Research, IX, COSPAR, Akademie-Verlag (Berlin), May 1971, pp. 65-68.
19. Wilkins, Judd R.: Man, His Environment, and Microbiological Problems of Long-Term Spaceflight. Biotechnology. NASA SP-205, 1967, pp. 133-143.

TABLE I.- SAMPLE COLLECTION AREAS

Sample no.	Sample designation	Sample area
1	Hair	20-cm ² area of hair (and scalp) on top of head
2	Ears	Right and left external auditory canals with 2 revolutions of each swab in each ear canal
3	Neck	20-cm ² area below hairline at base of neck
4	Nose	Internal area of both nostrils
5	Throat swab	Surfaces of tonsils and posterior pharyngeal vault
6	Hands	20-cm ² area on right and left palms
7	Axilla	20-cm ² area below hair on each side
8	Groin	5-cm strip from rear to front on right and left inguinal area between legs
9	Toes	Area between the two smallest toes of each foot
10	Gargle	10 cm ³ of phosphate buffer used as gargle and washed through oral cavity 3 times

TABLE II.- ISOLATION MEDIA

Sample designation	Media	Number of plates	Dilution range
Back of neck Hair Hands Axilla Ears	Blood agar	2	^a 10 ⁰ to 10 ⁴
	Mannitol salts agar	3	10 ⁰ to 10 ¹
	Cornmeal, malt-extract, yeast-extract agar (CMMY)	4	10 ⁰
	Sabouraud's dextrose agar (SAB)	5	10 ⁰
Gargle (natural)	Blood agar	2	10 ⁰ to 10 ⁵
	Mannitol salts agar	3	10 ⁰ to 10 ¹
	CMMY	4	10 ⁰ to 10 ²
	Rogosa agar	3	10 ⁰ to 10 ³
	Chocolate bacitracin agar (Choc)	3	10 ⁰ to 10 ⁵
Gargle (centrifugate)	CMMY	4	10 ⁰
	SAB	5	10 ⁰
Nose	Blood agar	2	10 ⁰ to 10 ⁴
	Mannitol salts agar	3	10 ⁰ to 10 ³
	CMMY	4	10 ⁰
	SAB	5	10 ⁰
	Choc	3	10 ⁰ to 10 ¹
Toes and groin	Blood agar	2	10 ⁰ to 10 ⁵
	Mannitol salts agar	3	10 ⁰ to 10 ¹
	CMMY	4	10 ⁰
	SAB	5	10 ⁰
Throat swab	Blood agar	2	10 ⁰ to 10 ⁵
	Mannitol salts agar	3	10 ⁰ to 10 ¹
	CMMY	4	10 ⁰ to 10 ²
	SAB	5	10 ⁰
	Choc	3	10 ⁰ to 10 ⁵
	Rogosa agar	3	10 ⁰ to 10 ³

^aSample in 5 cm³ of phosphate-buffered physiological saline solution.

TABLE III. - RECOVERY OF GRAM-NEGATIVE RODS FROM ASTP APOLLO PRIME CREWMEMBERS
(GENERA OTHER THAN HAEMOPHILUS)

Genus and species	Crew-member	Location and quantity of bacteria, log ₁₀ : colony-forming units/cm ³ of gargle or swab diluent												
		Days before flight					Days after flight							
		F-43	F-29	F-18	F-5	F-0	R+0	R+18	R+29					
<u>Enterobacter aerogenes</u>	ACDR ^a	0	0	0	0	0	0	0	0	0	0	0	0	
	CMP ^b	Axilla Nose Mouth	1.95 1.84 1.60	Nose Mouth	2.23 4.00	Nose Mouth	1.47 3.00	Nose Mouth	3.30 2.20	Nose Mouth	2.78 2.60	Nose Mouth	2.48 2.00	Axilla Nose
	DMP ^c	Nose Mouth	2.00 1.48	Mouth	2.07	Nose Mouth	1.47 1.47	Nose Mouth	2.78 2.00	Nose Mouth	1.00 1.00	Nose Mouth	2.40	Mouth
<u>Escherichia coli</u>	ACDR	0	Mouth	2.00	0	0	0	0	0	0	0	0	0	0
	CMP	Groin	2.60	Groin	1.60	0	0	Groin	2.30	0	0	0	0	0
	DMP	0	0	Nose	2.07	0	0	0	0	0	0	Nose	1.78	Nose
<u>Moraxella species</u>	ACDR	Hands	1.00	0	0	0	0	0	0	0	0	0	0	0
	CMP	Mouth	5.00	Ear	1.30	0	0	0	0	0	0	0	0	0
	DMP	Mouth	2.43	0	0	Mouth	3.77	Mouth	2.30	Mouth	2.00	0	0	0
<u>Proteus mirabilis</u>	ACDR	Nose	2.85	Nose	2.00	Nose	2.84	Nose	2.56	Nose	1.30	Nose	1.00	Nose
	CMP	0	0	0	0	0	0	0	0	0	0	0	0	0
	DMP	0	0	0	0	0	0	0	0	0	0	0	0	0
<u>Acinetobacter calcoaceticus</u>	ACDR	0	0	0	0	0	0	0	0	0	0	0	0	0
	CMP	0	Neck Hand	2.00 2.60	0	0	0	0	0	0	0	0	0	Hair Neck Mouth
	DMP	0	0	0	0	0	0	0	0	0	0	0	0	0

^a Apollo commander.

^b Command module pilot.

^c Docking module pilot.

TABLE IV. - RECOVERY OF GRAM-NEGATIVE RODS FROM ASTP APOLLO BACKUP CREWMEMBERS

(GENERA OTHER THAN HAEMOPHILUS)

Genus and species	Crew-member	Location and quantity of bacteria, log ₁₀ colony-forming units/cm ³ of gargle or swab diluent					Day of recovery
		Days before flight					
		F-45	F-32	F-18	F-5	F-0	
<u>Enterobacter aerogenes</u>	ACDR	Nose 1.00	Nose 1.00	Nose 1.00	0	0	Nose 1.60
	CMP	0	0	0	0	0	0
	DMP	Nose 2.00 Mouth 2.47 Neck 1.00 Groin 1.69	Mouth 2.69 Groin 3.69	0	Mouth 2.00	0	Axilla 1.78
<u>Escherichia coli</u>	ACDR	0	0	0	0	0	0
	CMP	0	0	0	0	0	0
	DMP	0	0	0	0	0	0
<u>Moraxella</u> species	ACDR	Hair 2.11 Neck 3.17 Axilla 2.86 Hands 2.17	0	Neck 1.00	0	0	Nose 2.00 Axilla 1.00
	CMP	0	0	0	0	0	0
	DMP	0	0	0	0	0	Toes 2.00
<u>Proteus mirabilis</u>	ACDR	0	0	0	0	0	0
	CMP	0	0	0	0	0	0
	DMP	0	0	0	0	0	0
<u>Acinetobacter calcoaceticus</u>	ACDR	0	0	0	0	Groin 3.00	Ear 3.00
	CMP	0	0	0	0	0	Neck 2.00 Axilla 1.60
	DMP	Axilla 1.30 Hands 1.60	0	0	0	Nose 2.30 Axilla 1.30 Hands 1.70	0
<u>Klebsiella pneumoniae</u>	ACDR	0	0	0	0	0	0
	CMP	0	0	0	0	0	0
	DMP	0	0	Mouth 3.00 Groin 3.38 Hands 1.30	Mouth 3.48 Groin 3.38 Hands 1.00	Mouth 3.78 Groin 4.00	Mouth 1.49 Groin 3.30

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TABLE V. - RECOVERY OF GRAM-NEGATIVE RODS FROM ASTP APOLLO PRIME
CREWMEMBERS (GENUS HAEMOPHILUS)

Crew-member	Species of <u>Haemophilus</u>	Quantity of bacteria, log ₁₀ colony-forming units/cm ³ of gargle or swab diluent							
		Days before flight					Days after flight		
		F-43	F-29	F-18	F-5	F-0	R+0	R+10	R+29
Throat swab									
ACDR	<u>H. haemolyticus</u>	0	0	4.47	0	0	0	0	0
	<u>H. influenzae</u>	0	0	4.90	0	0	3.34	0	0
	<u>H. parahaemolyticus</u>	0	0	0	0	0	0	0	0
	<u>H. parainfluenzae</u>	4.50	5.04	5.36	5.17	5.20	3.49	4.62	2.18
CMP	<u>H. haemolyticus</u>	5.60	5.47	0	0	0	3.30	0	0
	<u>H. influenzae</u>	5.77	0	0	0	0	0	0	0
	<u>H. parahaemolyticus</u>	0	0	4.00	5.90	0	0	0	0
	<u>H. parainfluenzae</u>	5.69	6.04	5.14	5.90	5.36	3.84	5.87	5.49
DMP	<u>H. haemolyticus</u>	0	0	4.47	0	0	0	0	0
	<u>H. influenzae</u>	0	0	0	0	4.00	0	0	0
	<u>H. parahaemolyticus</u>	0	0	0	0	0	0	5.11	0
	<u>H. parainfluenzae</u>	5.41	5.23	4.84	6.46	5.45	4.77	4.90	4.00
Gargle									
ACDR	<u>H. haemolyticus</u>	0	0	6.49	0	0	5.30	0	0
	<u>H. influenzae</u>	0	4.90	5.77	0	0	0	0	1.48
	<u>H. parahaemolyticus</u>	0	0	0	0	0	5.00	0	0
	<u>H. parainfluenzae</u>	5.54	5.99	6.77	6.53	6.53	5.95	5.08	4.18
CMP	<u>H. haemolyticus</u>	0	6.30	0	6.90	0	0	0	0
	<u>H. influenzae</u>	5.69	0	0	0	0	0	0	0
	<u>H. parahaemolyticus</u>	0	0	6.30	7.11	0	0	0	0
	<u>H. parainfluenzae</u>	6.23	7.08	7.20	7.39	6.48	5.15	6.48	7.00
DMP	<u>H. haemolyticus</u>	0	0	5.77	0	0	0	5.78	0
	<u>H. influenzae</u>	0	0	0	0	0	0	0	0
	<u>H. parahaemolyticus</u>	4.69	0	0	5.30	0	5.30	5.00	0
	<u>H. parainfluenzae</u>	5.44	6.04	6.43	6.80	6.28	5.52	6.25	5.46

TABLE VI. - RECOVERY OF GRAM-NEGATIVE RODS FROM ASTP APOLLO BACKUP

CREWMEMBERS (GENUS HAEMOPHILUS)

Crew-member	Species of <u>Haemophilus</u>	Quantity of bacteria, log ₁₀ colony-forming units/cm ³ of gargle or swab diluent					
		Days before flight					Day of recovery
		F-45	F-32	F-18	F-5	F-0	
Throat swab							
ACDR	<u>H. haemolyticus</u>	0	4.60	0	0	0	0
	<u>H. influenzae</u>	0	0	0	0	0	0
	<u>H. parahaemolyticus</u>	4.30	0	4.11	0	0	0
	<u>H. parainfluenzae</u>	5.32	4.95	7.95	5.81	6.05	5.38
CMP	<u>H. haemolyticus</u>	0	0	0	0	0	0
	<u>H. influenzae</u>	0	0	0	0	0	0
	<u>H. parahaemolyticus</u>	0	0	4.65	4.65	4.57	0
	<u>H. parainfluenzae</u>	4.34	2.30	3.95	3.60	4.48	4.57
DMP	<u>H. haemolyticus</u>	0	0	0	5.04	0	0
	<u>H. influenzae</u>	0	4.00	0	3.00	3.00	0
	<u>H. parahaemolyticus</u>	0	0	0	0	0	0
	<u>H. parainfluenzae</u>	2.44	5.20	4.41	4.69	4.04	4.15
Gargle							
ACDR	<u>H. haemolyticus</u>	5.47	0	0	0	0	5.00
	<u>H. influenzae</u>	0	0	0	0	0	0
	<u>H. parahaemolyticus</u>	0	0	0	0	0	0
	<u>H. parainfluenzae</u>	5.95	6.54	6.60	0	6.49	6.36
CMP	<u>H. haemolyticus</u>	0	0	0	0	0	0
	<u>H. influenzae</u>	0	0	0	0	0	0
	<u>H. parahaemolyticus</u>	0	0	6.00	6.77	0	5.30
	<u>H. parainfluenzae</u>	4.70	0	6.43	6.36	5.86	6.41
DMP	<u>H. haemolyticus</u>	0	0	0	0	0	0
	<u>H. influenzae</u>	3.00	0	0	0	0	0
	<u>H. parahaemolyticus</u>	4.25	0	0	0	4.00	4.30
	<u>H. parainfluenzae</u>	0	6.73	7.43	7.43	5.81	5.00

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TABLE VII. - RECOVERY OF GRAM-NEGATIVE RODS FROM ASTP SOYUZ PRIME CREWMEMBERS

Microbial group	Crewmember	Location and quantity of bacteria, log ₁₀ colony-forming/swab (gargle in colony-forming units/10 cm ³ buffer)									
		Days before flight					Days after flight				
		F-48	F-33	F-15	F-7	F-0	R+0	R+7	R+15		
Enterobacteriaceae	SCDR ^a	0	0	Gargle 1.30	0	0	Gargle Groin	Gargle 1.30	0		
	FE ^b	Gargle 1.30	Gargle 3.78 Groin 1.70	Gargle 2.05	Gargle 2.39	Gargle 1.78 Groin 2.79	Gargle Nose Throat swab	Gargle 2.96	NS ^c		
<u>Acinetobacter calcoaceticus</u>	SCDR	0	0	0	0	0	0	0	0		
	FE	0	0	0	0	Groin 1.70	Gargle 2.36	0	NS		
<u>Aeromonas</u> species	SCDR	0	0	0	0	0	Gargle 3.06	Gargle 1.30	0		
	FE	0	0	0	0	0	0	0	NS		

^a Soyuz commander.

^b Flight engineer.

^c No sample taken.

TABLE VIII.- RECOVERY OF GRAM-NEGATIVE RODS FROM ASTP SOYUZ BACKUP CREWMEMBERS

Microbial group	Crewmember	Location and quantity of bacteria, log ₁₀ colony-forming units/swab			
		Days before flight			
		F-45	F-29	F-14	F-6
Enterobacteriaceae	SCDR	Groin 3.10	0	0	0
	FE	0	0	0	0
<u>Alcaligenes</u> species	SCDR	Groin 2.18	0	0	0
	FE	0	0	0	0

TABLE IX. - RECOVERY OF CANDIDA ALBICANS FROM THE
MOUTHS OF APOLLO CREWMEMBERS

Crewmember	Days before flight				In flight	Days after flight				
	F-43	F-29	F-18	F-5		F-0	F+5	R+0	R+18	R+29
Prime crew										
ACDR	A ^a	A	A	A	A	A	P ^b	A	A	A
CMP	P	P	P	P	P	P	P	P	P	P
DMP	A	P	P	P	A	P	P	P	P	P
Backup crew										
ACDR	A	A	A	A	A	A	NS ^c	A	NS	NS
CMP	P	P	P	P	P	P	NS	P	NS	NS
DMP	A	A	A	A	A	A	NS	A	NS	NS

^aAbsence of C. albicans.

^bPresence of C. albicans.

^cNo sample taken.

**TABLE X. - STAPHYLOCOCCUS AUREUS RECOVERY FOR ASTP APOLLO
CREWMEMBERS FROM PREVIOUS SPACE FLIGHTS**

Crewmember	Flight	Sampling period	Sample area	Phage type		
Prime ASTP crew						
ACDR	Apollo 10	F-30	Hands	NA ^a		
CMP	Apollo 15	F-30	Scalp Nose	NT ^b 85		
		F-5	Nose Mouth	85 NT		
		F-30	Groin Nose	NT 75/85		
	Skylab 3	F-14	Groin	NT		
		F 5	Mouth Nose	75/85 NT		
		F-30	Groin Nose	NT 75/85		
		F-14	Nose, groin Mouth	NT 75/85		
	Skylab 4	F-5	Ear, groin Nose Mouth, scalp	NT 29/75 29/47/75		
		DMP Clinic - - Mouth 83-A				
		Backup ASTP crew				
ACDR	Apollo 12	F-30	Scalp, ears, axilla, hands, nose, inguinal	NA		
		F-14	Nose	NA		
		F-0	Toes, nose	NA		
		R+0	Nose	NA		
	Skylab 3	F-45	Nose, mouth	3-A		
		F-14	Scalp, navel, nose, groin	3-A		
		F-5	Nose	3-A		
		F-0	Nose	3-A		
		R+0	Hands Nose	3-A 3-A/3-C		
		R+8	Nose, hands, navel Groin	3-A 3-A/3-C		
		R+15	Scalp Nose, axilla, hands Navel	3-A/3-C 3-A 3-C		
		CMP	Apollo 14	F-14	Nose, hands	6/47/53/54/77/ 79/80/81
		DMP	Skylab 3	F-45	Scalp Groin Nose	29/79 29/79/81 29/53/54/79/80
F-14	Nose			29/53/54/79/80		
F-5	Hands Nose			29/79 NT		
R+0	Mouth, scalp, feces Hands Nose			29/79 3-A 29/53/54/79/80		
R+8	Mouth			29/53/79/80		
R+18	Nose Mouth			29/79 29/53/79/80		

^aNot applicable (typing not done).

^bNontypable.

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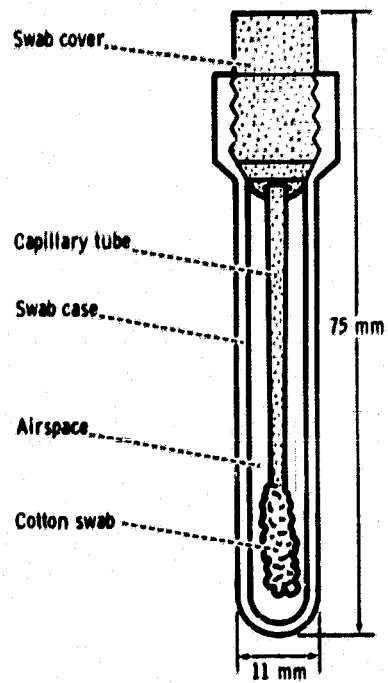
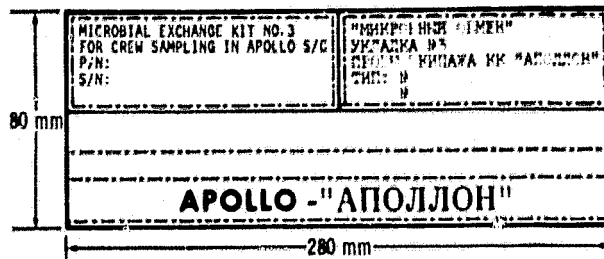
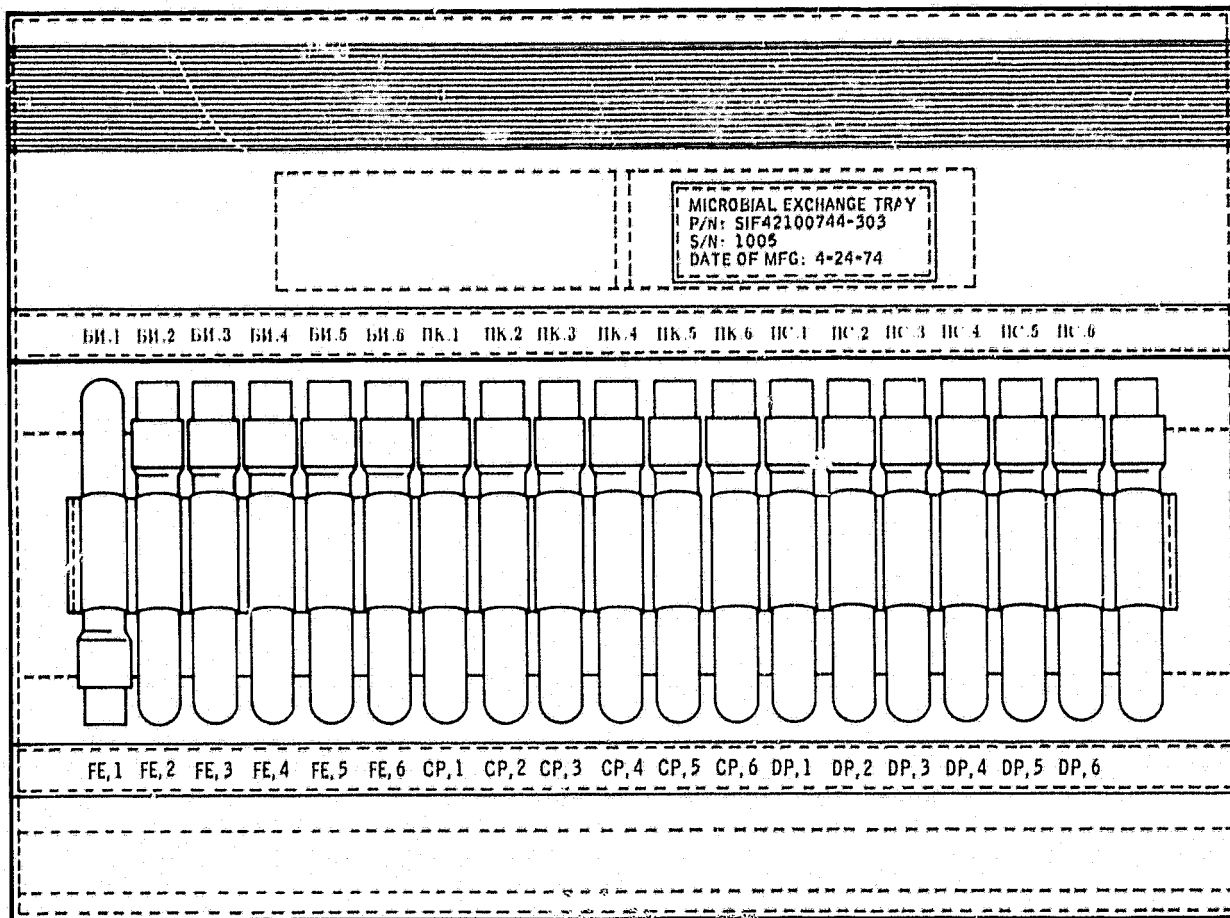


Figure 1. - In-flight microbial sample collection device.



(a) Closed kit.



(b) Open kit showing sample collection devices in place.

Figure 2.- Beta-cloth sample collection kit used for crewmember samples in the Apollo spacecraft.