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TECHNICAL MANUSCRIPT 209

MICROBIAL CONTAMINATION  
IN CLEAN ROOMS

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TECHNICAL MANUSCRIPT 209

MICROBIAL CONTAMINATION IN CLEAN ROOMS

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#### ABSTRACT

A study to determine the level of microbial contamination in an industrial clean room was undertaken as part of the over-all study of spacecraft sterilization. The level of microbial contamination obtained on a steel surface after exposure to aerial fallout for 52 weeks was essentially the same as the level obtained after the first week. Microbial contamination in the clean room was about one-tenth of that in the adjacent factory area. The airborne microbial contamination in a clean room increased when it was occupied by personnel and rapidly decreased when the room was vacated. Personnel appeared to have little or no effect on the level of inanimate particulate contamination present in the clean room.

## I. INTRODUCTION

Clean-room assembly facilities, or white rooms as they are sometimes called, are becoming more commonplace as electronic and other industries are producing miniaturized equipment whose performance is impaired by entrapped small dust particulates. The interplanetary space program,\* however, places another requirement upon space vehicles, namely that they be sterile, or contain no viable microorganisms that could contaminate solar bodies and negate subsequent search for extraterrestrial life forms.<sup>1-8</sup> Such a requirement arouses interest in the number of viable microorganisms in clean assembly areas, as well as interest in the total inert particulates. The present Air Force specifications for clean rooms<sup>9</sup> are based only upon this latter point. Clean rooms are not designed to be sterile assembly areas, such as are used in the pharmaceutical industries,<sup>7</sup> nor could they be expected to be sterile when personnel are working in them.

However, the precautions taken to reduce inert particulates should also lower the biological contamination of articles assembled in them and make subsequent sterilization treatments simpler and more reliable. In this study, which extended for one year, biological contamination was measured in clean-room facilities at the Martin Plant in Middle River, Maryland. When this study was begun no information was available on this subject, although a related paper on air contamination in clean rooms appeared shortly after it started.<sup>9</sup>

Two separate clean-room facilities were used in the two-part study. The prime purpose of Part I was the determination of the number of viable aerobes and anaerobes that accumulate on a stainless steel surface during one year. Also determined was the resistance of these microorganisms to heat shock, a technique that kills sensitive vegetative microorganisms but does not kill resistant organisms such as bacterial spores. To give an index of the aerial microbial contamination in the area, the air and aerial fallout were periodically sampled for one or two hours. In addition, the microbial contamination on stainless steel was measured after it was handled by gloved clean-room personnel. Part II consisted of a comparative study of the level of aerobic aerial microbial contamination in a clean room occupied by personnel under three different circumstances: (i) when personnel wearing clean room clothing and masks were sitting, (ii) when they were active, and (iii) when they were in street clothes and active.

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## II. MATERIALS AND METHODS

### A. CLEAN ROOMS

Standard clean rooms with nonlaminar air flow (Class II and III according to old designation fitting the Air Force definition)<sup>8</sup> were used in this study. For Part I, clean room A was essentially a complete two-room structure (50 by 100 by 12 feet) with entry locks, erected in a corner of a very large factory (400 by 1000 by 49 feet). The study was conducted in the clean-room facility (which included both a cleaning area and an assembly area) and also in the adjacent non-clean factory area, a few feet from the clean room. The observed number of people present in the clean-room facility under regular working conditions during the various test periods was always far below the allowable number, 75, based on a ratio of 1.5 persons per 100 square feet. Usually, there were less than ten present in the two clean areas. There was also relatively little activity in the factory area near the clean-room facility.

For Part II, idle clean room B (24.6 by 33 by 9 feet) in a six-room complex located in another large factory area was used. To conduct this study, one wall of the room was modified for direct access to the factory area. The clean room was occupied by the maximum allowable number of persons, in this instance, one per 100 square feet or a total of eight. Only Martin Company personnel certified for clean-room work participated as subjects in this special test.

### B. TEST PROCEDURE

For Part I, a total of 120 sterile 1- by 2-inch stainless steel strips for each test site were placed horizontally on sterile stainless steel trays to accumulate microorganisms from aerial fallout. At intervals throughout the year, eight strips were assayed to determine the number of viable microorganisms before and after heat shock. Five of these strips were used to enumerate aerobes and the remaining three to enumerate anaerobes. Also, at each sampling period, four sterile 1- by 2-inch stainless steel strips were handled by an individual wearing gloves while working in the clean room. Two strips were used to determine the number of viable aerobes or anaerobes before and after heat shock. Each of the strips was shaken in a bottle containing sterile 0.05% Tween 20 solution and then assayed for viable microorganisms by the pour plate method, both before and after the sample was heat shocked at 60 C for 30 minutes. Each sample was either cultured in tryptose agar under aerobic conditions or in anaerobic agar under anaerobic conditions. All plates were incubated at 37 C for 72 hours before colony counts were made.

Periodically throughout the year, at each test site, a Fort Detrick slit sampler<sup>3</sup> was used for an hour to collect microorganisms in the air. At the same interval, agar settling plates were used to determine the number of microorganisms falling from the air. Tryptose agar plates were used for the enumeration of aerobes and anaerobic agar plates were used for the enumeration of anerobes.

Part II of this study consisted of two tests that measured the effect of personnel activity upon the number of aerobes in the air, the number of microorganisms falling from the air per unit time, and the number of particulates in the air for each of five consecutive one-hour test periods. The subjects followed standard procedures in entering the clean room, which included a dust removal step both before and after donning a clean-room uniform over street clothes. In addition, a disposable surgical mask was worn to prevent gross biological contamination from the oral and nasal passages. The specific circumstances concerning each test period were:

Period 1. Clean room was vacant during air sampling.

Period 2. Eight subjects, wearing clean-room uniforms over street clothes and breathing through a disposable surgical mask, entered the clean room and worked while in a sitting position throughout the hour test period.

Period 3. This period differed from the second only in activity. The eight subjects walked around the room for one minute then sat for three minutes; this procedure was repeated every four minutes throughout the one-hour test period.

Period 4. Without leaving the room, the subjects removed their masks and clean-room uniforms (except for plastic boots that covered their street shoes). The same activity described above was performed by the subjects, now in street clothes.

Period 5. At the end of Period 4, the subjects left the clean room through the special door directly to the factory area so that their street clothes would not contaminate the other areas in the six-room structure. A one-hour air sample was again taken in the vacated clean room.

The Fort Detrick slit sampler, which measures the number of particulates in the air containing viable microorganisms, the Andersen sampler, which also measures microbial particulates, and the all-glass impinger (AGI), which tends to break up bacterial clumps and measures the total number of viable microorganisms present, were all used to determine the number of microorganisms in the air.<sup>5</sup> The Fort Detrick slit sampler was operated throughout each one-hour test period; the Andersen sampler and the AGI were used during the last 15 minutes of each test period. Agar

settling plates were used to determine the number of microorganisms settling from the air during each of the one-hour test periods. Microbial samples were taken at four sites. Three sites were at a seven-foot level, near a ventilation opening, the fourth was at the three-foot level near where the subjects sat. Appropriate plates used for the slit and Andersen samplers contained tryptose agar, as did the settling plates. Tryptose saline diluent was the collecting medium for the AGI samples, which were subsequently assayed by the pour plate method and cultured in tryptose agar. All plates were incubated at 37 C for 48 hours before colony counts were made.

The clean room was monitored for airborne particulates with an electronic sampling device<sup>10</sup> and by collecting particles on a membrane filter. The electronic sampling device attached to an automatic recorder outside the clean room continuously counted particles throughout each test period. During the second, third, and fourth test periods, the clean room was also monitored by collecting the airborne particles on a membrane filter that was subsequently removed from the clean room for microscopic examination and counting.

### III. RESULTS AND DISCUSSION

The results of the one-year study in clean room A and the adjoining factory area are presented in Figure 1 and in Tables 1 and 2. Data from the special test involving personnel activity in clean room B are given in Tables 3 and 4.

The data given in Figure 1 show the mean and the range of five determinations obtained for total aerobic count on stainless steel for each time period and test area. The level of surface contamination reached in both the clean and factory areas was fairly constant from the first through the last sampling period throughout the year no matter how many weeks the samples had remained exposed. A statistical analysis of the data substantiated the observation. The same phenomenon had been observed earlier with similar steel strips exposed in our own laboratory building. The apparent higher contamination level obtained in the factory area at 47 and 52 weeks, respectively, appears to be a seasonal phenomenon<sup>7</sup> occasioned by a substantial increase in mold population during the late summer months. On the 47th week, a new set of sterile steel strips was placed in the factory and cleaning areas. These strips were assayed on the 52nd week along with those that had been in the test areas for the entire 52 weeks. Table 1 shows that the aerobic contamination on steel after five weeks' exposure to air was comparable to the contamination that accumulated on steel during the entire year.

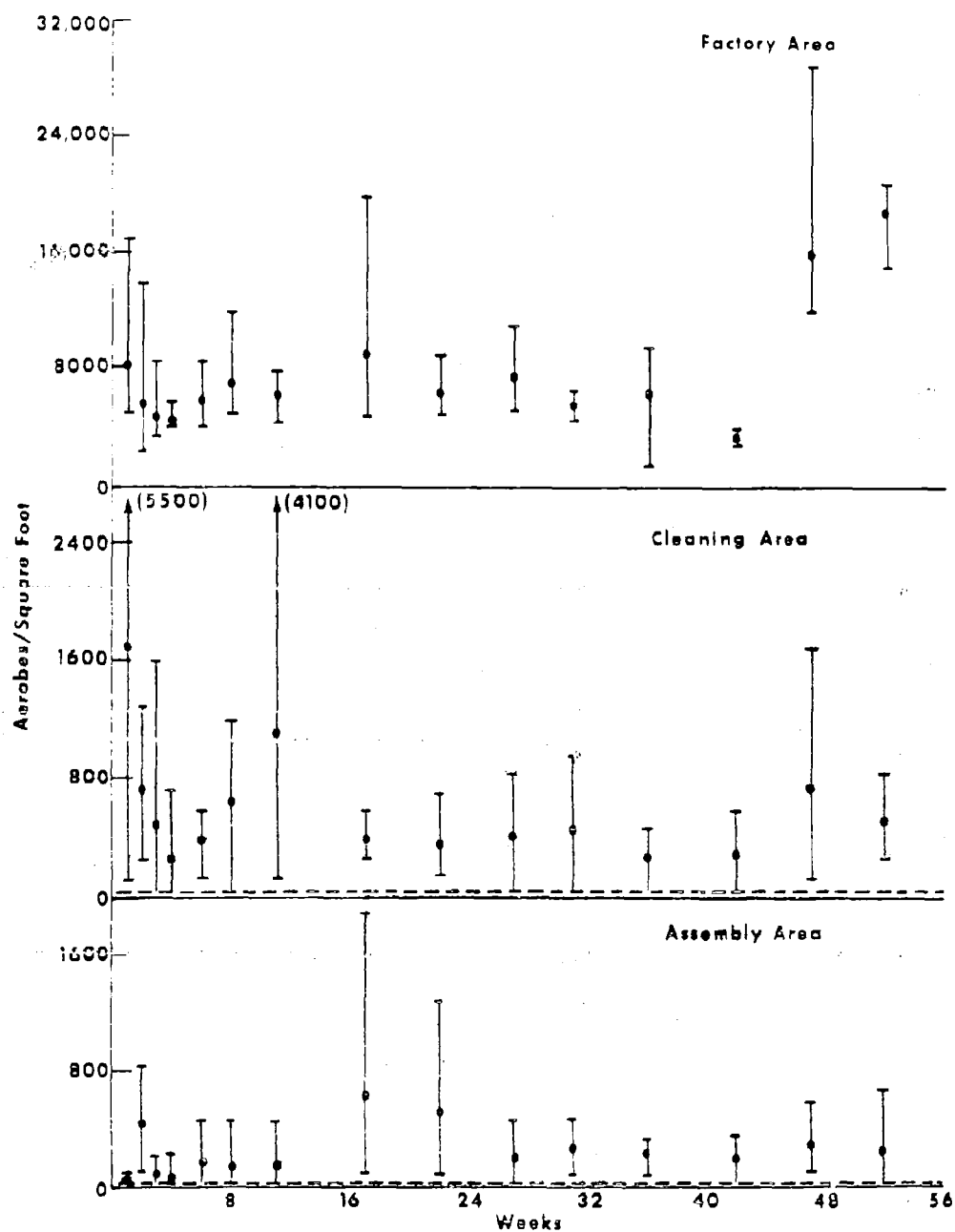


Figure 1. Number of Aerobes on Stainless Steel as a Function of Time. (Each bar shows the range of five determinations; the mean of each is represented by a dot on the bar. The dotted line indicates the lowest limit of contamination detected by the assay method.)

TABLE 1. COMPARISON OF AEROBIC CONTAMINATION ON STAINLESS STEEL  
AFTER 5 AND 52 WEEKS' EXPOSURE TO AIR  
IN FACTORY AND CLEANING AREAS

	Weeks Exposure	Aerobes per Square Foot
Factory Area	5 <sup>a</sup> /	19,000
	52	19,000
Cleaning Area in Clean Room A	5 <sup>a</sup> /	600
	52	510

a. Exposure period only during 47th to 52nd week of this study.

About five times more aerobes than anaerobes were present on the steel surface and, although not shown in the graph, often half or more of these microorganisms survived heat shock. In addition to spore-forming bacteria and the occasional molds recovered after heat shock, some cocci, which were probably protected by extraneous material such as dust, also survived the heat shock treatment. On the basis of colony formation, a great variety of species were represented.

An index of the level of airborne microbial contamination present in the factory and clean room during the years' study is given in Table 2. Michaelson and Vesley<sup>8</sup> reported that the number of airborne microorganisms in the various industrial white rooms studied ranged from 0.05 to 7.4 per cubic foot. The data reported here also fall within that range. In this part of the study all determinations of the air and aerial fallout were taken during regular working hours when the room was occupied, so that the steel samples were probably exposed to lower levels of contamination for more than two-thirds of their exposure time when no personnel were present.

The results in Table 3 indicate that when clean room B was vacant (test Period 1) there was very little microbial contamination in the air, but when the clean room was occupied by personnel the microbial contamination greatly increased even when the personnel wore surgical masks in addition to the clean-room clothing (test Period 2). Personnel movement (test Period 3) and personnel wearing street clothes (test Period 4) also increased microbial contamination. However, the level of microbial contamination was rapidly reduced immediately after the personnel left the clean room (test Period 5). The viable airborne microbial population seemed to be distributed primarily as individual cells rather than as

TABLE 2. LEVEL OF AIRBORNE MICROBIAL CONTAMINATION PRESENT IN FACTORY AND CLEAN ROOM A

Test Sites	Slit Sampler, Microorganisms per Cubic Foot				Settling Plate, Microorganisms per Square Foot per Hour			
	Aerobes		Anaerobes		Aerobes		Anaerobes	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Factory Area	3.4	1.2 - 7.3	0.7	0.03-2.4	225	79-376	37	2-102
Clean Room								
Cleaning Area	0.4	0.08-0.8	0.06	0 - 0.3	23	4-66	6	0- 34
Assembly Area	0.5	0.07-1.2	0.07	0 - 0.2	24	5-71	5	0- 24

TABLE 3. EFFECT OF PERSONNEL AND THEIR MOVEMENT UPON LEVEL OF MICROBIAL CONTAMINATION  
IN CLEAN ROOM B

Test Period	Clean Room Activity	Microorganisms		Microorganisms	
		per Sq Ft per Hr Settling Plate	per Cubic Foot of Air Slit Sampler	per Sq Ft per Hr Settling Plate	per Cubic Foot of Air Andersen AGI
1	None, room vacant	4	0.08		
2	Eight persons, in uniforms and masks, sitting	26	0.5	1.3	1.4
3	Eight persons, in uniforms and masks, moving	90	1.4	2.0	2.9
4	Eight persons, in street clothes, moving	680	6.6	12.1	28.3
5	None, room vacant	30	0.5		
	Factory area, immediately outside clean room, during performance of above test	394	5.2		8.6

clumps, since the results of the AGI samplers, which measure the total number of microorganisms, were not appreciably different from the results of the Fort Detrick slit samplers and the Andersen samplers, which measure particulates containing one or more microorganisms.

The particulate count remained fairly constant throughout the entire study (Table 4). Clean room B was designed to meet the requirement of not more than 20,000 particles per cubic foot of air 0.5 micron and larger, with not more than 4,000 particles per cubic foot of air 1.0 micron and larger. The data indicate that the standard set forth for this clean room is not exceeded even when the maximum allowable number of persons were moving about the clean room in their street clothes.

Personnel not only increase the airborne microbial contamination in a room by their presence and movement, but they also may increase the microbial population on a surface by handling. Several hundred microorganisms (range <50 to 840) per square foot were deposited on a previously sterilized stainless steel surface by handling only once with a gloved hand. More aerobes than anaerobes were obtained and high percentages of these survived heat shock.

The results of this study indicate that the microbial contamination in the Martin Company clean room used for the year-long study was about one-tenth that in the adjoining factory area. However, the number of personnel working in this room averaged only about ten per cent of the maximum allowable in such an area. In the short-range activity study conducted in clean room B, for which the maximum allowable personnel were utilized, the microbial contamination in the air increased greatly with activity. Thus the surface accumulation in clean room A (Figure 1) could well have been higher, if the room had been more utilized. Nevertheless, the level of microbial contamination in a clean room in full operation probably would be appreciably less than the level attained in the factory. The results shown in Table 3 indicate that the microbial contamination was about one-tenth that in the factory when personnel were sitting and about one-fifth during maximum activity.

The most striking observation of this study is that the microbial contamination on a stainless steel surface due to aerial fallout rapidly reached a maximum level and remained more or less constant throughout the year. A second observation, is that although human activity in a clean room can be undertaken without noticeably increasing the total number of particulates, it greatly affects the biological contamination.

TABLE 4. PARTICULATE CONTAMINATION IN CLEAN ROOM B DURING VARIOUS ACTIVITIES

Test Period	Clean Room Activity	Particles/Cubic Foot <sup>a</sup>					Fibers
		0.4-0.5 $\mu$	0.5-1.0 $\mu$	1.0 $\mu$	5-10 $\mu$	15-25 $\mu$	
1	None, room vacant	29,800	3,640	756			
2	Eight persons, in uniforms and masks, sitting	27,000	3,020	1,230	219.8	16.1	7.1 0.5
3	Eight persons, in uniforms and masks, moving	26,600	2,220	802	314.6	20.8	9.0 0.6
4	Eight persons, in street clothing, moving	16,800	1,340	1,140	611.5	66.5	46.9 1.8
5	None, vacant	13,800	778	224			

a. For particles 0.4 to 1.0 $\mu$ , an automatic electronic apparatus was used. Particles 5 to 25 $\mu$  were collected on a membrane filter.

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