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EFFECT OF ENVIRONMENT ON BIOLOGICAL BURDEN DURING SPACECRAFT ASSEMBLY

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ABSTRACT

A test program was conducted to determine the effect of environment on the accumulation of bio-burden on spacecraft during assembly operations. Three environments were selected: a typical high-bay manufacturing area having a Class 100,000 cleanliness, a 16 x 16 ft Class 100 laminar downflow tent, and the Sterilization Assembly Development Laboratory (SADL) 30 x 40 Class 100 laminar downflow assembly room which is replete with personnel and equipment entry control systems.

The test item, assembled under these varying conditions, was a 14-ft-dia capsule mechanical training model, consisting of mockups of the major subassemblies which are expected to comprise a typical spacecraft capsule. The complete encapsulation of the capsule in a bio-barrier was the terminal step of the assembly process.

The results of the study program showed that there were no significant effects on the aerobic spore accumulation due to environment; however, there was an effect on the control of aerobic vegetative cells. It was found that there was a noticeable reduction in the aerobic vegetative population in the Class 100 Sterilization Assembly Development Laboratory assembly room when compared to the Class 100 tent and the typical, manufacturing environment. The configuration of the spacecraft surfaces and their exposure to the environment had an effect on the bio-burden accumulation in all three test environments.

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SECTION I

INTRODUCTION

1.1 SCOPE

A planetary quarantine policy has been established by NASA to prevent the introduction of Earth microorganisms to other planets. The Sterilization Assembly Development Laboratory (SADL) was built as a pilot plant to develop and demonstrate spacecraft assembly and sterilization procedures which could meet the microbiological burden requirements imposed by the NASA quarantine standards. The fundmental task of SADL was to determine the effect of environment on microbiological burden accumulation during spacecraft assembly and to establish minimum requirements for equipment, procedures, and facilities, necessary to assemble, test, encapsulate, and sterilize a capsule which would satisfy the NASA quarantine constraints (Ref. 1).

1.2 PURPOSE

The purpose of this study was to perform multiple assemblies of a capsule in the following assembly environments* to evaluate their effect on microbial burden accumulation:

1) A typical high-bay assembly area (approximately Class 100,000 clean when unoccupied).

2) A portable, laminar downflow Class 100 cleanroom.

3) The SADL Class 100 clean assembly room complete with personnel entry and exit airlocks and passthroughs for tools and equipment.

Management of the SADL task was organized to carry out the assemblies in the project-oriented manner. The operating responsibilities of SADL were vested in the project manager and his supporting staff, which consisted of four technical groups: Facilities, Assembly, Microbiology, and Quality

*"NASA Standards for Clean Rooms and Work Stations for the Microbially Controlled Environment," NHB 5340.2, August, 1967.

Assurance. This organization made possible the development of technical interfaces necessary for the assembly of the capsule using standard manufacturing techniques and skills (Ref. 2).

Since the purpose of this effort was to investigate environmental effects, it was necessary to maintain strict control over those other variables that might mask the environmental effect. For example, stringent personnel dress procedural controls were invoked in order to minimize microbial contamination that could be attributed to personnel. Also, hardware cleaning procedures were rigorously defined and closely followed so that the microbial burden on the hardware prior to each capsule assembly would be uniform.

The results reported are meaningful only in light of the tight controls which were exercised over extraneous potentially contaminating sources. To extend the results of this test program to an actual capsule assembly, consideration must be given to applying similar controls to the actual situation.

SECTION II

TEST MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Test Facilities

The three environments in which assembly operations were performed for this study were the High Bay area, a portable laminar downflow room (tent) located in the High Bay area, and the SADL assembly room. These environments comprised the SADL facility (Ref. 3).

The High Bay area (Room 118) (Fig. 2-1) was an open room, 60 x 70 x 50 ft high, containing an overhead bridge crane. It was a typical manufacturing/assembly area with exposed pipings, ducting, and structural members. The room was air-conditioned (roughing filters only), and had a tile floor.

The portable laminar downflow room (tent) measured 16 x 16 ft and could be varied in height from 12 to 18 ft. It was a mobile stand supporting a bank of High Efficiency Particulate Air (HEPA) filters and lights and had sides of transparent, antistatic plastic. Blowers with roughing filters were mounted on opposite sides of a plenum which made up the roof of the stand. The side curtains were positioned 18 in. above the floor. The tent assemblies were performed in the High Bay area. The tent did not contain a hoist; therefore, during capsule assembly, two major subsystem assembly/mating operations (installation of impact limiter and aeroshell) had to be performed outside the tent in the High Bay area and then the assemblage was wheeled back into the tent.

The SADL assembly room was a 30 x 40 x 35 ft high laminar downflow room and was adjacent to the High Bay area. Large air locks were present for personnel entry and exit. For handling large subassemblies, there were two fixed, two-ton electric hoists in the room. Each was enclosed in a plastic sleeve, externally vented, to eliminate particulate contamination due to flaking. The walls of the room were covered with cloth-backed vinyl plastic with epoxy-resin-sealed joints. The floor consisted of 2 x 2 ft perforated panels installed on structural supports over a 6-ft deep return air plenum. The ceiling contained

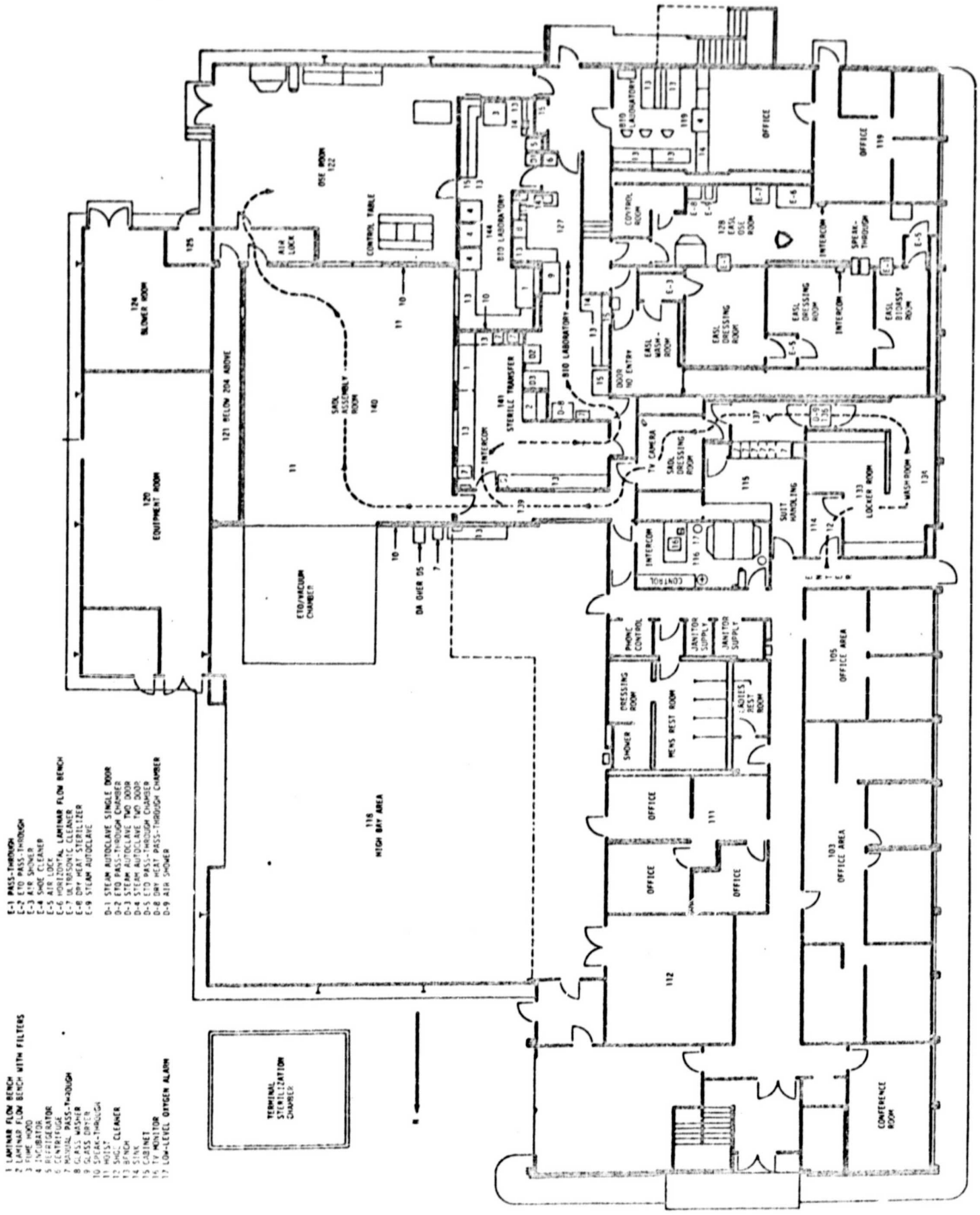

Fig. 2-1. Sterilization Assembly Development Laboratory
floor plan (showing personnel flow plan)

ninety-two, 9 x 4 ft HEPA filter modules interspersed with rows of lighting fixtures.

2.1.2 Hardware

The capsule used in this study was the Capsule Mechanical Training Model (CMTM); a mechanical mockup of the major subassemblies which might be expected to comprise a typical landing capsule (Fig. 2-2). It consisted of a 14-ft-diameter aeroshell in which was installed a payload section (bus) of the Mariner C type, containing eight electronic subassemblies (spares from the Ranger series), a 4-ft-diameter impact limiter, a parachute canister, a deorbit motor, and a relay link antenna. A sterilization canister as well as all CMTM assembly fixtures and handling equipment were also provided. Figure 2-3 gives a breakdown of the CMTM, its subsystems, and the assembly support and handling equipment.

2.2 METHOD

2.2.1 Assembly Procedure

The capsule assembly, test, and encapsulation procedures defined the capsule assembly and test operations in a step-by-step fashion and integrated the requirements of microbiological sampling and Quality Assurance monitoring into an optimum plan for assembling hardware in accordance with reliability requirements while meeting the planetary quarantine constraints. The procedures delineated the following:

1) The preparation of tools, hardware, and the assembly area.

2) The capsule assembly/disassembly operations.

3) The microbiological sampling.

4) The point of inspection by Quality Assurance during assembly.

Several trial assemblies were performed to validate the procedures and to establish the protocol for the capsule assembly operation. Janitorial services were scheduled for the assembly areas so that 48 hours of quiescence would occur before each assembly.

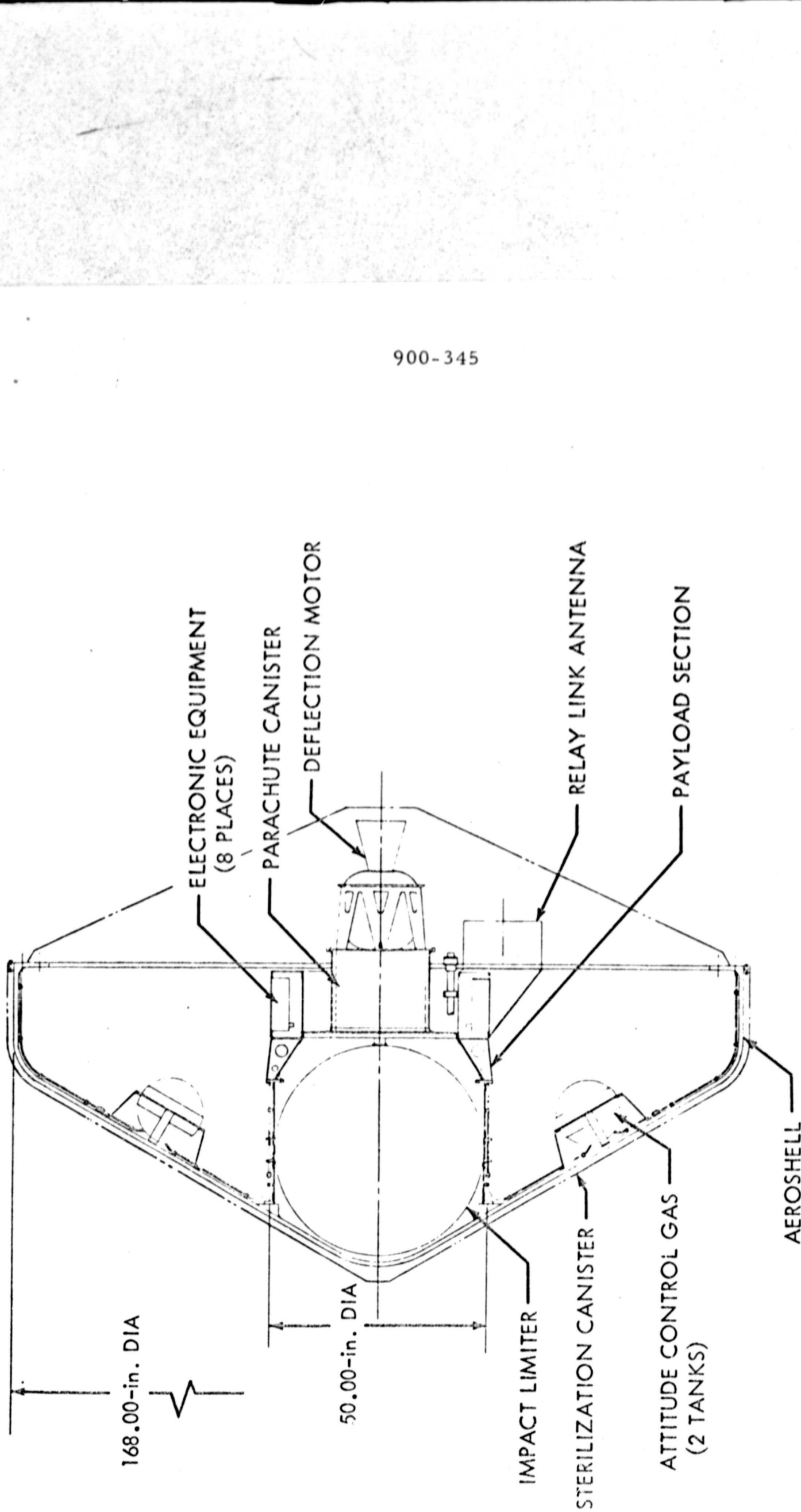

Fig. 2-2. Capsule Mechanical Training Model (CMTM)

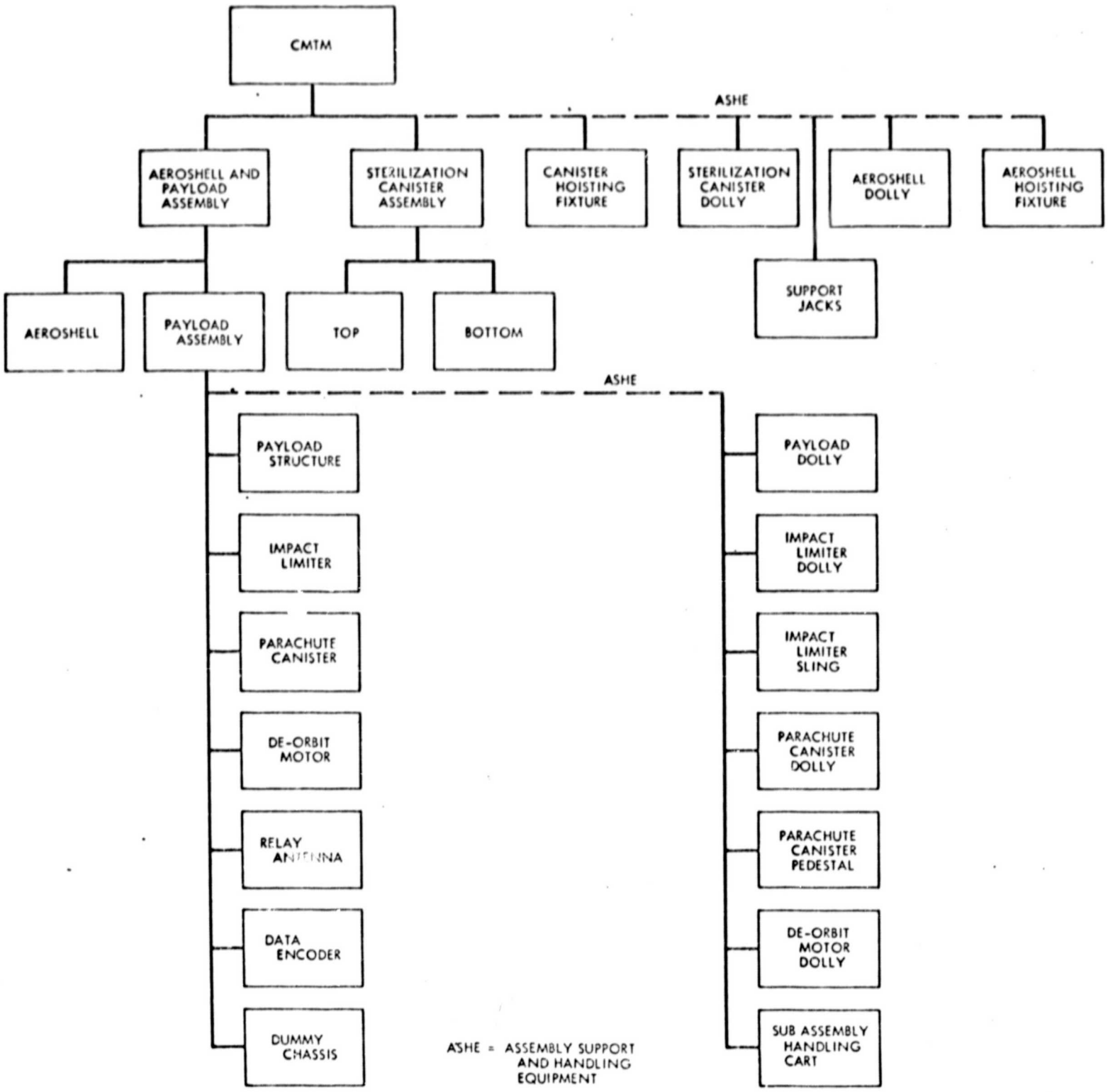

Fig. 2-3. Capsule Mechanical Training Model assembly configuration and interfaces

Table 2-1. CMTM daily assembly — events schedule

Day	Activity
1	Preparation of CMTM assembly area
2	Hardware wiped down with isopropyl alcohol, stainless steel coupons placed on designated subassemblies and covered with antistatic plastic cover
3	Same as Day 2
4 and 5	All CMTM hardware under cover
6	Installation of chassis to payload structure. Installation of impact limiter
7	Installation of aeroshell to payload assembly. Installation of parachute canister to aeroshell/payload assembly
8	Installation of deorbit motor to aeroshell/payload assembly. Installation of relay antenna to aeroshell/payload assembly
9	Removal of pre-quarantine coupons
10	Placement of CMTM in quarantine
11 and 12	CMTM remains in quarantine
13	CMTM out of quarantine and post-quarantine coupon samples taken

The preparation of the CMTM subsystems for assembly, and quarantine of the assembled CMTM encompassed 13 days (see Table 2-1). The actual assembly operation buildup of the CMTM from subsystems, covered three days.

2.2.2 Environmental Monitoring

A microbial baseline was established in each of the three assembly areas involved in this study. Fallout samples (collection of fallout on 1- x 2-in.

stainless steel coupon) were taken in the High Bay and tent environments at 40 in. above floor level. Twenty coupons were placed in trays at eight different sites in the High Bay to give good room coverage; four sample sites were used in tent environment. Five coupons were taken from each tray per day starting on the third day of exposure, with the last five coupons being removed on the seventh day (one week) of exposure. The microbial baseline for SADL Assembly Room was obtained by the use of fallout and Reyniers samples (placed at 72 in. above floor level). Twelve fallout and 17 Reyniers sites were evenly distributed over the 1,200-ft^2 area to construct a profile of the microbial burden of the assembly room.

In addition to the microbial baseline conducted in each of the assembly areas, microbial monitoring was conducted concurrently with each assembly. As in the microbial baseline study, fallout samples were taken in the High Bay (40 in., 6 sites) and in the tent (40 in., 4 sites; 72 in., 4 sites) while both Reyniers (72 in., 5 sites) and fallout (72 in., 5 sites) samples were taken in SADL Assembly Room.

2.2.3 CMTM Monitoring

The total CMTM external surface was divided into 75 sampling zones (Ref. 4). These zones were defined by first determining those areas which would be contacted by the assemblers during the assembly of the capsule. This was determined by dusting the assemblers' gloves with fluorescent dye during a trial assembly of the CMTM and mapping the contacted areas using an ultraviolet light. Secondly, the non-contacted areas were divided as to their angle of exposure to the environment: horizontal upward facing, slanted or vertical, and horizontal downward facing surfaces.

With the resulting four zone types based on handling (contact) and environmental exposure, it was assumed that the level of microbial burden accumulation would be different during CMTM assembly. Therefore, the number of samples per unit of area was varied on the different zone types so as to get the same degree of precision of burden estimate. Approximately 1300 sampling sites were identified.

Six hundred stainless steel (1 x 2 in.) coupons were used for sampling. These coupons were attached at the specified sites on each zone using

double-back silicone-based tape. In addition to the 600 samples, 700 "dummy coupons" were attached so that the assemblers would not know which were to be sampled and to assure that the coupons would be handled (contacted) due to the high density. Three different sampling matrices were used so that the sample coupons would vary for each assembly in each environment.

The removal schedule of the sample coupons during CMTM assembly was as follows:

1) Immediately prior to the assembly of any subassembly, to serve as a control to identify initial burden.

2) Before and after the eight chassis were assembled to payload assembly.

3) Before and after the impact limiter was lowered onto the payload structure.

4) Before and after the aeroshell was assembled onto the payload structure.

5) Before and after the parachute canister was assembled onto the payload structure.

6) Before and after the deorbit motor was assembled to the payload structure.

7) Before and after the relay link antenna was assembled to the payload structure. Note: At this point, the CMTM is completely assembled.

8) Before and after a quarantine period. In this case, representative samples were removed from all the exposed surfaces of the CMTM.

9) Before and after the CMTM was lowered into the lower half of sterilization canister.

10) Just prior to mating the two halves of the sterilization canister.

2.2.4 Bioassay Procedures

Twenty percent of the sampling coupons removed from the CMTM were assayed for aerobic and anaerobic vegetative cells and spores. The remaining

80% were assayed for aerobic vegetative cells and spores only. Except for this modification, all samples were assayed in accordance with the NASA Standards for Microbiological Examination of Space Hardware (NHB 5340.1, August 1967 edition). The reasons for the modification were as follows:

1) Prior experience had shown that a negligible anaerobic population would be detected; i.e., aerobic forms would predominate in the samples.

2) By reducing the number of samples to be assayed for anaerobes it was possible to increase both the relative size (volume of aliquot) and number of samples to be assayed for aerobic populations.

The environmental fallout coupons were assayed using the same procedures as for the coupons taken from the CMTM. The Reyniers samples were assayed in accordance with the NASA procedures cited above.

2.2.5 Test Constraints

Since the objective of this study was to evaluate the effect of environment on the microbial burden accumulation during the assembly of the CMTM, it was important that extraneous variables affecting microbial burden be controlled. Sources of variables affecting microbial burden fell into two categories, those associated with the hardware (CMTM and assembly support equipment) and assembly procedures and those associated with the control of personnel activity.

The hardware was degreased with toluene before the start of the test program to assure uniform surface characteristics. After the initial degreasing of the hardware with toluene, only spot-degreasing was required between assemblies where violations occurred which would have contaminated the surface with oil.

To establish a baseline level of contamination on the hardware for each assembly, all subassemblies were wiped down with 90% isopropyl alcohol before placing the sampling coupons on the hardware. Swab samples (47) were taken from all subassemblies before and after alcohol wipedown to establish a baseline for initial microbial burden on the hardware for each assembly. Immediately after the coupons were placed on a given subassembly, the unit was

covered with a decontaminated (Ethylene Oxide treatment, ETO) antistatic plastic cover. The subassembly remained covered until required in the assembly operation. In addition, tools and equipment used in assembly were bagged in plastic containers and decontaminated with ETO, and were stored in these bags until used.

All personnel associated with the assembly operation, including microbiological laboratory personnel, underwent a defined dressing procedure. This included a surgical-type scrubbing of the hands and the wearing of decontaminated hoods, smocks, booties, surgical latex gloves, and face masks.

Other major constraints that were imposed to enhance the control of extraneous variables, were that:

1) All facility operations, mechanical assembly, biological monitoring, etc., were performed in accordance with established written procedures only.

2) Equipment maintenance and cleaning of the assembly areas were scheduled so that sufficient time for contamination monitoring of the areas was available prior to the next scheduled assembly to assure that the intramural environment was not altered.

3) Access to the assembly areas was strictly limited to personnel with assigned responsibilities in performing the assembly, thereby assuring the same personnel activity associated with each assembly.

Quality assurance monitors certified adherence to the capsule assembly and test requirements as well as the microbiological constraints (Ref. 5).

2.2.6 Data Handling

The microbiological assay monitoring program generated more than 10,000 data points. To facilitate the retrieval and analysis of this massive amount of information, the Biological Assay Data Storage and Retrieval computer program was written. This program established a master file for the biological assay data from which data points could be selected and/or from which test routines for data analysis could be performed. A typical listing from the file is shown in Table 2-2. Data could be selected 1) to establish burden by zone type and/or assembly step, etc., 2) to perform the

Table 2-2. Typical listing in master file

ASSEMBLY STEP / ZONE / TYPE OF ZONE / SAMPLE SITE / QA CONTROL NOTATION / ASSAY PROCEDURE / CMTM ASSEMBLY NO.	AEROBIC VEGETATIVE COUNTS			AEROBIC SPORE COUNTS			ANAEROBIC VEGETATIVE COUNTS			ANAEROBIC SPORE COUNTS			TOTAL AEROBES	TOTAL VEGETATIVE	TOTAL SPORES	TOTAL POPULATION	CARD NO.
3.15A01C42 2S01	0	0	0	0	0	0							0	0	0	0	4600
3.15A01C49 1S01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4610
3.15A01C55 2S01	0	0	0	0	0	0							0	0	0	0	4620
3.15A01C56 1S01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4630
3.15A01C60 2S01	0	0	0	0	0	0							0	0	0	0	4640
3.15A01C70 2S01	0	0	0	0	0	0							0	0	0	0	4650
3.15A01C74 2S01	0	0	0	0	0	0							0	0	0	0	4660
3.15A01C83 2S01	0	0	0	0	0	0							0	0	0	0	4670
3.15A01C85 2S01	1	0	1	0	0	0							0	0	0	0	4680
3.15A01C88 2S01	0	0	0	0	0	0							0	0	0	0	4690
3.15A01C92 2S01	0	0	0	0	0	0							0	0	0	0	4700
3.15A01C94 1S01	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	4710
3.15A01C97 2S01	0	0	0	0	0	0							0	0	0	0	4720
3.15A02C23 2S01	0	0	0	0	0	0							0	0	0	0	4730
3.15A02C39 2S01	0	0	0	0	0	0							0	0	0	0	4740
3.15A02C43 2S01	0	0	0	0	0	0							0	0	0	0	4750

Kolmogorov-Smirnov test using two or three subsets of data, 3) to perform total burden calculations, or 4) to perform other appropriate statistical tests. Data that appeared to be the result of either assembly procedure violations or laboratory accidents, as noted in the Quality Assurance assembly log and laboratory accident logs, respectively, was identified in the master file with a code and was not utilized in subsequent data analyses.

Statistical hypotheses were formulated for evaluating the effect of environment and assembly conditions on microbial burden accumulation on the CMTM. The statistical hypotheses are stated in the form of questions in Table 2-3. The statistical methods applied to answer these questions are also shown in Table 2-3. To obtain significant results in the statistical testing, a value of 5% ($\alpha = 0.5$) for the risk of rejecting a true hypothesis was assumed for all tests. Questions 1-3 in Table 2-3 will be discussed subsequently. The analyses results for the test controls, burden accumulation, and post-quarantine (i.e., Questions 4-10) will be discussed in the next section.

Table 2-3. Data analysis

Question	Statistical Methods Applied	CMTM Data Analysis
Distributions		
1. Were the data normally distributed?	Graphical	All
	χ^2 test for goodness fit	Selected samples
2. Could the data be transformed to obtain a normal distribution?	Log, square, square root and linear transformations	Selected samples
3. Did the data follow the Poisson distribution?	χ^2 test for goodness fit	Selected samples
Test Controls		
4. Were the controls, i.e., post-alcohol swabs, subassembly control coupons, and assay control coupons, statistically comparable throughout the experiment?	Kolmogorov Smirnov Test for observed difference between two Poisson variables	Post alcohol swabs Subassembly control coupons Assay control coupons
5. Was there a detectable difference in the environmental controls?	Kolmogorov Smirnov Test for observed difference between two Poisson variables t test F test	Environmental coupons
Burden Accumulation		
6. Was the spacecraft burden accumulation data from three assemblies in a given environment statistically comparable?	Kolmogorov Smirnov Test for observed difference between two Poisson variables	Assembly data
7. Was there a significant difference in the accumulation of burden on a capsule when it is assembled in the high bay area, a laminar downflow tent, or in the SADL assembly room?	t test F test confidence interval	Spacecraft burden numbers (sigma calculation)
8. Was there a significant difference in the accumulation of burden on different zone types of the spacecraft?	Analysis of variance	Weighted zonal counts
Post-Quarantine		
9. Were the quarantine data (pre-quarantine or post-quarantine data) statistically comparable for a given environment.	Kolmogorov Smirnov Test for observed difference between two Poisson variables Kruskal Wallis one way analysis of variance	Pre-quarantine coupons Post-quarantine coupons
10. Did the post-quarantine period result in a significant reduction of microbial burden in a given environment?	χ^2 test using contingency table	Pre-quarantine coupons vs Post-quarantine coupons

The data could not be plotted linearly on normal probability paper and could not be transformed to achieve normality. Attempts to fit the data to Poisson distribution also failed. This problem was attributed to the large percentage of zero counts (60-90%) occurring in the data. The high percentage of zero counts made it necessary to use 1) nonparametric methods such as the Kolmogorov-Smirnov test to compare counts between different subsets and 2) dichotomous population methods using attribute data,* such as the χ^2 test using the contingency table.

Another approach for surmounting the zero count problem was to assume for calculated data (e.g., burden on spacecraft and weighted zonal counts) that the error in the calculated measurement was made up of a large number of small errors from various sources (a reasonable assumption). Then, the central limit theorem indicates that the composite error will be normally distributed. Hence, under this assumption, parametric methods, such as confidence intervals, t tests, and analysis of variance, could be and were applied.

The sigma calculation was the name given to the method for determining the amount of burden accumulated on the spacecraft at the completion of assembly. Only data from procedural steps involving actual assembly were used in the sigma calculation. The following equation was applied to each zone of the spacecraft, and the burden from each zone was summed to give a total burden for the spacecraft:

$$\text{Burden on spacecraft} = \sum_{i=1}^{75} \frac{n_i A_i}{A_{s_i}}$$

where

i = zone number

A_i = area of i^{th} zone

A_{s_i} = effective area sampled on i^{th} zone

n_i = number of colonies counted on assays from the i^{th} zone

*A coupon with a count equal to zero was classified negative; a coupon with a count greater than zero was classified as positive.

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The weighted zonal burden represents the numbers that were compared to determine if there existed a significant difference in accumulation of burden on different spacecraft zone types. The following equation was used:

$$\overline{N}_{r,s} = \sum_{j=1}^{3} \frac{K\, X_{rsj}}{Y_{rsj} A_r}$$

$\overline{N}$ = weighted zonal burden = average number of organisms (weighted counts) on r^{th} zone type in the s^{th} environment for three assemblies each environment

r = zone type; a = 1; b = 2; c = 3; d = 4

s = environment; High Bay = 1; tent = 2; SADL = 3

j = assembly number = 1, 2, 3

Y_{rsj} = total number of assayed coupons on i^{th} zone type of j^{th} assembly in s^{th} environment

X_{rsj} = number of colonies counted on assays from the r^{th} zone type of the j^{th} assembly in the s^{th} environment (number of organisms)

A_r = total area that is represented by r zone type

K = constant = 4×10^6 (coupons x ft^2)

Because the zone types were not of equal areas and the number of assayed coupons from the same and different zone types was not constant, the use of a weighting factor became necessary for a comparison of zone types.

SECTION III

RESULTS AND DISCUSSION

3.1 FACILITY CERTIFICATION

A baseline study of the three environments evaluated in this paper was conducted to establish the microbial burden level in the areas without personnel activity. The results of this baseline study indicated that the High Bay was in the order of a Class 100,000 room while the tent exhibited a Class 100 environment. The SADL Assembly Room was certified as a Class 100 clean room, meeting or exceeding such requirements.

3.2 TEST CONTROLS

The post-alcohol swabs were statistically comparable ($\alpha = 0.05$) throughout the experiment as were the subassembly control coupons and the assay control coupons. These results were verified by two independent tests (Table 2-3, Question 4).* Since the post-alcohol swabs and subassembly control coupons were comparable throughout the experiment, it was implied that the initial burden on the CMTM at the beginning of all assemblies was similar. Although the assay control coupons were comparable from an overall assembly-to-assembly basis, there was considerable variation in the proportion of controls contaminated on a day-to-day basis (Ref. 5). The overall assembly comparisons were within permissible statistical limits (i.e., the "background" contamination contributed by laboratory assay procedures was comparable on an assembly-to-assembly basis), hence the assay control data was not considered an important factor that would affect the results of the other analyses. Therefore, other data, specifically environmental data, burden accumulation and pre- and post-quarantine data were not corrected for biological laboratory induced contamination. It must be pointed out that the fundamental objective of the assemblies was to ascertain the effect of different environments on microbial population levels detected on the CMTM. The experiment was designed so as to identify relative numbers of microorganisms; not absolute.

*These statistical tests were made within and between assembly groups (i.e., High Bay, tent and SADL).

The environmental control coupons (fallout coupons) for each of the three assemblies in a given environment were compared using total population data. The three assemblies in the High Bay area were not statistically comparable, but the three assemblies in the tent were comparable as were those in the SADL Assembly Room. Division of the data into aerobic vegetative cells and aerobic spores and consideration of the first three days of actual assembly only, gave results as shown in Figs. 3-1 and 3-2. These results are not extrapolated to organisms/ft^2 but are expressed as organisms per 0.8 square inches.*

It should be noted that each point represents the mean value of test site means for a given day (e.g., for assembly 202, Day, there were 4 test sites with 5 coupons assayed for each test site, and test site means were averaged to get the value that was plotted). The estimated mean fallout (micro-organisms/1 ft^2) and 95% confidence limits for the true mean value of fallout are given in Table 3-1.

Since fallout from Days 1 and 2 accumulated on those coupons removed on Day 3, and comparison of fallout at the end of assembly in each of the environments was also obtained (by using the t test and F test) from Day 3 data, no statistical conclusions can be made for the aerobic vegetative data between the High Bay and tent, or the High Bay and SADL (variances are not homogeneous). However, since the tent mean fallout (i.e., aerobic vegetative) is an order of magnitude less than the High Bay mean fallout, this may indicate that the tent more effectively controls microbial burden levels. The t test on the Day 3 aerobic vegetative fallout (means) for the tent and SADL environments indicates that a difference in fallout environments between the tent and SADL Assembly Room did exist. A one-log difference in vegetative fallout was noted between the tent and SADL Assembly Room. For the aerobic spores, the t test indicates that there was no significant difference in the average fallout accumulation for the three environments.

*The 0.8 square inches is an effective area and takes into account the dilutions involved in the assay of the coupon samples. Multiplication by a factor of 180 will yield organisms/ft^2.

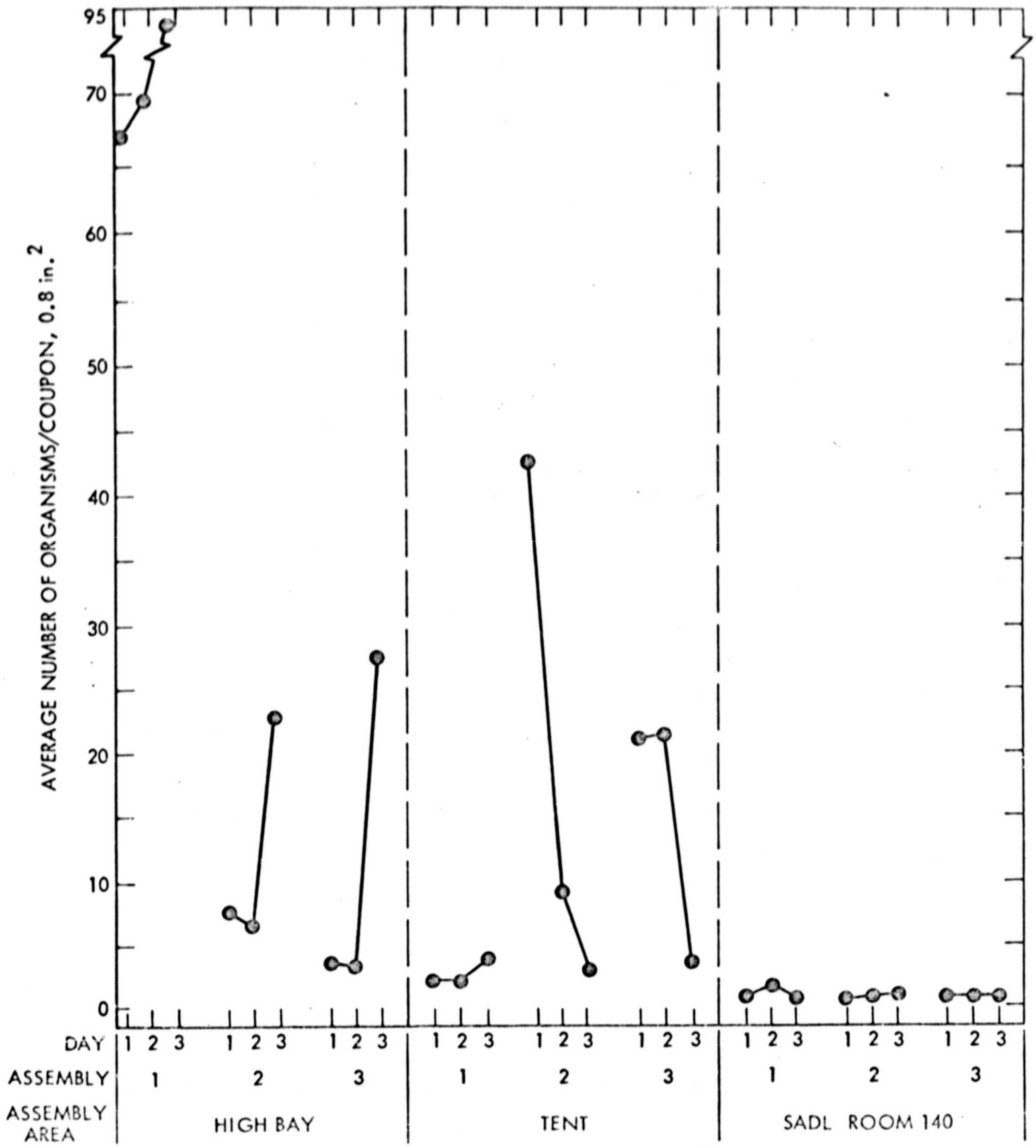

Fig. 3-1. Environmental burden for three-day assembly (aerobic vegetative)

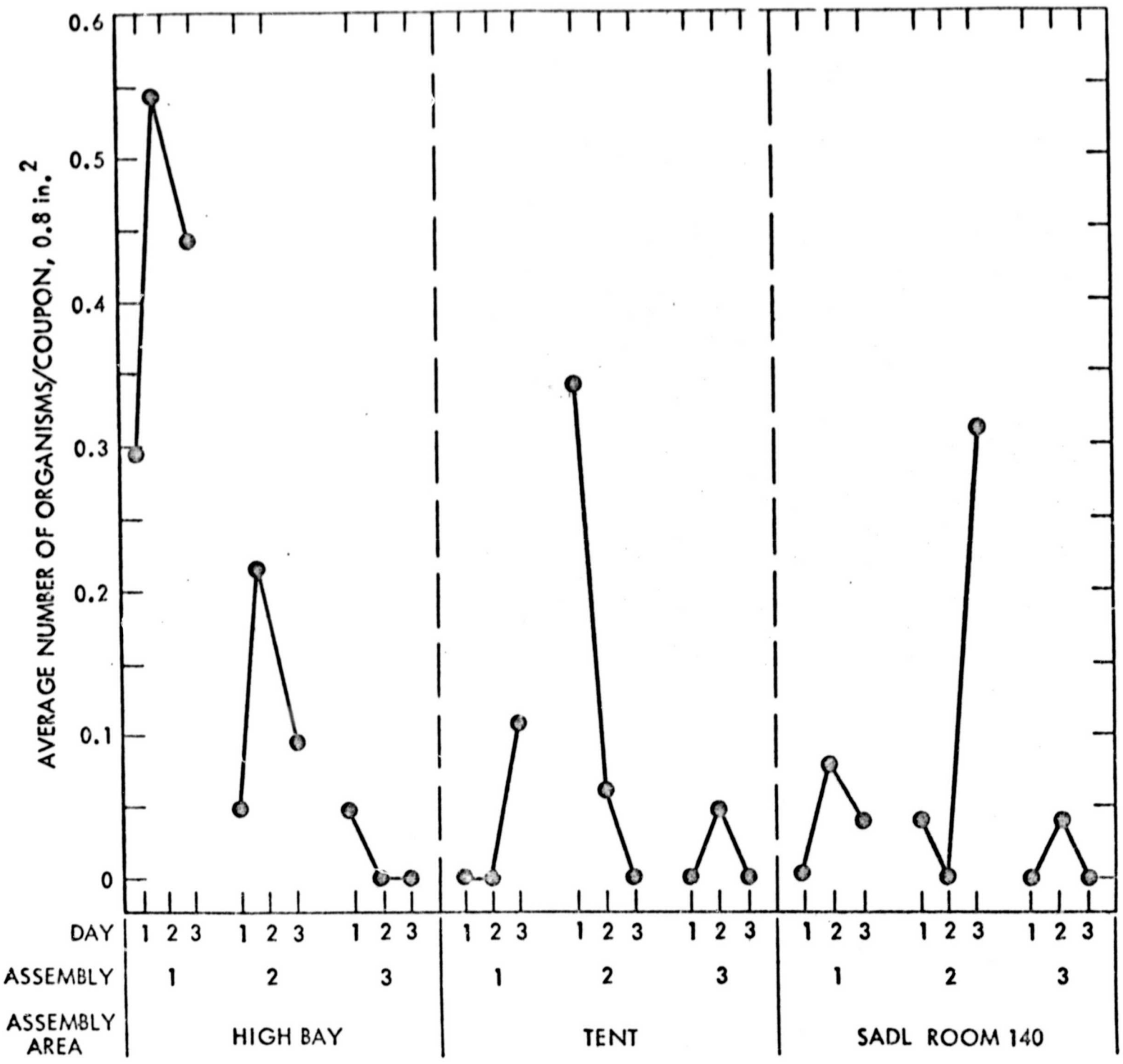

Fig. 3-2. Environmental burden for three-day assembly (aerobic spores)

Table 3-1. Environmental control fallout data (aerobes)

Environment	Mean Fallout Microorganisms/ft^2	95% Confidence Limits[a] Microorganisms/ft^2	
		Lower Limit	Upper Limit
High Bay			
Vegetative	8,676	549	16,812
Spore	32	0	83
Tent			
Vegetative	500	408	592
Spore	7	0	20
SADL			
Vegetative	23	5	41
Spore	22	0	58

[a]Confidence limits that would be negative were set equal to zero. Hence, the confidence intervals are asymmetric.

3.3 CMTM BURDEN ACCUMULATION

As discussed in the Method and Materials section, three assemblies of the CMTM were performed in each of the three environments (High Bay, tent, and SADL Assembly Room). A sigma calculation was performed on each assembly to estimate the microbial burden accumulation on the CMTM during the 3-day assembly period. The application of the Kolmogorov-Smirnov test to the data used for the sigma calculations indicated that the samples taken for the three assemblies in a given environment were from the same population or populations with the same distribution. The sigma calculations were based on

Table 3-2. CMTM burden accumulation sigma calculation (aerobes)

Environment	Mean CMTM Burden Micro-organisms	95% Confidence Limits[a] Microorganisms	
		Lower Limit	Upper Limit
High Bay			
Vegetative	3.46×10^5	1.75×10^5	5.17×10^5
Spores	1.22×10^4	8.27×10^3	1.62×10^4
Tent			
Vegetative	4.37×10^5[b]	0	1.61×10^6
Spores	3.34×10^3	5.50×10^2	6.13×10^3
SADL			
Vegetative	1.27×10^4	8.70×10^3	2.14×10^4
Spores	1.06×10^4	4.00×10^2	2.08×10^4

[a] Confidence limits that would be negative were set equal to zero. Hence, the confidence intervals are asymmetric.

[b] Range of sigma values for three tent assemblies were 7.94×10^4 to 1.07×10^6.

approximately 150 coupons from each assembly. The estimated mean value and the 95% confidence limits for the true mean value of the CMTM burden are given in Table 3-2.

For the aerobic vegetative burden, the F test indicated that only the variances from the High Bay and tent assemblies were homogeneous. Thus, the application of the t test to those two sets of sigma calculations showed that the two means were statistically equivalent, indicating no difference in amount of burden accumulation between the two environments. As can be seen in

Table 3-2, there was a one-log reduction in aerobic vegetative accumulation in SADL Assembly Room compared with the High Bay and tent environments. For the aerobic spore burden all variances were homogeneous; thus application of the t test on the aerobic spore sigma calculation indicated that there was no significant difference in the means of spore burden accumulation in the three environments. That is, the spore burden on the CMTM during assembly in the three environments was essentially the same.

In examining the results of the study of microbial burden accumulation on the CMTM an understanding of assembly conditions is necessary. The assembly procedures developed for the CMTM required very little assembly activity over the spacecraft hardware. Most of the work was carried out by assemblers working under the aeroshell, or from the side, thereby allowing the vertical laminar air to have the maximum effect in controlling dissemination of organisms due to assembly activity. The space available for personnel during the tent assemblies was limited due to the space occupied by the CMTM when it was placed within the tent. This condition may explain the level of vegetative burden found on the CMTM during tent assemblies. Assembly procedures were also designed so as to control both the microbial burden entering assembly areas and the translocation of microbial burden within these areas.

The surface of the CMTM was divided into zones which were expected to have different microbial burden accumulation. The effect of handling and surface exposure is shown in Table 3-3. The data presented in Table 3-3 have been normalized to account for a differing number of samples per unit area (sampling density) taken from each zone type (see definition of weighted zonal counts in Sec. 2.2.6). Therefore, the sum of the burden from all areas for a given assembly is not equal to the total burden estimated for that assembly. As presented in Table 3-3, handling and surface exposure appear to have an effect on microbial burden accumulation. In all three environments, the horizontal upward facing surfaces had the highest burden accumulation followed by the areas contacted by the assemblers. This difference was very small in the SADL Room compared with the tent and High Bay environments. The results show that surfaces which are not exposed directly to the environment (downward facing surfaces) had the least burden accumulation.

Table 3-3. Effect of surface exposure on bio-burden (aerobes)[a]

Assembly Environment	Handled surface	Non-handled surfaces		
		Horizontal upward	Vertical and slanted	Horizontal downward and inside
High Bay				
Vegetative	1086	48, 617	131	102
Spores	411	866	3	2
Tent				
Vegetative	1673	35, 709	111	34
Spores	24	40	2	2
SADL				
Vegetative	109	362	8	12
Spores	25	25	2	2

[a]Data presented as the average number of organism (weighted counts) for three assemblies each environment.

For statistical verification, the analysis of variance was applied to the three assembly groups and four zone types. The test applied separately to the vegetative weighted zonal counts and spore weighted zonal counts. Since only the variances from two zone types, namely the vertical and slanted surfaces and the horizontal downward and inside surfaces, were homogeneous (vegetative case as well as spore case), comparisons using the analysis of variance could only be made for these two types and cases. The analysis indicated that the mean vegetative weighted counts of all assemblies for these two zone types were statistically equivalent. That is, there was no statistical differences that could be attributed to the differences in zone types for the three assembly groups. The same analysis, however, did indicate that there were statistical differences between the assembly groups (i.e., a detectable difference in environments was noted). For the mean spore weighted counts, the two zone types were also shown to be statistically equivalent. However,

Contrast to the vegetative case, no statistical differences were noted between the environments for the spore case. This again verifies the point that was made previously that significant differences exist in vegetative burden accumulation when the CMTM is assembled in the three environments, but no significant difference can be noted for spores insofar as this series of tests is concerned.

4 POST-QUARANTINE

The test program included an evaluation of the effect of a 4-day quarantine period in which the assembled CMTM was placed in a Class 100 laminar flow environment. The quarantine data used for the analysis resulted in histograms that were statistically similar for a given environment. To determine a significant difference between the pre- and post-quarantine data, the proportion of the number of coupons with counts was compared. Assuming a risk of 5% for rejecting a true hypothesis, the analysis indicated that proportion with counts for a given environment were statistically equal for the pre- and post-quarantine periods.

Sigma calculations for the pre-quarantine data and for the post-quarantine data were compared by inspection and showed that there was very little, or no reduction, in either vegetative cell or spore burden which resulted from the assembly of the CMTM in SADL Assembly Room or the tent. There was from one-half to one-log reduction in the vegetative cell burden that accumulated in the High Bay environment; however, very little reduction was noted in spore accumulation.

SECTION IV

SUMMARY AND CONCLUSIONS

A test program was carried out to evaluate the effect of environmental cleanliness on the microbial burden accumulation during the assembly of the CMTM. The three environments evaluated were 1) a High Bay assembly area, Class 100,000, 2) a portable cleanroom (tent), Class 100, and 3) the SADL Assembly Room, Class 100. Three assemblies of the CMTM were performed in each environment.

The environmental monitoring (i.e., environmental fallout coupons) of the CMTM showed a one-log reduction in aerobic vegetative cells in the tent as compared to the High Bay assembly area, and two-log reduction in the SADL Assembly Room as compared to High Bay. However, because of the lack of homogeneity in the variances, no statistical conclusions could be made for the aerobic vegetative data between the High Bay and tent, or the High Bay and SADL Assembly Room. The t test did indicate a difference in the fall-out environment (vegetative cells) between the tent and SADL Assembly Room. The t test showed no significant difference in the mean aerobic spore fallout for the three environments.

The aerobic vegetative cell accumulation on the CMTM, as determined by the sigma calculation, was found to be of the same order of magnitude for both the High Bay and tent environments. The SADL assemblies showed a one-log reduction in aerobic vegatatives on the CMTM when compared to the High Bay and tent assemblies. The t test, when applied to the sigma calculations for the High Bay and tent, showed that the two means were statistically equivalent. However, no statistical conclusions could be drawn between the sigma values for the tent and SADL Assembly Room, or the High Bay and SADL Assembly Room. The t test applied to the aerobic spore sigma calculation showed no significant difference in the means of spore accumulation on the CMTM in the three environments, insofar as this series of tests is concerned.

The configuration of the CMTM surfaces, and their exposure to the environment, was found to affect the microbial burden accumulation in all three test environments. The horizontal upward facing surfaces were found to have the highest burden accumulation (spores and vegetative cells), followed

by the areas contacted by assemblers. Although no statistical conclusions could be made between certain zonal areas because of a nonhomogeneity of variances, statistical comparison was made between vertical or slanted surfaces and horizontal downward and inside surfaces. The analysis indicated that the means of the two zone types were statistically equivalent for both vegetative and spore burden.

The evaluation of a 4-day quarantine program showed very little or no reduction in vegetative cell or spore burden. No significant difference was found between the pre- and post-quarantine data when the proportion of the number of coupons with counts was used in the comparison.

The applicability of these test results to an actual capsule assembly has not been included in this discussion. It must be understood that the scope of this study was to evaluate only one parameter, that of the cleanliness level of the environment on microbiological burden accumulation. To extend these results to the actual assembly of flight articles, parameters such as clothing, precleaning of hardware, limited personnel access, limited assembly duration, assembly procedures and Quality Assurance monitoring (which were carefully controlled for this study) must be evaluated as to their effect on burden accumulation. If these parameters are controlled on a flight program in an analogous manner as used for this study, then these results can be directly applied.

SECTION V

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APPENDIX A

ADDITIONAL SADL PROGRAM STUDIES

1. INTRODUCTION

A number of minor studies were conducted in the SADL program in addition to the primary study described in the body of this report. The objective of these studies was to better define the effect of various assembly constraints on microbial accumulation during capsule assembly. The assembly constraints studied included 1) requiring all assembly support equipment entering the tent environment to be decontaminated with 90% isopropyl alcohol (Modified Tent Study), 2) determining the effect of untrained assembly personnel in the SADL Assembly Room environment (Untrained Personnel Study), and 3) reducing the protective clothing requirement in the SADL Assembly Room environment (Clothing Study).

Two assemblies of the CMTM were performed for each of the above studies. Except for the condition being tested, the assembly procedures and constraints were the same as those described in the body of this report.

This appendix presents the modification made to the basic assembly conditions described in the body of this report and gives the results of these studies. It is emphasized that the tests covered in this appendix were not conducted in such an exhaustive manner as those described in the body of this report. The results given here are therefore presented as trends or indications rather than results which can be rigorously defended.

2. MODIFIED TENT STUDY

Both the tent and the SADL Assembly Room were Class 100 cleanrooms, but for the basic assemblies conducted in them, different constraints applied to the decontamination of material entering these areas. Two additional assemblies were therefore carried out in the tent environment with microbial decontamination of items entering the assembly area under the same control as for items entering the SADL Assembly Room. This was accomplished by decontaminating (wiping with 90% isopropyl alcohol) all support equipment and microbial sampling material prior to being passed into the tent assembly environment to simulate the use of ETO, autoclaves, and mechanical passthroughs

available for controlling microbial contamination on items entering the SADL Assembly Room.

The results of these tests indicated that the additional decontamination constraints had little or no effect on microbial burden accumulation. The data did indicate, however, that the number of samples with greater than 10 organisms per sample was reduced which may mean that the translocation of contamination from the support equipment was being controlled.

3. UNTRAINED PERSONNEL STUDY

Two assemblies of the CMTM were performed in the SADL Assembly Room using untrained assemblers. It was found that the use of untrained assemblers had no detectable effect on microbial burden accumulation under conditions of this test. It should be pointed out that the personnel chosen as "untrained personnel" were from the Facility Operations Group within the SADL program and they were aware of the objectives of the test and had prior knowledge of the assembly procedures. These considerations may have had a significant effect on the results.

4. CLOTHING STUDY

The final study carried out as part of the SADL Program was to perform two assemblies of the CMTM in the SADL Assembly Room with reduced clothing requirements. For these assemblies, personnel were not required to wear face masks or booties except for the bio-technicians taking samples. Also, dacron wrist-length gloves were substituted for the surgical latex gloves previously used, and paper hair covers were substituted for the hoods.

The results, when compared to data from the other SADL Assembly Room assemblies, showed that the reduced clothing requirements did not significantly affect the microbial burden accumulation on the CMTM. This indicated that under the assembly procedures and constraints defined for the SADL program, the clothing requirement could have been reduced. However, it is important to fully understand the assembly conditions in order to evaluate the meaning of the results. First, the assembly procedures developed for the CMTM required very little assembly activity over the spacecraft hardware. Most of the work was carried out by having the assemblers

work under the aeroshell or work from the side, thereby allowing the vertical laminar flow air to have the minimum effect in controlling the dissemination of microorganisms generated by assembly activity. It seems clear that for assembly conditions in which assemblers may be above the hardware, that clothing constraints (such as boots) become extremely important.