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BIOLOGICAL MONITORING OF THE CAPSULE MECHANICAL TRAINING MODEL DURING ASSEMBLY IN THE STERILIZATION ASSEMBLY DEVELOPMENT LABORATORY

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SECTION I

INTRODUCTION

A major concern in planetary exploration for extraterrestrial life is the possibility of introducing Earth organisms to other members of the solar system via impacting spacecraft. To minimize this hazard, NASA establishes maximum contamination levels for planetary-bound vehicles. The task of developing spacecraft assembly procedures wherein the microbiological burden meets a planetary quarantine standard is a function of JPL's Sterilization Assembly Development Laboratory (SADL).

The SADL facility has a floor area of 20,000 sq ft which includes two laminar down-flow, Class 100 bio-clean rooms; one is 20 ft x 20 ft x 8 ft high for microbiological experimentation and the development of subsystem sterilization assembly techniques and the other is 30 ft x 40 ft x 35 ft high and is used as the capsule assembly area. [1] A microbiology laboratory, high bay receiving area, ETO/vacuum chamber, Operations Support Equipment area, terminal sterilization chamber, and personnel support areas comprise the balance of the facility.

The tool used for the development of minimum burden assembly procedures is the Capsule Mechanical Training Model (CMTM), a 14-ft diameter mechanical mock-up of the major subassemblies which may be expected to comprise a typical spacecraft capsule. It consists of an aeroshell in which is installed a payload section (bus) of the Mariner C type, eight electronic subassemblies (spares from the Ranger series), a 4-ft diameter impact limiter, a parachute canister, an unloaded deorbit moter, and a relay-link antenna. [1, 2]

A primary requisite for the development of low microbial burden assembly procedures is a rigorous monitoring program capable of providing estimates of the accumulation of microbiological burden on the hardware during assembly. The resulting data provides a basis for the selection of optimum assembly processes and the design and operation of support facilities.

The biological monitoring plan for the hardware assembly is integrated with the CMTM Assembly Procedure outline and the final CMTM Assembly Procedure is then prepared. The resulting document delineates the procedure for:

1) Preparation and decontamination of tools, hardware, and the assembly area.
2) CMTM assembly/disassembly operations.
3) Microbiological sampling.
4) Quality assurance points of inspection during CMTM assembly.

The assembly procedure is significantly impacted by the interim steps required to permit the biological sampling and, on occasion, is modified so that the assembly sequence will optimize the taking of samples rather than an efficient assembly process. In this sense, the biological monitoring plan exerts a control on the assembly process, since it must supply information relative to:

1) Initial biological contamination of the CMTM subassemblies.
2) The amount of burden added during the various assembly steps.
3) The total burden on the CMTM at the completion of assembly.

SECTION II

DEVELOPMENT OF THE BIOLOGICAL MONITORING PLAN

The purpose of the biological monitoring of the CMTM assembly operations is to estimate the biological burden existent on the CMTM hardware "as received," the burden buildup at various stages of assembly, the burden indigenous to mated areas made inaccessible by the assembly, and the total biological burden accumulated during the assembly. The biological monitoring plan is based on work performed during Phases I and II of the SADL Test and Operations Project by the AVCO Corporation under JPL Contract Number 951624.[3, 4]

The sampling technique used to estimate biological burden was to attach sterile 1 x 2 inch stainless steel strips (coupons) to the selected sampling sites on the CMTM hardware (Figure 1) and remove them at specified intervals. The procedure conformed with those set forth in Reference 5.

The monitoring plan was designed to establish:

1) where to sample,
2) the proportion of the total area of the CMTM that should be sampled, and
3) when to sample.

A. SELECTION OF SAMPLING AREAS

One of the major factors in the biological monitoring of spacecraft assembly is the distribution of burden relative to capsule configuration. To determine the area to be sampled, the surface areas of all CMTM subassemblies were divided into expected burden level zones, which are categorized as the following types:

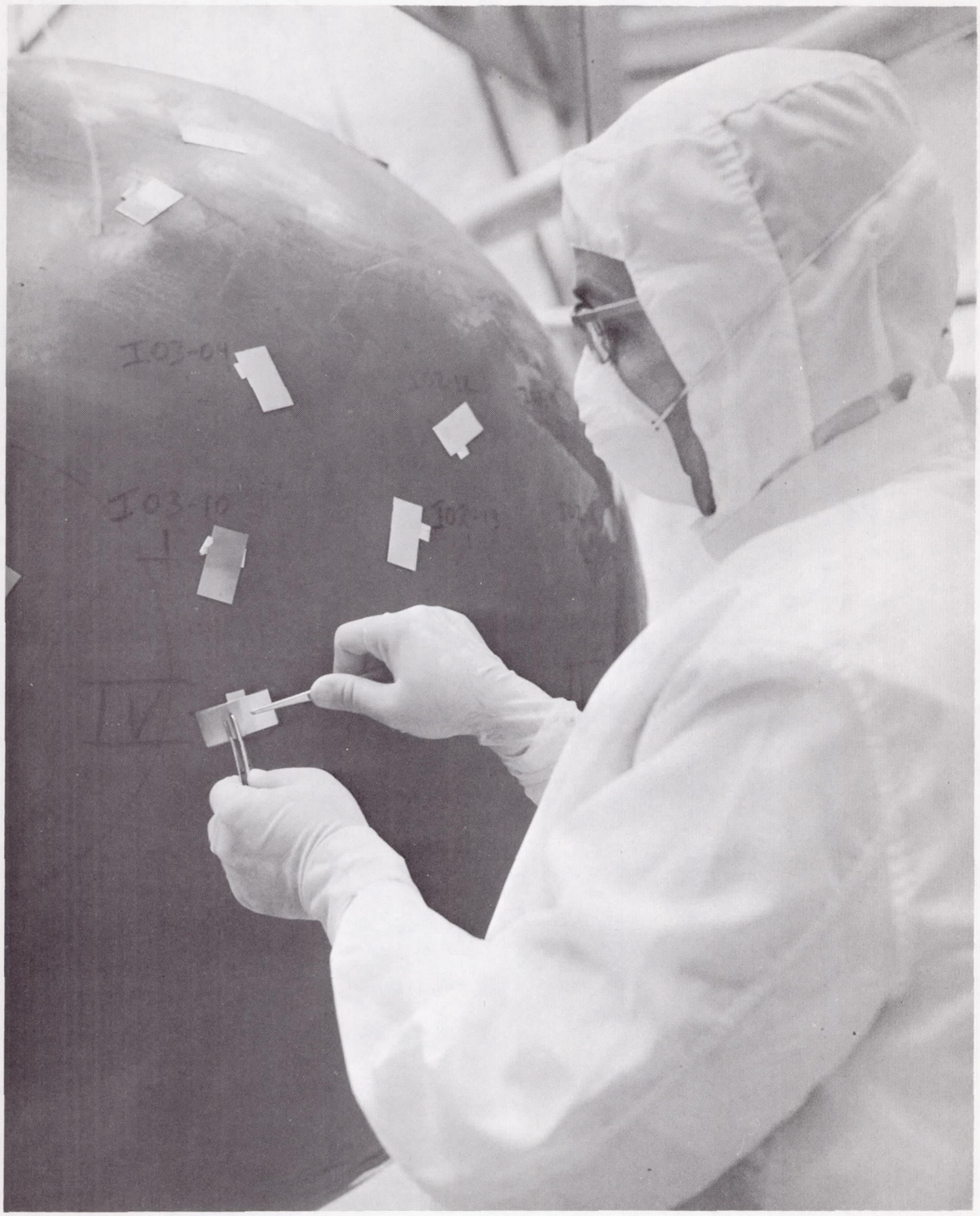

Figure 1. Bioengineer Placing Stainless Steel Sampling Coupons on Impact Limiter

Type

1) Handled areas: areas contacted by assemblers.
2) Direct fallout areas: areas which are horizontal upward facing flat surfaces, ridges, flanges, etc.
3) Indirect fallout areas: areas which are vertical or slanted surfaces.
4) Extremely low burden areas: areas which are downward facing or inside surfaces.

All zone types, except Type 1, were established by a simple visual examination of the physical configuration of the hardware. The Type 1 zones were identified by having the assemblers assemble the CMTM while wearing gloves dusted with fluorescent tracer powder. All subassemblies of the CMTM were periodically scanned with an ultraviolet lamp (during assembly) and all visible areas of fluorescence were mapped and photographed. Prior to each assembly operation, the wrists of the assemblers were scanned under an ultraviolet lamp by Quality Assurance (QA) to assure particle density for the purpose of providing an adequate fluorescent signature. The assembly procedures used in this test were followed strictly in subsequent tests thereby establishing, through repetition, a true index of contamination resulting from personnel contacts.

B. ALLOCATION OF SAMPLING SITES

The following conditions and definitions were established as the statistical basis for allocating the number of coupons to the different zone types:

1) The open CMTM surface areas at a given assembly stage were defined to be identifiable areas which were exposed and accessible within the normally defined operations of the assembly process.

2) The various segments of the open CMTM surface areas at a given assembly stage were categorized into one of the 4 expected burden level zones.

3) Independent burden estimates were to be derived relative to the total open assembly CMTM surface areas at 16 defined assembly stages.

4) The total number of coupons to be assayed in a given CMTM assembly in the SADL facility were limited to approximately 600.

5) A minimum of 3 completed CMTM assemblies were to be performed for each test condition.

The objective of the coupon allocation plan was to apportion the 600 coupons in such a manner that the precision of estimates would be maximized. The precision of burden estimates is greatly influenced by the sample size or number of coupons associated with a given estimate and the inherent variability of the burden distribution relative to the open CMTM surface areas sampled (the greater the variability, the less precise the estimate).

A stratified, randomized sampling procedure was selected for the coupon allocation plan. This procedure consisted of subdividing (zoning as described) the respective surface area population into subpopulations and selecting a given number of coupons (subsamples) from the respective subpopulations. The following formula was used to allot coupons to the identified zones of the subassemblies:

$$n_i = \frac{a_i \cdot s_i}{\sum_{i=1}^{k} a_i \cdot s_i} \cdot 600$$

where n_i = the number of coupons to be apportioned to the i^{th} subpopulation (surface areas of like zone at a given estimation point).

a_i = the area size of the i^{th} subpopulation.

s_i = the standard deviation (measure of burden variability of the i^{th} subpopulation).

k = the number of identified subpopulations.

i = the identifying number of a given subpopulation.

It is noted that the defined allocation formula requires known standard deviations (s_i) values. Estimates of these values were not available, therefore, relative variability factors were assigned on the basis of biological factors. This consisted of assigning a relative variability factor of 1 to 8 to the various subpopulation zone categories. Each Zone Type 1 (contact areas) was assigned a variability factor of 5 to 8, depending on how soon the subpopulation was sampled following contact by personnel; Zone Type 2 (direct fallout) was assigned a variability factor of 4; Zone Type 3 (indirect fallout) a factor of 2; and Zone Type 4 (minimal fallout) a factor of 1. Table 1 gives the distribution of coupons to the various zones and subassemblies.

In addition to the 600 coupons assigned by the coupon allocation plan, 700 dummy coupons were allocated for the following reasons:

1) Personnel assembling the CMTM could bias the results by their conscious or subconscious awareness of coupons.
2) In case some coupons fell from their sites or were damaged during assembly, other coupons were available for substitution.
3) Additional coupons may be desired for special studies.

A five-character code was used for identifying each coupon (sampling site) by subassembly and location. The first character identifies the subassembly site as follows:

A - aeroshell
B - band assembly clamp
C - sterilization canister
D - eight electronic subassemblies
I - impact limiter
M - deorbit motor
O - motor clamp
P - parachute canister
R - relay-link antenna
S - payload structure
U - umbilical cord

Table 1. CMTM Assembly Coupons

Subassembly	Number Zones	Number Pre-Assembly Coupons	Number Post-Assembly Coupons	Number Pre-Quarantine Coupons	Number Post-Quarantine Coupons	Total Coupons Taken	Total Coupons Available
Payload (S01-S14) (U01-U04)	18	41, (7), 6^{S}	9, 6^{S}	14, 6^{S}	14, 6^{S}	109	227
Chassis (D01-D10) (B01-B04)	14	30, (7)	15	13	13	78	282
Impact Limiter (I01-I05)	5	18, (3)	13	0	0	34	72
Aeroshell (A01-A16)	16	5, (17), 48^{C}	82	79	79	310	663
Parachute Canister (P01-P05)	5	8, (3)	2	0	0	13	37
Motor (M01-M04) (Ø01-Ø03)	8	6, (3) 2^{S}	6, 8^{S}	6, 5^{S}	6, 5^{S}	47	47
Relay Antenna (R01-R04)	4	6, (3)	12	7	7	35	35
Sterilization Canister (C01-C05)	5	3, (10)	8^{C}	0	0	21	64
TOTALS	75	169	161	130	130	647	1427

( ) = Wraps off coupons (controls)
S = Swab samples
C = Prior to final encapsulation

The next two characters are a two-digit number identifying the zone. The final two-digit number identifies the sample site number within a given zone. For example, sampling site A04-07 is the seventh coupon of the fourth zone of the aeroshell assembly (Figure 2).

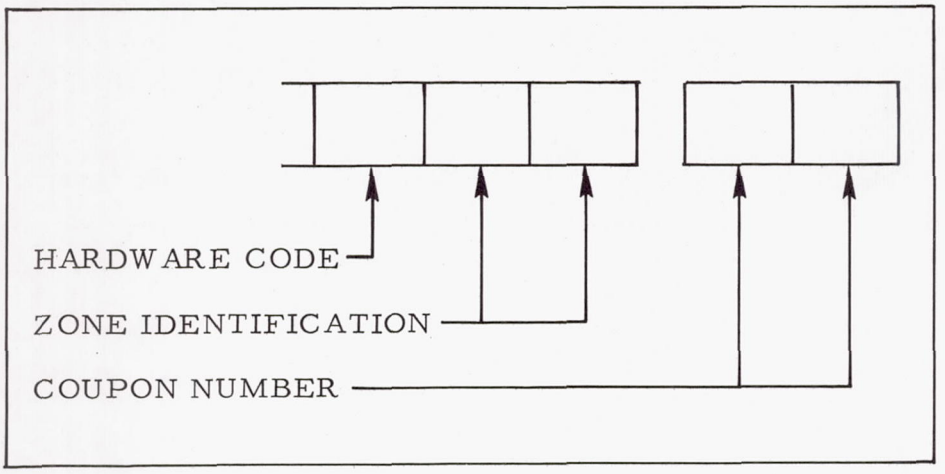

Figure 2. Identification of Coupons

Zones 1 and 2 of the aeroshell are the only zones which were assigned more than 100 coupons. An X as the second character indicates a coupon number between 100 and 199, and a Y indicates a coupon between 200 and 299. For example, the first coupon of Zone 1 of the aeroshell assembly is identified as A01-01, the one hundredth as AX1-00, and the two hundredth as AY1-00.

The allocation of sampling sites on the CMTM is illustrated in Figure 3. After all the sampling sites were selected, the sample numbers were electro-etched on the metal and the ink markings were removed to maintain surface characteristics.

C. SAMPLE REMOVAL SCHEDULE

The final step in the development of the monitoring plan was to determine a schedule for removing coupons. The schedule, which was developed,

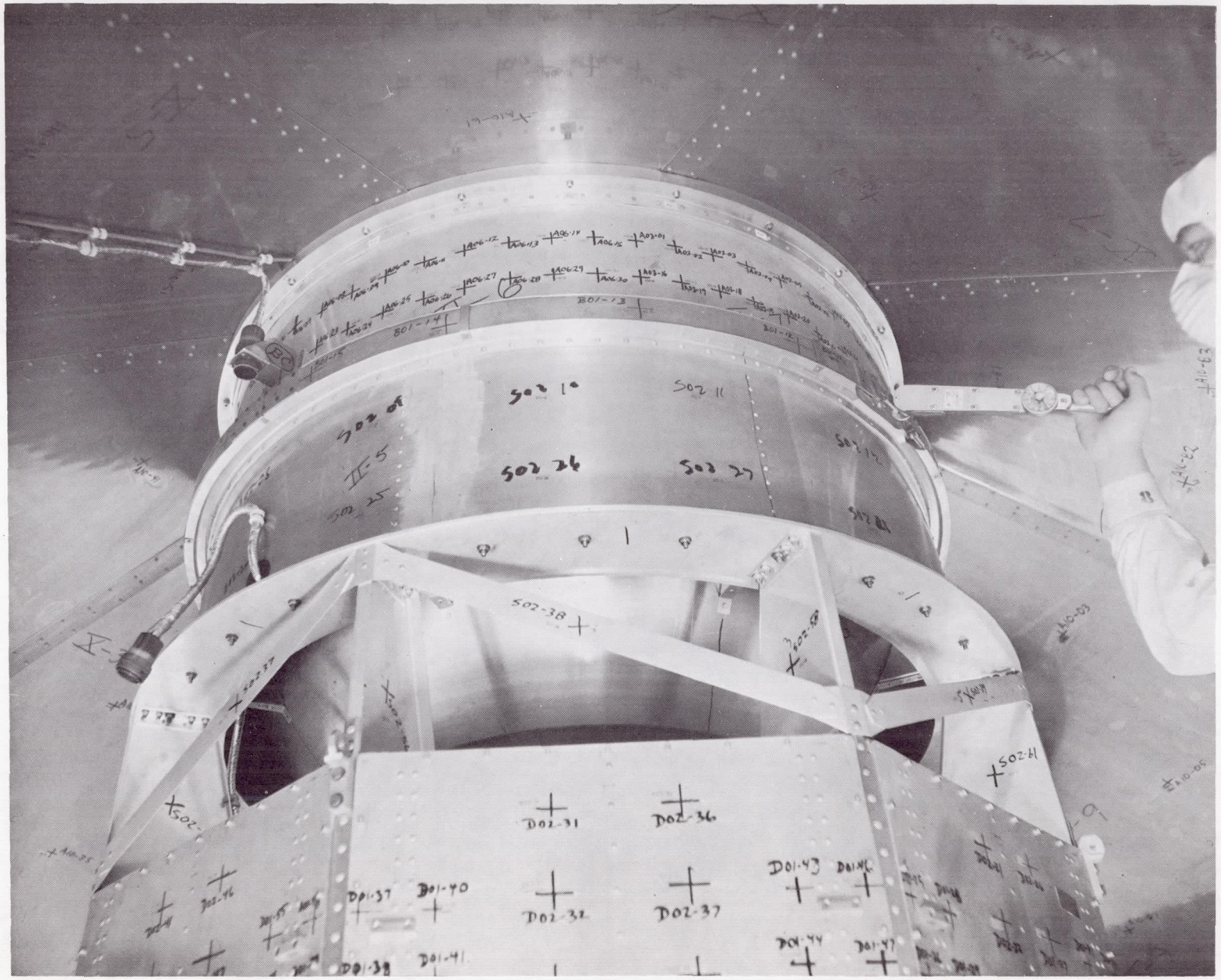

Figure 3. Payload Structure with Electronic Subassemblies Installed
Showing Surface Sampling Sites

identified the following ten steps in the assembly procedure at which coupons were to be removed:

1) Immediately prior to the assembly of any subassembly, to serve as a control for the identification of the initial burden.
2) Before and after the eight chassis were assembled to the 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.
8) Before and after a quarantine period. In this case, representative samples were removed from all the exposed surfaces of the entire 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.

The coupon removal schedule was developed to determine the burden accumulation associated with the installation of each subassembly as well as the total burden accumulation for the entire assembly of the CMTM. The sample coupons removed before each assembly step were taken from those areas which would become occluded, mated, or made inaccessible by the installation of the subassembly (Figure 4) and the coupons removed after each assembly step sampled the external surface of the installed subassembly. Therefore, to determine the burden accumulation associated with the installation of any subassembly, the sample data taken before installation was added to the post installation sample data.

Figure 4. Sampling of Impact Limiter Prior to Being Lowered onto Payload Structure Bioengineer is Placing Coupon into Sterile Jar Held by Assembly Technician.

The coupon removal schedule allowed two methods for determining the total burden accumulation associated with the entire assembly operation. The first method was to simply total the burden accumulation of all the subassemblies to get a grand total. The second method was to total the burden that was made inaccessible by the installation of the subassemblies (sample coupons removed before installation) and add to this total the burden determined by sampling the exposed surface of the assembled CMTM as described in Step 8 (before the quarantine period). The second method of determining total burden has the advantage of accounting for burden accumulation or dieoff on the exposed surfaces which had been sampled earlier in the assembly operation.

A sampling schedule for the CMTM assembly was developed from the coupon distribution tables which had been prepared and integrated with the Assembly Procedure.[6] Table 2 shows a composite of these tables. The following table is a typical sampling schedule for an assembly cycle.

DAY	ACTIVITY	SAMPLES
1	subassembly prep.	48
2	subassembly prep.	48
3	assembly	101
4	assembly	142
5	assembly	123
6	pre-quarantine	65
7	quarantine	0
8	quarantine	0
9	quarantine	0
10	post-quarantine	130
11	assembly (pre-encap.)	78
	TOTAL	735

Table 2. Coupon Distribution and Removal Schedule for CMTM Assembly

Zone	1	2	72	73	74	75	
Zone Identification	A01, X1, Y1	A02, X2	U01	U02	U03	U04	Totals
Pre-Chassis Assembly							48
Post-Chassis Assembly				2^s	4^s		25
Pre-Impact Limiter Assy							15
Post-Impact Limiter Assy							13
Pre-Aeroshell Assembly	⑥	③					42
Post-Aeroshell Assembly	30	14					87
Pre-Parachute Can.Assembly							19
Post-Parachute Assembly							2
Pre-Motor Assembly							17
Post-Motor Assembly							14
Pre-Relay Antenna Assembly							15
Post-Relay Antenna Assembly							12
Pre-Quarantine	30	14		3^s	3^s		130
Post-Quarantine	30	14		3^s	3^s		130
Umbilical Cord Assembly			3		6^s		22
Prior to Final Encapsulation	32	16					56
TOTAL	128	61	3	8^s	16^s	0	647
Total Available Sites	210	108	21	8^s	16^s	0	1442
Area (in.^{2})	25345	12700	255	116	795	20	249346 sq in.

Note: 1. Circled numbers denote coupons which were used for control and the number of post alcohol swab samplings.

2. The symbol, s, indicates swab sampling.

D. TEST CONSTRAINTS

Extraneous variables affecting the biological burden must be controlled during assembly or the monitoring plan is subverted. For this reason, test constraints were applied to the program which controlled the handling of hardware, described personnel clothing requirements, and defined QA control activities.

All subassemblies were wiped down with 90% isopropyl alcohol and, after the coupons were placed, the subassemblies were covered with a decontaminated (ETO) antistatic plastic cover until required in the assembly operation. Swab samples were taken from all subassemblies before and after alcohol wipedown to establish a baseline for initial burden.[7] All personnel associated with assembly operation, including the bio-personnel, underwent a defined dressing procedure and wore prescribed clothing.[8]

Quality Assurance was assigned the responsibility to monitor all activities which could effect extraneous contamination during CMTM assembly operations and the subsequent biological assay of the coupons.[9] Of particular value were the QA reports written after each CMTM assembly noting all deviations from the mechanical assembly and biological assay procedures and recording any abnormal activity associated with the assembly operation.

SECTION III

SUMMARY AND CONCLUSIONS

The monitoring plan described above had two underlying assumptions:

1) The level of burden accumulation would be affected by the angle of exposure of a spacecraft surface to the environment due to gravity and laminar flow and by personnel (assemblers) handling.
2) That the inherent variability of spacecraft surfaces (subpopulations) would require varying the sample size in order to get the same precision of burden estimates.

These two assumptions required that the total allotment of samples be distributed to the different spacecraft surfaces (subpopulations) in such a way that the statistical confidence would be the same for all burden estimates. In order to accomplish this, a relative variability factor based on biological factors was assigned for the allocation formula.

The results of a study evaluating the effect of different environments on burden accumulation during the assembly of the CMTM when this monitoring plan was used, showed that the assumption that the level of burden accumulation would be affected by the angle of exposure of surfaces to the environment was correct. These results are shown in Table 3. The results show, as in the case of the CMTM Assembly Procedures, that when a capsule is assembled in a fixed position, a sampling plan must include the ability to sample these surfaces (subpopulations) differently so that the same degree of precision of measurement of each subpopulation would be acquired. That is, varying the number of samples per unit of area for the different subpopulations for the purpose of obtaining the same precision of burden estimates is a requirement in order for the total burden on the assembled capsule to be acquired, since the total is the sum of each individual subpopulation. Varying the number of samples per unit of area of the different subpopulations will also emphasize the sampling of those zones of high burden levels which again results in an increase in the precision of the final total burden estimate.

Table 3. Effect of Assembly Environment and Surface Orientation on Biological Burden (Aerobes)

Assembly Environment	Handled Surface	Nonhandled Surfaces		
		Horizontal Upward	Vertical and Slanted	Horizontal Downward and Inside
High Bay				
Vegetative	1,086*	48,617	131	102
Spores	411	866	3	2
Tent				
Vegetative	1,673	35,709	111	34
Spores	24	40	2	2
SADL				
Vegetative	109	362	8	12
Spores	25	25	2	2

*Data for each assembly environment is the average number of organisms (weighted counts) resulting from three assemblies of the CMTM in each environment.

The requirement for dividing a capsule into subpopulations based on the angle of environmental exposure may not hold on other capsule assemblies where the assembly procedures require rotating the capsule during buildup. In the monitoring program for the Mariner V Spacecraft[10], it was found that throughout the majority of the program, no significant differences were noted between horizontal and vertical sample data. The conclusion of this study was that this anomaly was due to the continual rotation and tipping of the spacecraft during assembly. That is, a surface that was horizontal at one point in the assembly would be vertical later in the assembly. The requirement for zoning a capsule and the unequal distribution of sampling sites used in the development of this monitoring plan applies only when the capsule assembly procedure requires the capsule to be held in a fixed position for an extended period of time.

However, as the data indicates, those areas contacted by the assemblers would be expected to have a different burden level than the non contacted areas and would still require special sampling allocation unless the handled areas were assumed to be uniformly distributed over the entire surface of the spacecraft.

The coupon (sample) allocation formula used in the development of the monitoring plan for distribution of total samples to the various subpopulations required knowing the standard deviation (measure of variability) of the expected biological burden. Since the standard deviation values required were unknown, a weighting factor based on biological factors was substituted. A refinement of the variability factors for each subpopulation can now be made based on the data from the environment evaluation study using this monitoring plan.

The results of the above study also indicated that the allocation of coupons to the different subpopulations, using the allocation formula bias the results toward those zones with large areas. This conclusion was attributed to the fact that the allocation formula was used to distribute the samples to each individual zone. If a zone has a very large area, this value tends to nullify the effect of the selected variability factors.

SECTION IV

RECOMMENDATIONS

The following recommendations are made as the result of the development and use of this monitoring plan.

1. Biological monitoring plans for a spacecraft which will be assembled in a fixed position should include zoning of the surface of the spacecraft. Zoning should be based on the orientation of various parts of the spacecraft to the environment and on the degree of handling (contact) by assemblers. When monitoring a spacecraft which is periodically rotated during assembly, zoning of the surfaces with respect to the environment is less important than if the spacecraft is fixed, but contact areas should still be identified and special sampling of these areas should be considered.
2. The allocation of samples required to estimate the burden on each zone should be made by using the allocation formula first so that the number of samples per zone type may be determined and then reapplying the same formula to distribute the samples allocated to each zone to individual subpopulations within a given zone.
3. The total number of samples used should be rigorously examined. For a capsule the size of the CMTM (approximately 1,200 ft^2 of surface area), 600 coupons were felt to be inadequate.
4. The sample removal schedule and controls were found to be satisfactory and are recommended for use in conjunction with future spacecraft biological monitoring plans employing coupons and possibly for other sampling methods.
5. The use of coupons (1 x 2 inch stainless steel strips) presented a number of problems such as: a) they are hard to attach satisfactorily; b) the size of the sample is very small; c) they cannot be attached when the capsule is undergoing some environmental testing; and d) they cannot be attached to some components such as cable, etc. It is recommended that other sampling methods be used wherever possible.

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