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Technical Report 32-1207

The Microbiological Aspects of Sterilization Assembly Development Laboratories EASL and SADL

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Abstract

Industrial clean rooms are used to reduce particulate contamination upon hardware. The accompanying reduction in microbial contamination has led to the investigation of laminar flow clean rooms for future use in spacecraft assembly. Minimizing the microbial contamination on spacecraft during assembly will enhance any sterilization process used to satisfy planetary quarantine requirements.

Microbiological studies conducted in the Experimental Assembly and Sterilization Laboratory (EASL) indicated the feasibility of assembling spacecraft hardware in vertical laminar flow clean rooms.

The advancements in technology and procedural knowledge gained from the EASL are continually being integrated with efforts within the Sterilization Assembly and Development Laboratory (SADL). Space hardware to be assembled in the SADL will be microbiologically monitored. This information will aid in the formulation of a final terminal sterilization process for spacecraft hardware.

The Microbiological Aspects of Sterilization Assembly Development Laboratories EASL and SADL

I. Introduction

A planetary quarantine requirement has been established for orbiting and impacting space vehicles by the National Aeronautics and Space Administration (NASA), Ref. 1. These requirements have been established to preclude microbial contamination of the planets. In order to fulfill the goals set forth by the NASA it is necessary for some space flight hardware to be sterile. Several alternate methods could be used in the final production of a sterile capsule.

In the past, sterilization has been achieved through the use of radiation, chemical agents, dry and moist heat, and various other methods. Due to the complexity of materials within a space vehicle, many of the known sterilization processes cannot be considered for use because of their deleterious effect upon the materials. The method presently under consideration for use in the production of a sterile space capsule is dry heat.

It is important that the reliability of both the spacecraft and the mission be maximized; therefore, the terminal process should be the minimum necessary to achieve the desired probability of sterility. Since sterilization time/temperature relationships are partially dependent upon the total microbial load present upon the test item, it may be possible to minimize the final terminal sterilization process by reducing the microbial load and yet still confidently achieve the desired condition of sterility.

It has been demonstrated that conventional and laminar flow clean rooms reduce particulate contamination, and that with this reduction of particulate matter, the levels of microbial contamination may similarly be reduced, Refs. 2–4.

II. Experimental Assembly and Sterilization Laboratory Microbiological Studies

In 1965, a facility was constructed at the Jet Propulsion Laboratory (JPL) in Pasadena, California, which was a full experimental implementation of the requirements set forth within the NASA "Interim Requirements for Bioclean Facilities," Ref. 5. This facility, the Experimental Assembly and Sterilization Laboratory (EASL), was designed with the objective of developing the procedures and techniques necessary for the assembly and test of sterile spacecraft components and subsystems.

The EASL facility, as has been previously reported, possesses a 300-ft² Class-100 vertical laminar flow clean room, Ref. 6. An adjacent laminar flow area is provided for microbiological monitoring and assay work. The EASL also contains a horizontal-flow dressing room, locker and wash area in addition to an operational support equipment area. It has been demonstrated that small cordwood modules, consisting of electronic components, can be assembled within the Class-100 EASL assembly area with relatively low to no microbial contamination, Ref. 7. Table 1 contains the results from the microbial assay of six such modules. Three of the modules were fabricated within the EASL while the remaining three were assembled in a non-laminar flow spacecraft assembly area, Bldg. 18 of JPL. As can be seen in Table 1, no viable particles were recovered from those modules assembled in the EASL. In comparison, the three modules assembled within a non-laminar flow spacecraft assembly area in Bldg. 18 revealed varying degrees of surface contamination. All six modules were assembled under identical clothing and assembly constraints and were individually assayed according to the NASA "Standard Procedures for the Microbiological Examination of Space Hardware," Ref. 8.

Table 1. Results from the microbiological assay of modules assembled in the EASL and Bldg. 18

Module assayed	Number of microorganisms (surface)				
	Peptone water aliquot			Plated module	Total per module
	Aerobes	Anaerobes	Heat shocked		
No. 1 Bldg. 18	139	2	13	19	160
No. 2 Bldg. 18	46	1	9	3	50
No. 3 Bldg. 18	211	3	27	27	241
No. 4 EASL	0	0	0	0	0[a]
No. 5 EASL	0	0	0	0	0[a]
No. 6 EASL	0	0	0	0	0[a]

[a]Medium not bacteriostatic; confirmatory spore innoculum grew on all plates.

The next series of hardware assembly studies conducted within the EASL were designed to investigate the possibility of constructing larger and more complex subsystems with resultant low microbial burdens. A secondary objective of these studies was to evaluate the feasibility of using stainless-steel coupons as a method for estimating the microbial load upon subsystem assemblies.

The approach used to investigate these areas was to assemble, under monitored conditions, typical spacecraft electronic components and then to microbiologically assay the completed unit.

Figure 1 shows a typical completed unit, consisting of 37 different electronic components, a terminal board, wire harness and chassis. In one of the two modules assembled, stainless-steel coupons, as shown in Fig. 1, were substituted for several of the electronic components. The coupons were designed to simulate the exact surface area of the actual components they replaced. After completion of the assemblies, the individual components and coupons were removed and microbiologically assayed. These data indicated that subsystem hardware of this size and complexity can be assembled with microbial loads in the 10^2 to 10^3 range, and that stainless-steel coupons can be incorporated into subsystem hard-

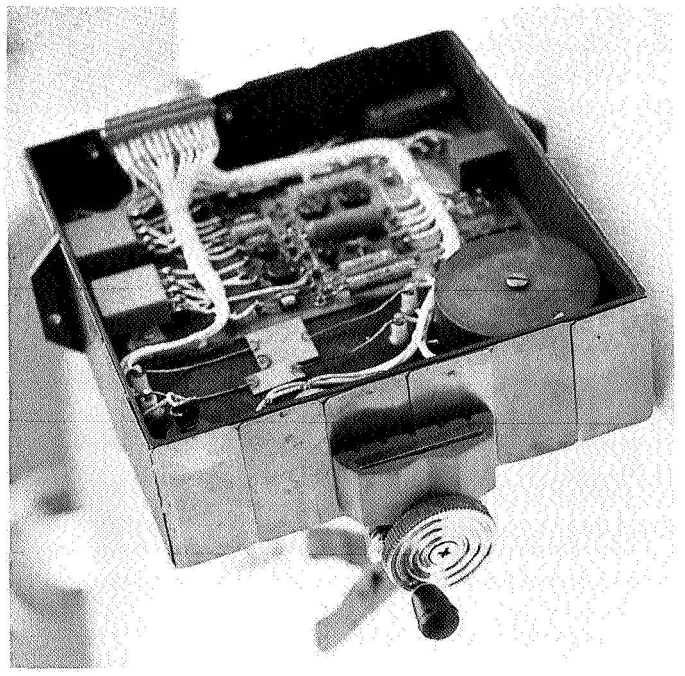

Fig. 1. Non-functional planar assembly (fabricated in the EASL) consisting of stainless-steel coupons and electronic components

ware providing a method for estimating the microbial burden upon the final assemblies.

Other associated tasks performed in the EASL have demonstrated that the clothing regimen imposed upon personnel within the assembly area controls the dissemination of microorganisms, Ref. 9. Tables 2–5 present data gathered during the assembly of hardware in the EASL vertical laminar flow area. The *sterile garments* portions of Tables 2–5 were obtained during an assembly series in which all personnel within the working area were garbed in sterile hoods, masks, coats and gloves. It can be seen that the microbial burden levels remained extremely low for all sampling, with exception of the clothing of the assemblers, which showed an increase in microbial contamination as the assembly progressed. The *street clothes* portions of the same tables were obtained during an identical assembly series, with the only introduced difference being that the personnel were garbed in street clothes. In almost all instances the microbial burden rates are increased. However, those areas not directly adjacent to the assembly working area remained relatively low and constant in burden levels. This may indicate that the inherent characteristics of laminar air flow tend to limit the dispersal of microorganisms by localization of contamination into the areas immediately adjacent to working personnel.

Table 2. Sampling of intramural air (Reyniers sampler, aerobic mesophilic viable particles/ft^3)

Time	Site 3	Site 10	Site 13
Sterile garments			
1/2 h before start	0.016	0	0
1st h	0	0.05	0
2nd h	0	0.03	0.016
3rd h	0	0	0.016
4th h after start	0	0	0
Street clothes			
1/2 h before start	0	0	0
1st h	0	0	0.016
2nd h	0	0	0.016
3rd h	0	0	0
4th h after start	0	0.03	0.03

In another effort designed to investigate the survival rates of microorganisms within vertical laminar air flow rooms, it was found that surface exposed microorganisms are rendered nonviable significantly faster within laminar air flow conditions than in a similar non-laminar air flow situation. The organisms were exposed on stainless steel coupons to the EASL vertical laminar air flow

Table 3. Sampling of biological burden beside assembler (stainless-steel strips, aerobic mesophilic viable particles/ft^2. Count is average of 3 strips)

Time	Left side of assembler			Right side of assembler		
	Spores	Vegetative cells	Total count	Spores	Vegetative cells	Total count
Sterile garments						
Controls	0	0	0	0	0	0
Start	0	0	0	0	8.6×10^1	8.6×10^1
1st h	0	0	0	0	0	0
3rd h	0	0	0	0	0	0
End of assembly	0	0	0	0	0	0
Street clothes						
Controls	0	0	0	0	0	0
Start	0	8.0×10^4	8.0×10^4	0	2.4×10^2	2.4×10^2
1st h	0	4.8×10^2	4.8×10^2	4.8×10^1	1.75×10^4	1.75×10^4
3rd h	0	1.4×10^4	1.4×10^4	0	8.0×10^3	8.0×10^3
End of assembly	2.9×10^2	2.9×10^5	2.9×10^5	0	4.0×10^2	4.0×10^2

Table 4. Sampling of clothing biological burden (Rodac plates, aerobic mesophilic viable particles/ft²)

Time	Left side of assembler						Right side of assembler					
	Smock sleeve	Trouser cuff	Smock chest	Smock hem	Smock shoulder	Glove	Smock sleeve	Trouser cuff	Smock chest	Smock hem	Smock shoulder	Glove
Sterile garments												
Start	0	0	0	0	0	0	0	0	0	0	0	0
End 1st h	0	0	76	0	0	38	228	76	0	38	38	0
End 4th h	912	72	38	266	38	0	2052	38	76	646	38	38
Street clothes												
Start[a]	0	0	0	0	0	0	0	0	0	0	0	0
End 1st h	228	456	114	912	152	3800	114	342	190	1370	304	3496
End 4th h	114	266	304	494	342	2660	684	342	608	494	418	1900

[a]Not sampled.

Table 5. Sampling of surface biological burden on work table (Rodac plates, aerobic mesophilic viable particles/ft². Counts are average of 5 Rodac plates)

Time	Left side of assembler		Right side of assembler	
	Site 1	Site 2	Site 3	Site 4
Sterile garments				
Controls	0	0	0	0
Start	0	0	0	0
1st h	0	0	0	0
3rd h	28	21	7	7
4th h (end of assembly)	4	7	7	0
Street clothes				
Controls	0	0	0	0
Start	0	0	0	0
1st h	0	0	0	0
3rd h	0	0	0	0
4th h (end of assembly)	0	0	0	0

environment and to a non-laminar air flow control condition of identical temperature and relative humidity (70°F and 45% RH). Figures 2 and 3 present the survivor curves for two of the microorganisms tested. No

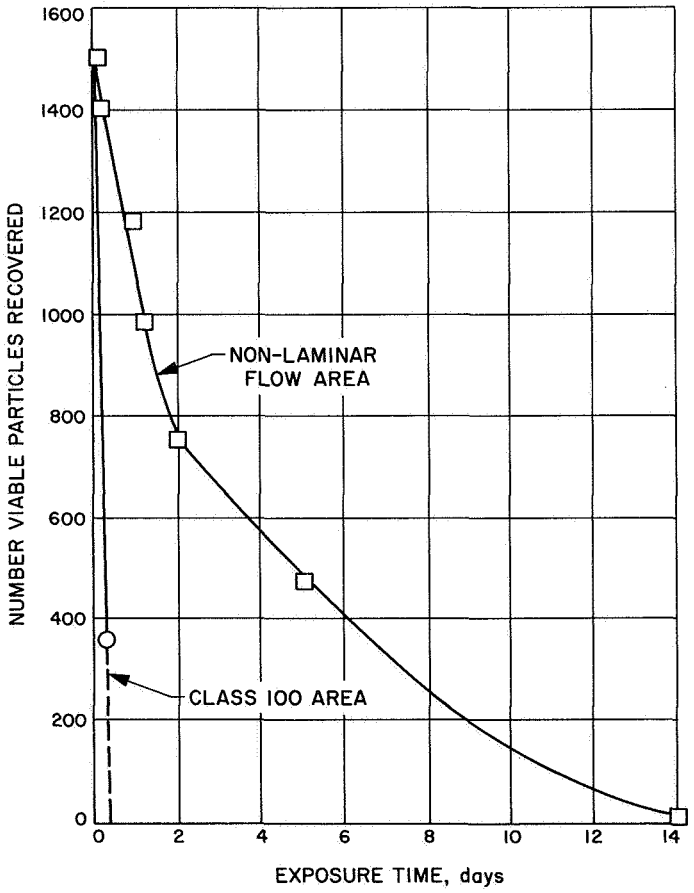

Fig. 2. Comparison of survival rates of *Staphylococcus aureus* FDA 209 exposed on stainless steel to the EASL and control situations

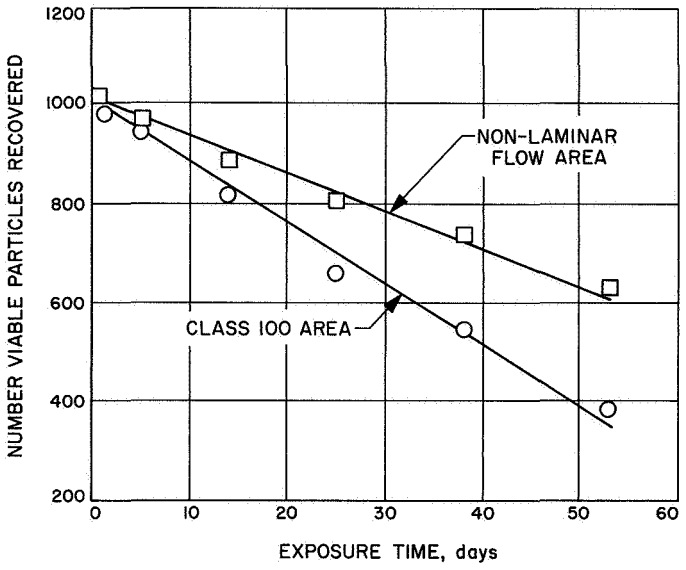

Fig. 3. Comparison of survival rates between spores of environmental isolate No. 48 (*Bacillus subtilis*) exposed on stainless steel to the EASL and control situations

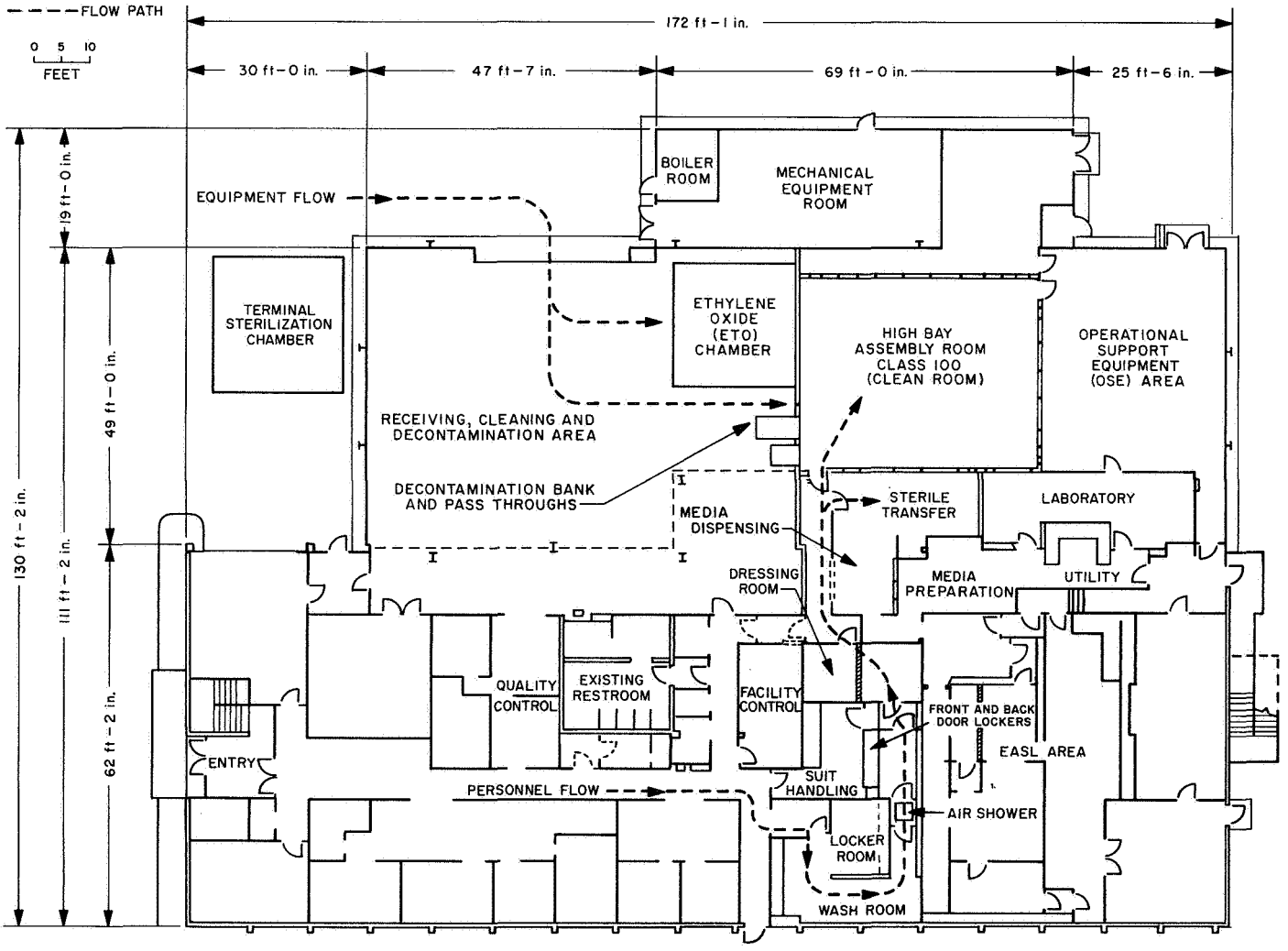

Fig. 4. Floor plan of the SADL facility

viable particles of *Staphylococcus aureus* FDA 209 were recovered after 6 h of exposure to the EASL vertical laminar air flow whereas in the non-laminar air flow control situation, viable particles were still recovered after 14 days (Fig. 2). Figure 3 presents the survivor curves for spores of an environmental isolate, later identified as a strain of *Bacillus subtilis*. The data indicated that these spores were more resistant to the effects of laminar air flow than were *Staphylococcus aureus* FDA 209 vegetative cells. Although viable spores were recovered in both the control and EASL laminar air flow areas after 53 days, the die-away rate for *Bacillus subtilis* spores was faster in the EASL situation.

These studies and other associated tasks suggest that laminar air flow clean rooms provide an environment, when exercised with proper constraints, that will enhance the opportunity of assembling space hardware with reduced levels of microbial contamination. The advancements in technology and procedural knowledge gained from the EASL are continually being integrated with efforts within the Sterilization Assembly and Development Laboratory.

III. Sterilization Assembly and Development Laboratory Studies

The Sterilization Assembly and Development Laboratory (SADL) was constructed at JPL to develop the techniques and methods necessary to perform the assembly, test, encapsulation and sterilization of a capsule that will meet engineering reliability and planetary quarantine requirements. The SADL, shown schematically in Fig. 4, consists of a 1200-ft^2 laminar down-flow clean room with a 35-ft-high bay area. Figure 5 shows this hardware assembly area, which possesses a specially designed 2700-ft^3 ethylene oxide (ETO) decontamination chamber. A dry-heat sterilization chamber of similar volume capability will be able to contain an entire capsule and its biological barrier during the dry-heat cycle necessary to achieve sterilization.

Adjacent to the hardware assembly area, a 1400-ft^2 microbiological assay laboratory will provide the space necessary for the implementation of monitoring, assay, and certification procedures. The total SADL facility occupies approximately 15,000 ft^2; this includes an operational support equipment area, a receiving and storage area, quality assurance area and facility control office in addition to the laminar down-flow assembly clean room and microbiological areas.

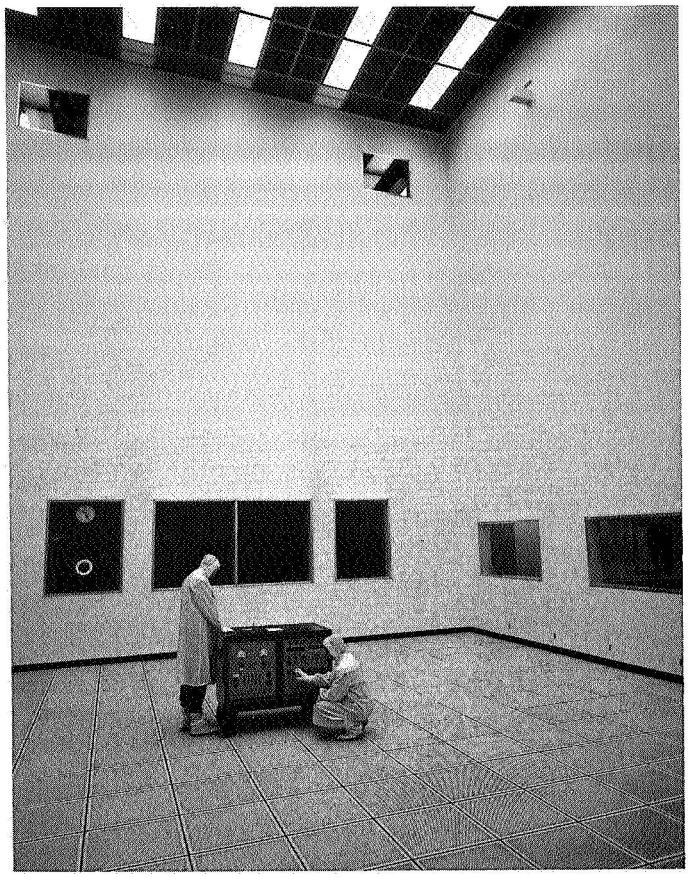

Fig. 5. SADL vertical laminar flow hardware assembly area

Space hardware to be assembled within the SADL clean room will be exposed to an ETO surface decontamination process prior to entrance into the hardware assembly area. The purpose of this decontamination process will be to reduce the microbial load upon the hardware surfaces. Our present hardware assembly efforts have principally utilized a capsule mechanical training model (CMTM), which can be seen in Fig. 6. This 14.5-ft-diam capsule model consists of eight basic subsystems: 1) impact limiter, 2) payload structure containing eight electronic chassis, 3) aeroshell, 4) parachute canister, 5) deorbit motor, 6) relay antenna, 7) umbilical cord, and 8) sterilization canister. These subsystems will be assembled into the final capsule model on which a biological load estimation will be made.

Microbiological monitoring of the CMTM assembly will be conducted through the use of stainless-steel assay

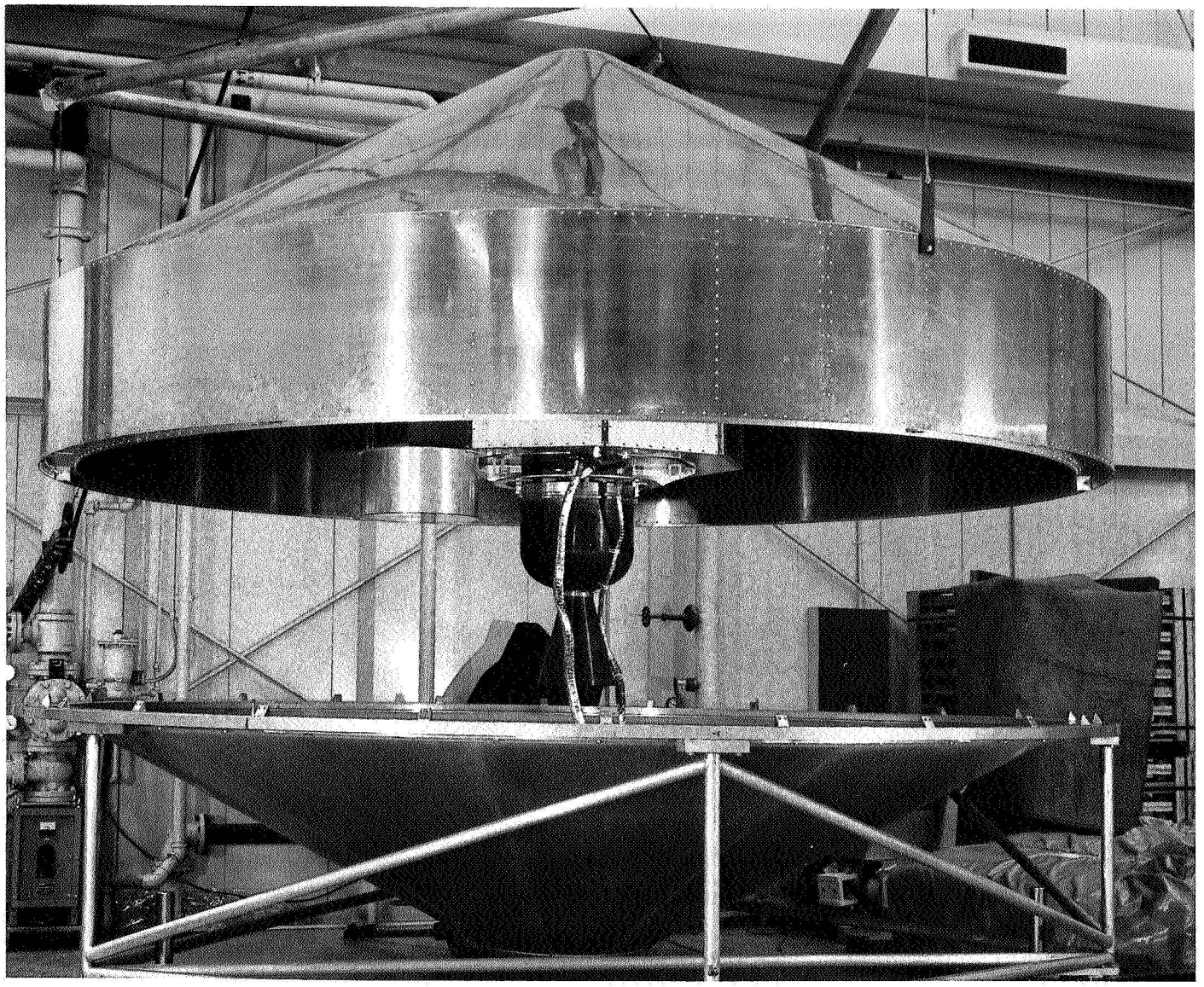

Fig. 6. Capsule mechanical training model (CMTM)

coupons, in addition to the routine microbiological methods of swabbing and Rodac assaying. These data will be used in the formulation of a microbial load estimation. This burden estimation coupled with an analysis of the thermal characteristics of the craft heating medium, in addition to knowledge of the heat resistance characteristics of the microorganisms, will aid in the formulation of a terminal dry-heat sterilization process for the CMTM.

The knowledge gained from EASL and SADL/CMTM operations will aid in the formulation of a final terminal sterilization process for spacecraft hardware.

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