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REPORT ON THE

"EFFECTIVENESS OF UTILIZING ASSAY COUPONS

FOR

BIOLOGICAL LOAD PREDICTION"

15 April 1967

Task 5.7

JPL CONTRACT 951624

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Prepared by

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SPACE SYSTEMS DIVISION
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for

JET PROPULSION LABORATORY

CALIFORNIA INSTITUTE OF TECHNOLOGY

PASADENA, CALIFORNIA

-NOTE-

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ABBREVIATIONS

°C	Degree Centigrade
D-Value	The time required to kill, at a given temperature, ninety percent of organisms exposed to the heat cycle
EASL	Experimental Assembly and Sterilization Laboratory
No.	Number
ml	Milliliter
min	Minute
TSA	Trypticase Soy Agar
C	Observed Count
DIL	Power of ten dilution factor
k	Index of experimental conditions
DF	Dilution factor
X	Count correction for dilution ($X = DF \cdot C$)
σ_{C_n}	Expected standard deviation in the n^{th} observed count
σ_n	Expected standard deviation corrected for dilution ($\sigma_n = DF \cdot \sigma_{C_n}$)
$\overline{X}_k$	Average corrected organism count for the k^{th} condition
T_k	Heating time associated with the k^{th} condition, defined so that $T_2 = 0$ corresponds to the time the sample attains temperature
σ_k	Expected error in $\overline{X}_k$
RMS	Root Mean Square

I. INTRODUCTION

The purpose of this study was to determine whether biological assay coupons could be realistically used to predict the biological burden on a subassembly.

To examine the validity of this technique, four subassemblies were built. The subassembly consisted of a printed circuit board on which was mounted resistors, capacitors, transistors, relays, modules, and diodes. All four subassemblies (boards) were built in the EASL vertical laminar flow assembly area.

Boards 1 and 2 were constructed by an assembler dressed in a sterile hood, smock, mask, and gloves. Boards 3 and 4 were constructed while the assembler was wearing street clothes. Boards 2 and 4 had bio-assay coupons attached to them; Boards 1 and 3 did not.

When the assembler mounted a component on the board, he would remove a bio-assay coupon (if attached), forward the coupon for biological assay, and then mount the component. This process would be continued until all the components were mounted. If coupons were not used, the assembler would mount the components in a specified sequence. (See Figures 1 and 2 for the numbers of components mounted, the sequence of mounting, and the location of the components and coupons.

The use of coupons for the predictions of biological burden has three fundamental problems: method of coupon attachment, area of attachment, and effectiveness of the technique.

A. METHODS OF COUPON ATTACHMENT

The methods of attachment examined were the use of (1) magnets, (2) adhesives, (3) cements, and (4) mechanical devices. During this investigation, it was found necessary to consider the following additional questions:

1. Does the method of attachment injure the surface to which the coupon is attached
2. Does the method of attachment securely anchor or fix the coupon
3. Could the coupons be easily removed after attachment
4. If cements or adhesives are used to attach the coupons, is there a residue left after the coupons have been removed
5. If a residue is left, can it be easily removed without damage to the surface to which the coupon was attached

B. AREAS OF ATTACHMENT

When selecting representative areas for coupon attachment, the selection of

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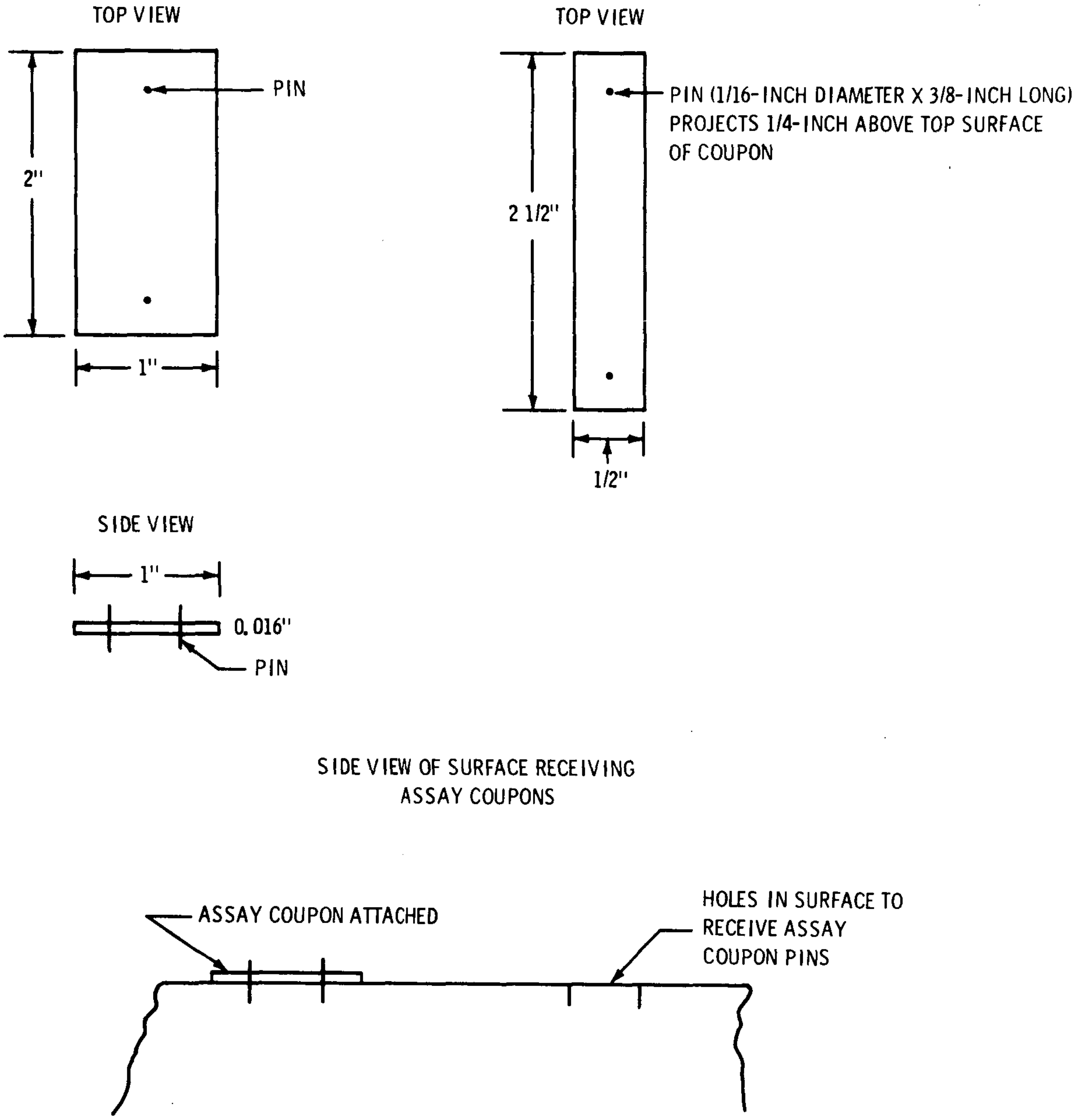

THESE FIGURES ARE NOT DRAWN TO SCALE.

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Figure 1. ASSAY COUPONS AND ATTACHMENT TECHNIQUE

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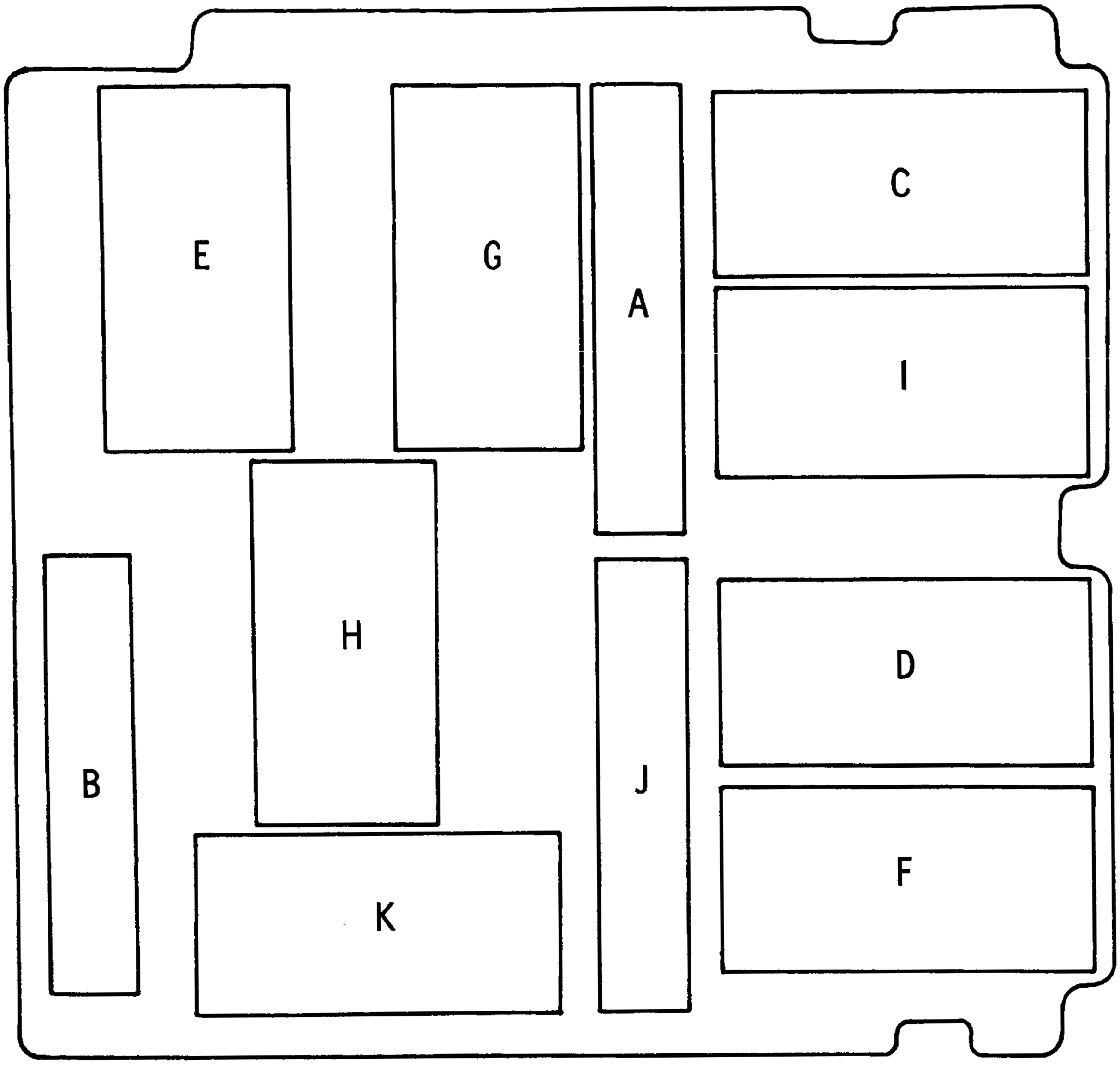

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Letters designate location of bio-assay coupons (Boards Nos. 2 and 4) and areas of component assembly on Board Nos. 1—4

Figure 2. LOCATION OF BIO-ASSAY COUPONS ON THE PRINTED CIRCUIT BOARDS

proper areas was of prime importance. The coupons should be placed in areas that receive representative contact with the environment and the various manipulations being performed. Improper location of the assay coupons could yield estimates of biological burden that could be inaccurate (too high or too low) due either to excessive or insufficient contact.

C. EFFECTIVENESS OF TECHNIQUE

When determining the effectiveness of the assay coupon technique in collection of the biological burden, it was necessary to compare the efficiency of the assay coupon technique with that of disassembling the subassembly in a horizontal laminar flow work station and bio-assaying each part separately. Ideally, a direct relationship of microbial population levels should be obtained when comparing the different techniques. If a proper relationship is not demonstrable, a correction factor might be obtained and applied.

II. TEST PLAN AND PROCEDURES

The experimental portion of this study was divided into two parts, A and B. Part A was concerned with (1) examining methods of attaching the assay coupons, (2) locating the assay coupons, and (3) determining the efficiency of the technique by comparing the biological burden obtained from coupons with that obtained when subassemblies were assayed by removing and assaying the components. Part B was concerned with the estimation of the biological burden of the capsule mechanical training model (CMTM). The CMTM is a mock-up of a capsule lander approximately 16 feet in diameter.

A. PART A: METHODS OF COUPON ATTACHMENT, LOCATION OF COUPONS, AND DETERMINATION OF COUPON EFFICIENCY

1. Bio-assay Coupons

   The bio-assay coupons were fabricated from stainless steel and were made in two sizes: 1 inch x 2 inches and 2-1/2 inches x 1/2 inch, both 0.016-inch thick.

2. Evaluation of Methods of Coupon Attachment

   a. Mechanical Attachment Techniques

   1) Bolts, clips, studs, channels, magnets, and pins were examined as means of attaching the bio-assay coupons to the hardware.

   2) Necessary holes were drilled in the subassemblies, and the required hardware for holding the coupons attached to the subassembly.

   3) After the coupons were attached, they were checked for firmness of attachment and ease of removal.

   4) The subassemblies (printed circuit board) and attached coupons were subjected to three cycles each of dry heat (175°C, 2 hours) and ETO decontamination (500 ± 50 mg ETO-F12, 6 hours).

   5) After the decontamination cycles, the coupons and subassemblies were examined for possible degradation of material and binding of coupons to the boards.

   6) See Figure 1 for an illustration of the pin technique of attaching the bio-coupons.

   b. Adhesives and Cements for Attachment of Coupons

   1) Cellulose cement, contact cement, epoxy cement, rubber cement, starch paste, Clearseal (a silicone rubber cement), cellophane tape, and double surface masking tape were evaluated.

2) After the coupons were attached to the printed circuit boards, the coupons were checked for firmness of attachment and ease of removal.

3) The coupons and printed circuit boards were then subjected to three cycles each of ETO and dry heat decontamination.

4) After the decontamination cycles, the coupons were re-examined for firmness of attachment and ease of removal and tested for sterility. Sterility testing was performed by (1) culturing the coupons in fluid thioglycollate medium at 37°C for 72 hours and (2) checking for growth.

5) The printed curcuit boards were visually examined for residual adhesive or cement after the coupons were removed.

6) The printed circuit boards were also examined for possible damage due to the action of the cements or adhesives.

3. <u>Areas of Coupon Attachment and Effectiveness of the Coupons as a Means of Determining Biological Burden</u>

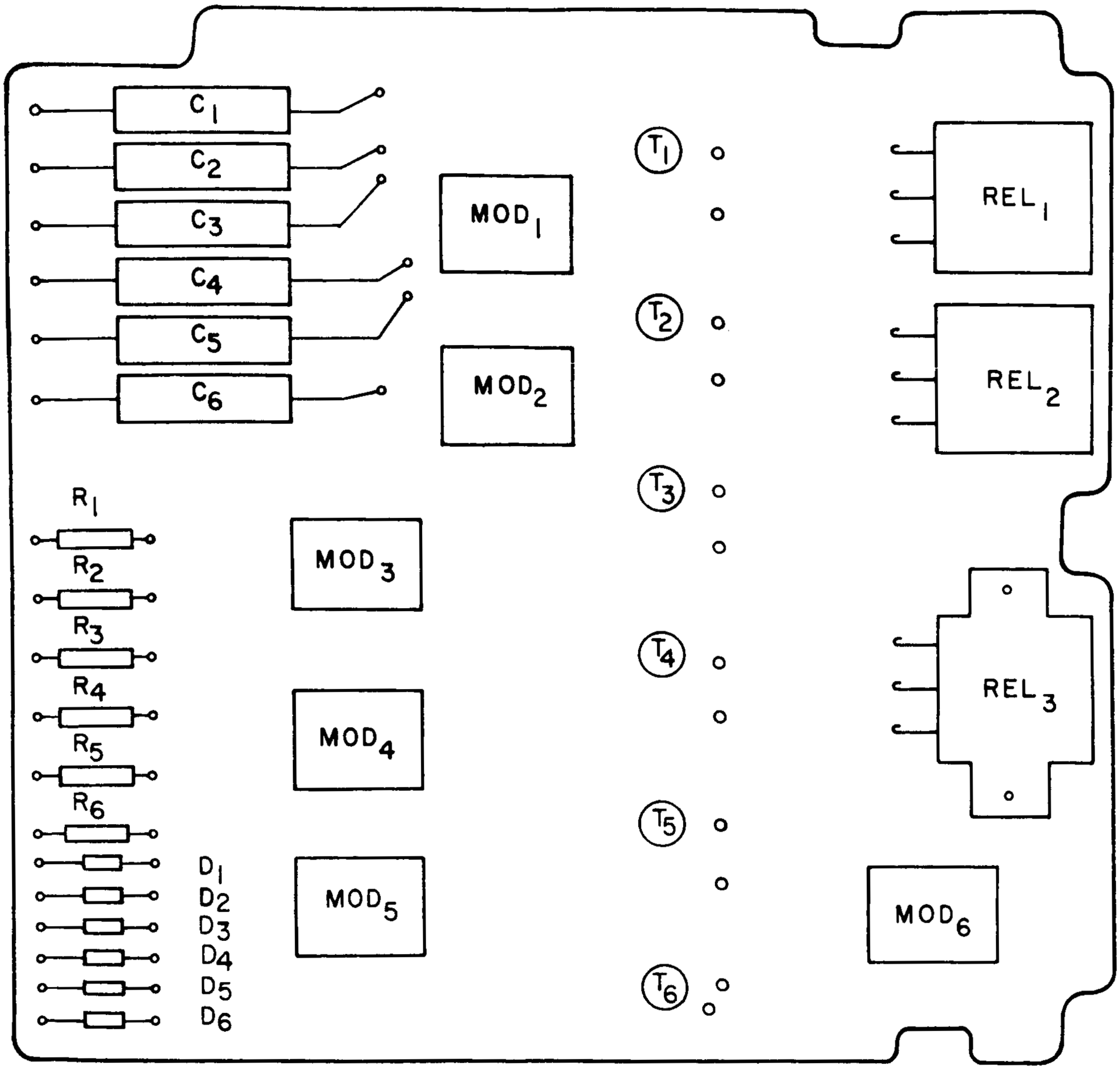
a) In order to determine the proper location of the biological assay coupons and their efficiency in determining biological burden, printed circuit board assemblies were built with attached assay coupons.

1) See Figure 2 for the locations of the bio-assay coupons, and Figure 3 for the types of components and their locations on the printed circuit board.

2) Two of the boards were built in the EASL facility with the assembler and bio-assayer clothed in sterile smocks, hoods, gloves, and masks. Two other boards were also built in the EASL, while the assembler was dressed in street clothes and the bio-assayer was dressed in the previously described sterile garments. In all, four boards were constructed, two with coupons and two without coupons:

1. Board 1 was built first in EASL, without coupons, while the assembler and bio-assayer were clothed in sterile protective clothing.

2. Board 2 was built second in EASL, with attached coupons, while the assembler and bio-assayer were clothed in sterile protective clothing.



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C	=	Capacitors	(six were used)	REL	=	Relays	(three were used)
D	=	Diodes	(six were used)	T	=	Transistors	(six were used)
MOD	=	Modules	(six were used)	R	=	Resistors	(six were used)

Figure 3. LOCATION AND TYPES OF COMPONENTS USED TO CONSTRUCT PRINTED CIRCUIT BOARD ASSEMBLIES 1 TO 4

3. Board 3 was built third in EASL, without coupons, while the assembler was dressed in street clothes and the bio-assayer was dressed in sterile protective garments as previously described.

4. Board 4 was built last in EASL, with attached coupons, while the assembler was dressed in street clothes, and the bio-assayer was protectively clothed.

b) Construction of the printed circuit board assemblies

1) The following procedure was used to assemble and mount the components on the printed circuit boards: diodes, transistors, modules, resistors, capacitors, and relays were mounted, using approved techniques, on the circuit board. The same sequence was used for all four boards.

2) As the components were mounted in a specific area, the assay coupons (when present) were removed and assayed for aerobic mesophilic organisms. See Figures 2 and 3 for the location of the assay coupons, the location of the construction areas, and the components to be mounted. Assembly of all the boards was started in A areas of the biocoupons, then continued to B, C, . . ., and, finally, the K, Areas:

1. All components, tools, and supplies used in the construction process were decontaminated by exposure to either dry heat or ETO decontamination process.

2. Air sampling using Reynier Samplers was performed during the assembly of Boards 1 to 4, using the procedures described in the NASA Preliminary Standard Procedures for the Microbiological Examination of Space Hardware.[1]

3. Biological burden on the assemblers clothing was determined using Rodac plates.

4. Biological burden to the immediate left and right sides of the assembler was determined using stainless steel fall out strips.[1]

5. Biological burden on the surface of the work table where Boards 1 to 4 were built was determined using Rodac plates[1].

6. See Figure 4 for the location of samplers, areas sampled, and the position of the assembler.

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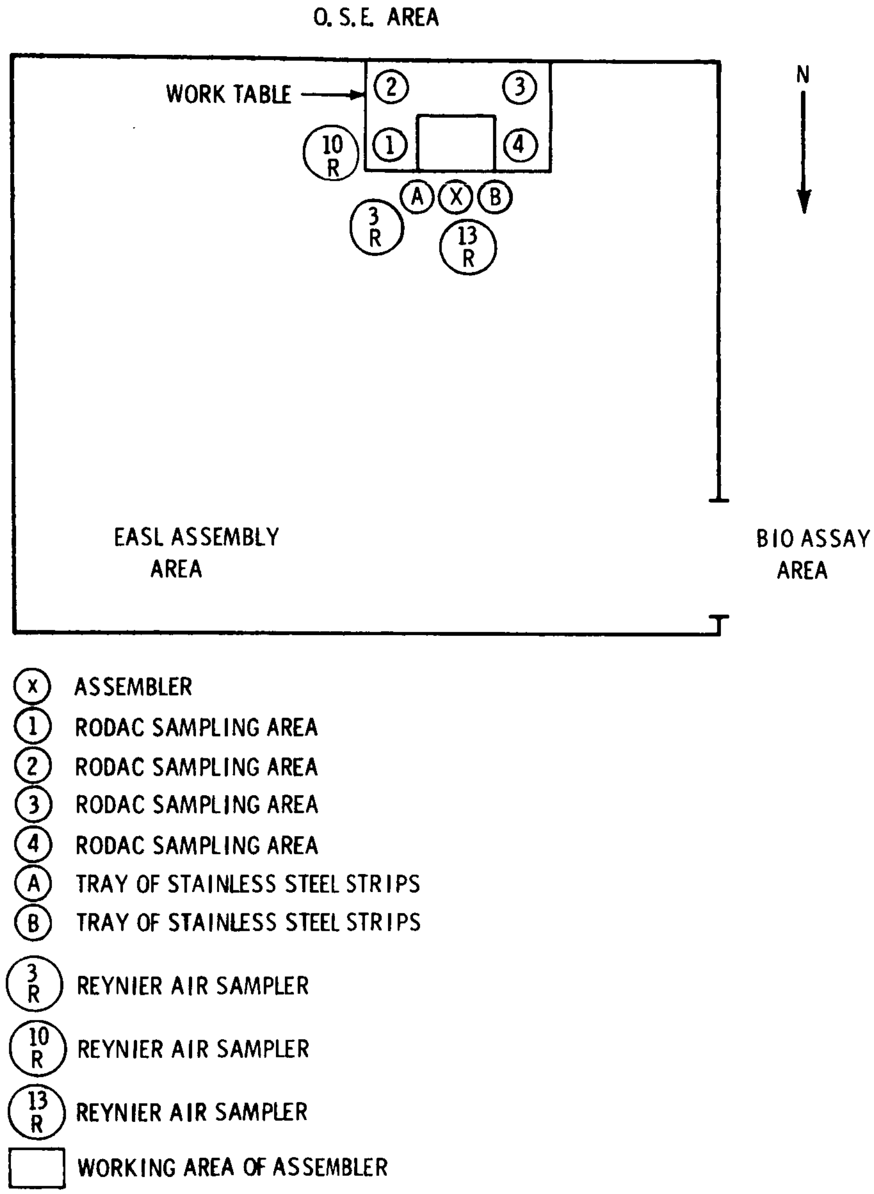

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Figure 4. SAMPLING AREAS FOR CLOTHING AND COUPON STUDY

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7. During the assembly of Boards 1 to 4 in the EASL assembly area, the facility temperature, pressure, hunidity, air velocity, and microbial burden (air and surface) were maintained at the levels prescribed in JPL specification XOY-50543-GEN.[2]

3) Correlation of the assembly process and microbial populations: A log was kept listing the assembly processes, the areas on which they were carried out,and the population levels found on each component.(See Table 1.)

4) Disassembly and assay of individual components to determine biological burden: The following general procedure was used: After the assemblies were completed the components were aseptically removed in a horizontal laminar flow work station and biologically assayed for aerobic mesophilic microorganisms--as described in the NASA Preliminary Standard Procedure for The Microbiological Examination of Space Hardware.[1]

5) A quality assurance log was kept listing all procedures carried our during the construction of Boards 1 to 4 in the EASL facility, violations of technique, and other pertinent information. See the report on Task 5., Clothing Study, Appendix A, for the complete QA log.

c) Comparison of Biological Burden Levels Obtained Using the Coupons Technique and the Disassembly Technique

Total populations and area populations obtained by the two procedures were compared; attempts were made to correlate burden levels with certain components. A study of the correlation between the two assay techniques was made.

B. PART B: BIOLOGICAL BURDEN ESTIMATES ON THE CMTM HARDWARE

1. Biological Burden Estimate

A biological burden estimate was made on the CMTM hardware subsystems and structure using the swabbing technique as described in Reference 1.

2. Areas Swabbed

Areas swabbed were the following:

a) Impact Limiter (3 areas swabbed)
b) Antenna relay canister (3 areas swabbed)
c) Parachute canister (3 areas swabbed)
d) Payload structures (4 areas swabbed)

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TABLE I

THE BIOLOGICAL BURDEN ON COMPONENTS, BOARDS 1 TO 4, AND THE MANIPULATIONS NEEDED TO MOUNT THEM

Component	Biological Burden	Manipulations
Board 1		
Capacitor (6)	(0) (0) (48*) (3*) (330*) (0) = 380* V.P.	Bend leads, mount on terminals, solder, cut excess lead.
Diodes (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	Bend leads, mount on terminals, solder, cut excess lead.
Modules (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	Modules cemented on board.
Relays (3)	(0) (0) (360)* = 360* V.P.	Two Relays cemented on board, one Relay on board.
Resistors (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	Bend leads, mount on terminals, solder, cut excess lead.
Transistors (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	Bend leads, mount on terminals, solder, cut excess lead.
Board 2		
Capacitors (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	Same as Board 1
Diodes (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	
Modules (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	
Relays (3)	(0) (0) (0) (0) (0) (0) = 0 V.P.	
Resistors (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	
Transistors (6)	(0) (0) (3) (0) (0) (0) = 3 V.P.	
Board 3		
Capacitors (6)	(0) (3) (201) (0) (61) (0) = 265 V.P.	Same as Board 1
Diodes (6)	(0) (4) (1) (0) (0) (39) = 44 V.P.	
Modules (6)	(25) (102) (150) (0) (0) (366)= 646 V.P.	
Relays (3)	(0) (0) (0) = 0 V.P.	
Resistors (6)	(0) (1) (0) (0) (0) (1) = 2 V.P.	
Transistors (6)	(0) (0) (0) (0) (1) (0) = 1 V.P.	
Board 4		
Capacitors (6)	(0) (54) (0) (3) (0) (0) = 57 V.P.	Same as Board 1
Diodes (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	
Modules (6)	(0) (2) (131) (0) (0) (0) = 133 V.P.	
Relays (3)	(1) (1) (1) = 3 V.P.	
Resistors (6)	(1) (0) (0) (0) (0) (0) = 1 V.P.	
Transistors (6)	(0) (0) (0) (0) (0) (0) = 0 V.P.	

a. The numbers in the component column indicate the number of components used; i.e., diodes (6).

b. The biological burden column lists the number of viable particles per component and the total for the components.

c. The * indicates abnormal situations that yielded high burden for the particular component. See Discussion for an explanation.

e) Electronics chassis (5 areas swabbed)
f) Sterilization canister (5 areas swabbed)
g) De-orbit motor (5 areas swabbed)

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III. RESULTS

A. EVALUATION OF TECHNIQUES FOR ATTACHED BIO-ASSAY COUPONS TO HARDWARE, SUBSYSTEMS, AND OTHER AREAS TO BE ASSAYED

1. Mechanical Means of Attachment

a. Assay strips held in place by magnets appeared at first to be promising but,upon further examination of experimental parameters, were rejected for three reasons:

1) Possible effect of magnets on instrument packages and electronic gear were unacceptable.
2) Assay coupons were not firmly fastened into position by the magnets.
3) Not all the material or areas to have coupons would hold magnets (plastic surfaces, etc.).

b. Assay strips held in position with channels. This technique was not acceptable for two reasons:

1) The channels for holding the coupons could not be placed in all the areas or subsystems that were to be monitored.
2) The channels would not be acceptable for flight hardware

c. Assay strips held in position with bolts. This approach was rejected for two reasons:

1) The need for too many holes to be drilled in and through the hardware.
2) Bolts tended (in some cases) to seize up when submitted to the dry heat decontamination cycle.

d. Assay strips held in position with clips. Clips were not acceptable due to the difficulty in attaching them to the surface to be assayed without producing damage to the surface.

e. Assay coupons held in positon by means of pins. (See Figure 1 for a diagram of this coupon with pin fasteners.) This technique was not acceptable for three reasons:

1) The protruding heads of the pins could puncture the assembly worker's protective gloves.
2) The assembler or worker might subconsciously avoid the coupon areas due to the protruding pins.

3) Holes have to be drilled into the hardware in order to mount the coupons.

2. <u>Cements or Adhesives Used to Mount the Assay Coupons</u>

a. Cellulose cement was found unacceptable:

1) After the ETO and dry heat decontamination cycles, the coupons were difficult to remove.

2) A residue of cement was left after the coupon was removed.

b. Clearseal (a silicone rubber cement) was found unacceptable as a means of attaching the coupons:

1) After the ETO and dry heat decontamination cycles, the coupons were difficult to remove.

2) A residue of silicone rubber was left after the coupon was removed

c. Rubber cement was found to be unacceptable as a means of attaching the coupons:

1) After the ETO and dry heat decontamination cycles, the coupons were difficult to remove.

2) A residue of rubber was left after the coupons were removed.

d. Epoxy cement was found to be unacceptable as a means of attaching the coupons:

1) After the ETO and dry heat decontamination cycles, the coupons were difficult to remove.

2) A residue of epoxy cement was left after the coupons were removed.

e. Starch paste was found to be unsatisfactory as a means of attaching the coupons:

1) The coupons were not held in position after the ETO and dry heat cycle.

2) A residue of paste remained after the coupons were removed.

f. Cellophane tape was found to be unsatisfactory as a means of attaching the coupons: After the ETO and dry heat decontamination cycle, coupons did not hold firmly to the surfaces to which they were attached.

g. Double-surface masking tape was found to be a satisfactory means of attaching the bio-assay coupons:

1) The coupons remained firmly attached in place.

2) The coupons could readily be removed after the ETO and dry heat decontamination cycles.

3) No residue of adhesive was observed after the coupons were removed.

4) Sterility testing of the coupons and the surfaces to which they were attached showed no growth in thioglycollate broth at 37°C after 72 hours.

5) Coupons were easily attached in a minimum of time without damage to metal and plastic surfaces and did not require holes, soldering, or associated procedures to anchor them into position.

B. EVALUATION OF THE BIOLOGICAL BURDEN ON THE COMPONENTS AND BIO-ASSAY COUPONS OF PRINTED CIRCUIT BOARDS 1 TO 4

Figure 5 lists the biological burdens found in the various components and on the top and bottom surfaces of the printed circuit boards used for the construction of Board 1.

Figure 6 lists the biological burden found on the various components and on the top and bottom surfaces of the printed curcuit boards used for the construction of Board 2.

Figure 7 lists the biological burden found on the various componenets and on the top and bottom surfaces of the printed curcuit boards used for the construction of Board 3.

Figure 8 lists the biological burden found on the various components and on the top and bottom surfaces of the printed curcuit bands used for the construction of Board 4.

Figures 9 and 10 list the position and the biological burden recovered from coupons <u>A</u> to <u>K</u> on Boards 2

Table II lists the niological burden found on the various componenets and on the top and bottom surfaces of the printed circuit used for the construction of Boards 1 to 4.

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TABLE II

BIOLOGICAL BURDEN ON COMPONENTS AND SURFACES OF BOARDS 1 TO 4

		Assembler in Sterile Garments		Assembler in Street Clothes	
		Board 1	Board 2	Board 3	Board 4
Capacitor	1	0	0	0	0
	2	0	0	3	54
	3	48*	0	201	0
	4	3	0	0	3
	5	330*	0	61	0
	6	0	0	0	0
Diode	1	0	0	0	0
	2	0	0	4	0
	3	0	0	1	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	39	0
Module	1	0	0	25	0
	2	0	0	102	2
	3	0	0	150	131
	4	0	0	0	0
	5	0	0	0	0
	6	1	0	369	0
Relay	1	0	0	0	1
	2	0	0	0	1
	3	360*	0	0	1
Resistor	1	0	0	0	1
	2	0	0	1	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	1	0
Transistor	1	0	0	0	0
	2	0	0	0	0
	3	0	3	0	0
	4	0	0	0	0
	5	0	0	1	0
	6	0	0	0	0
Surfaces					
Top		0	0	58	3
Bottom		0	0	49	1
Total Valves		4 Viable Particles	3 Viable Particles	1065 Viable Particles	197 Viable Particles

*See Discussion for explanation of values. (They are considered not valid.)

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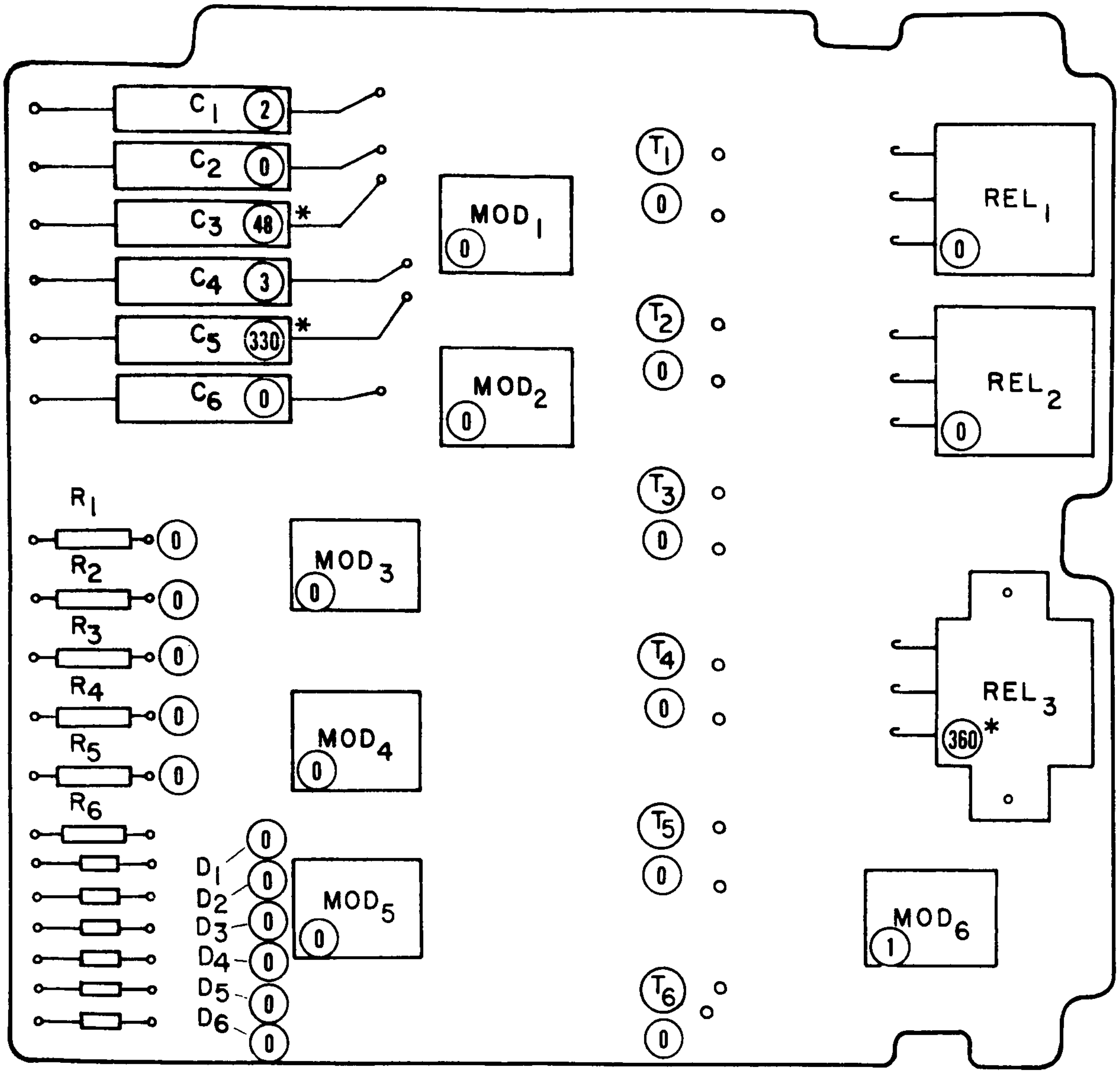

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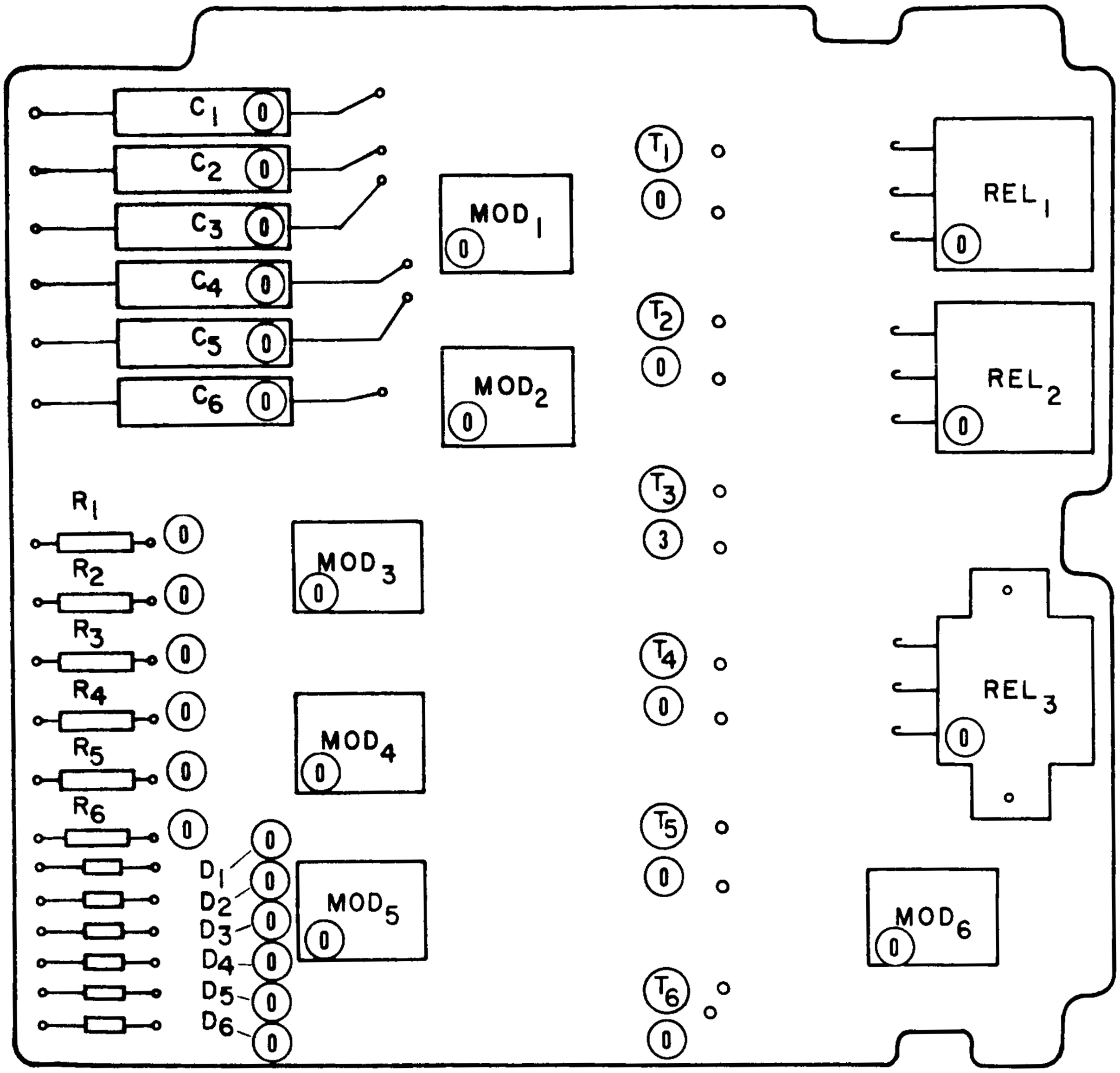
Numbers in circles indicate burden on components
Swab 1 to surface of board = 0
Swab 2 bottom surface of board = 0

C	=	Capacitor (six were used)	REL	=	Relay	(three were used)
D	=	Diode (six were used)	T	=	Transistor	(six were used)
MOD	=	Module (six were used)	R	=	Resistors	(six were used)

*See discussion for an explanation of these values.

Figure 5. BIOLOGICAL BURDEN ON COMPONENTS OF BOARD NO. 1 (COMPONENTS ONLY) AEROBIC MESOPHILIC MICROORGANISMS -- ASSEMBLER CLOTHED IN STERILE GARMENTS



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Numbers in circles indicate burden on component
Swab 1 top surface of board = 0
Swab 2 bottom surface of board = 0

C	= Capacitor	(six were used)	REL	= Relay	(three were used)
D	= Diode	(six were used)	T	= Transistor	(six were used)
MOD	= Module	(six were used)	R	= Resistors	(six were used)

Figure 6. BIOLOGICAL BURDEN ON COMPONENTS OF BOARD NO. 2 (COMPONENTS AND COUPONS) AEROBIC MESOPHILIC MICROORGANISMS -- ASSEMBLER CLOTHED IN STERILE GARMENTS

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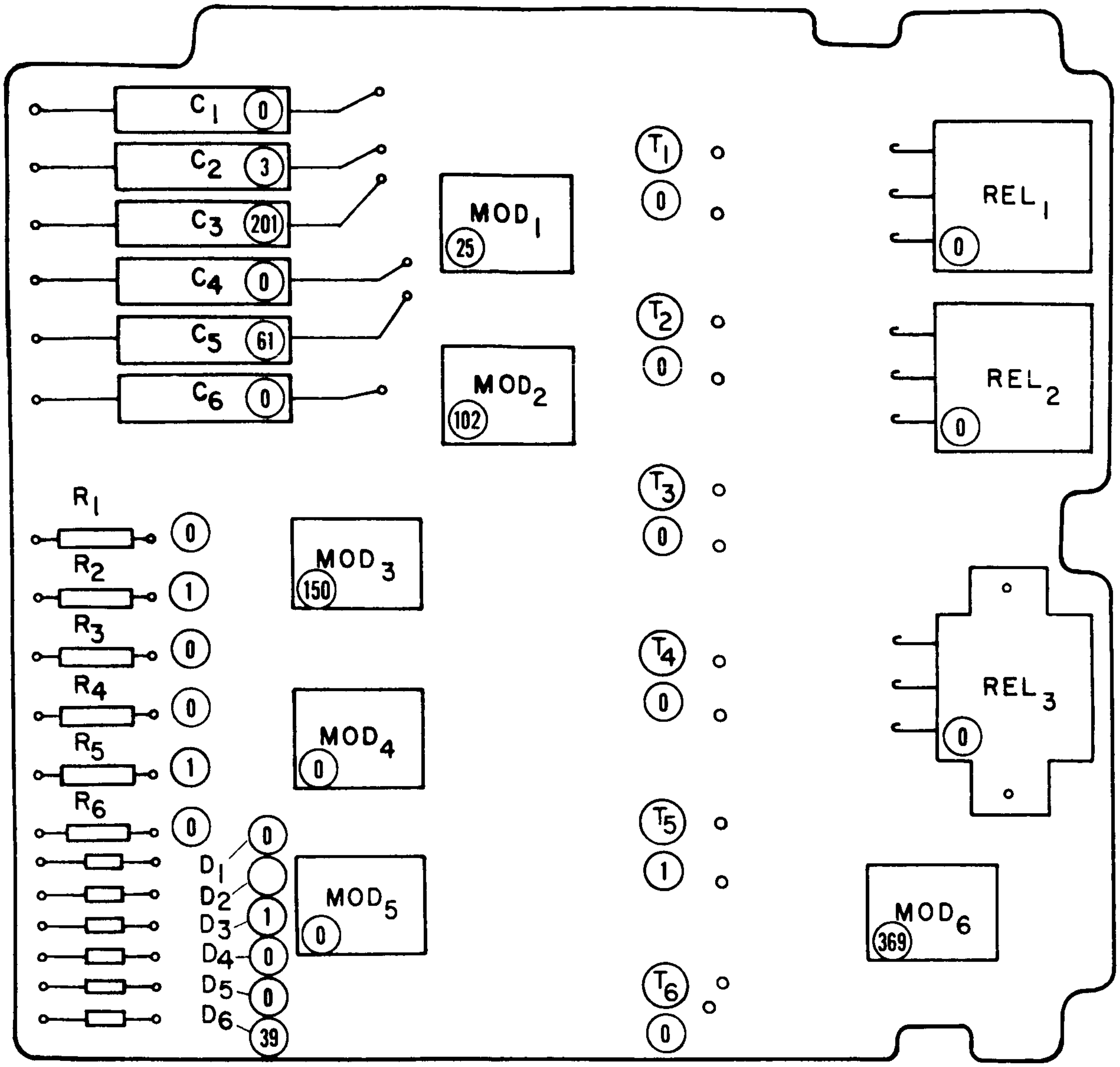

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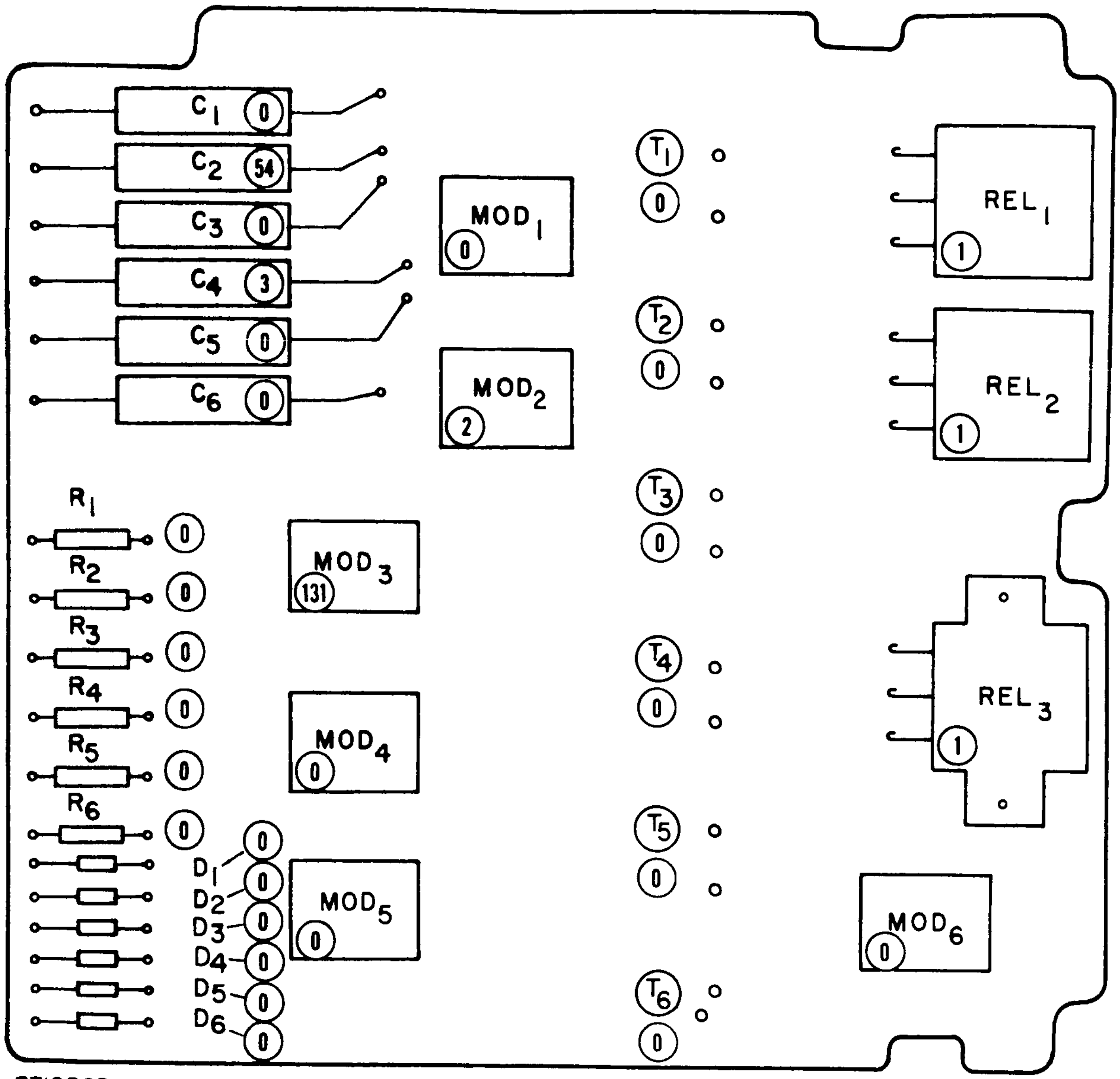
Swab 1 top surface of board = 58

Swab 2 bottom surface of board = 49

C	= Capacitor	(six were used)	REL	= Relay	(three were used)
D	= Diode	(six were used)	T	= Transistor	(six were used)
MOD	= Module	(six were used)	R	= Resistors	(six were used)

Figure 7. BIOLOGICAL BURDEN ON COMPONENTS OF BOARD NO. 3 (COMPONENTS ONLY) AEROBIC MESOPHILIC MICROORGANISMS -- ASSEMBLER CLOTHED IN STREET GARMENTS



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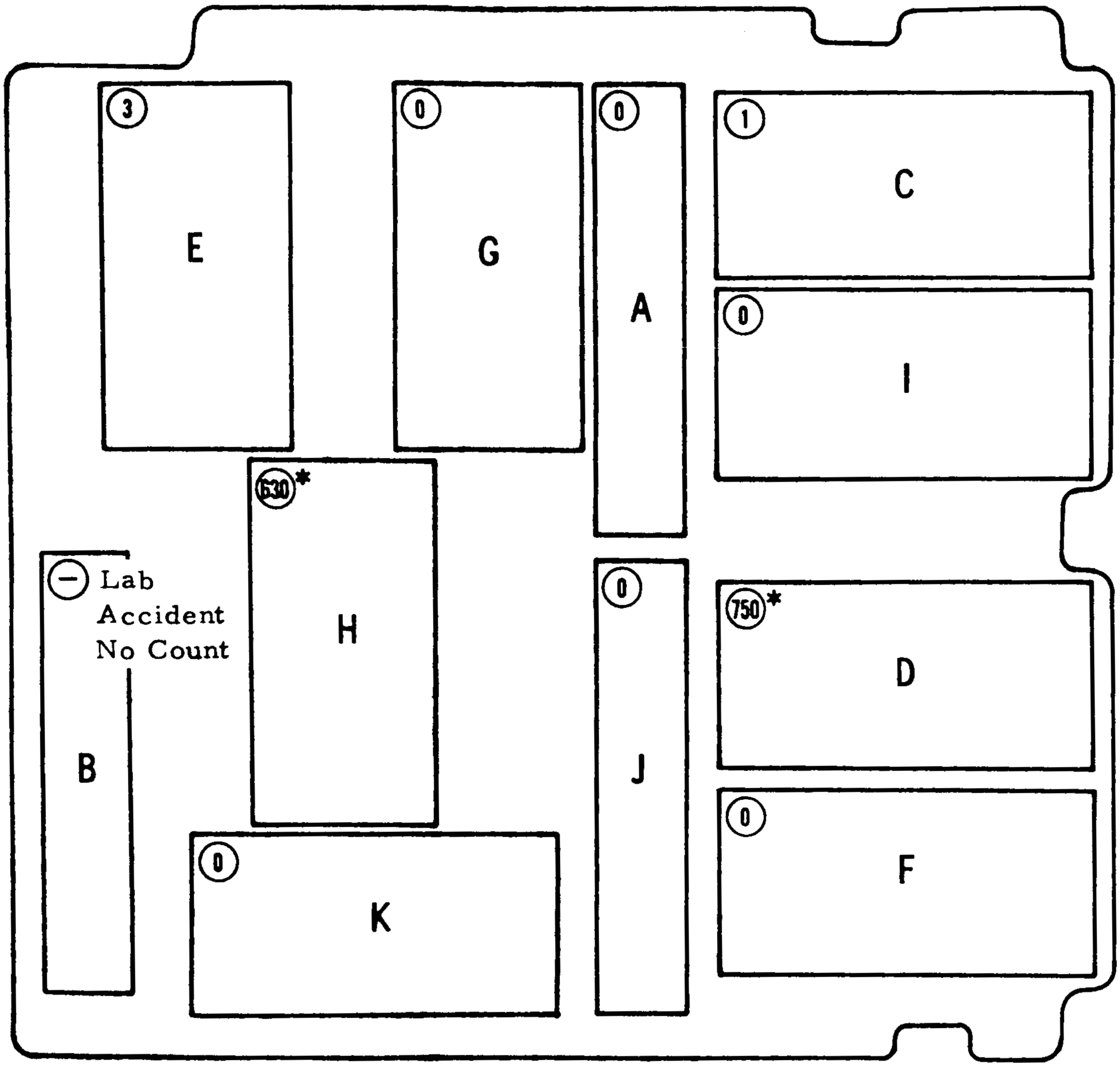
Numbers in cicles indicate burden on component
Swab 1 top surface of board = 3
Swab 2 bottom surface of board = 1

C	= Capacitor	(six were used)	REL	= Relay	(three were used)
D	= Diode	(six were used)	T	= Transistor	(six were used)
MOD	= Module	(six were used)	R	= Resistors	(six were used)

Figure 8. BIOLOGICAL BURDEN ON COMPONENTS OF BOARD NO. 4 (COMPONENTS AND COUPONS) AEROBIC MESOPHILIC MICROORGANISMS -- ASSEMBLER CLOTHED IN STREET GARMENTS



The circled number in each coupon indicates the number of viable particles per coupon

*See discussion for an explanation of these valves

Figure 9. BIOLOGICAL BURDEN LEVEL FOUND ON BIO-ASSAY COUPONS A TO K DURING THE CONSTRUCTION OF BOARD NO. 2

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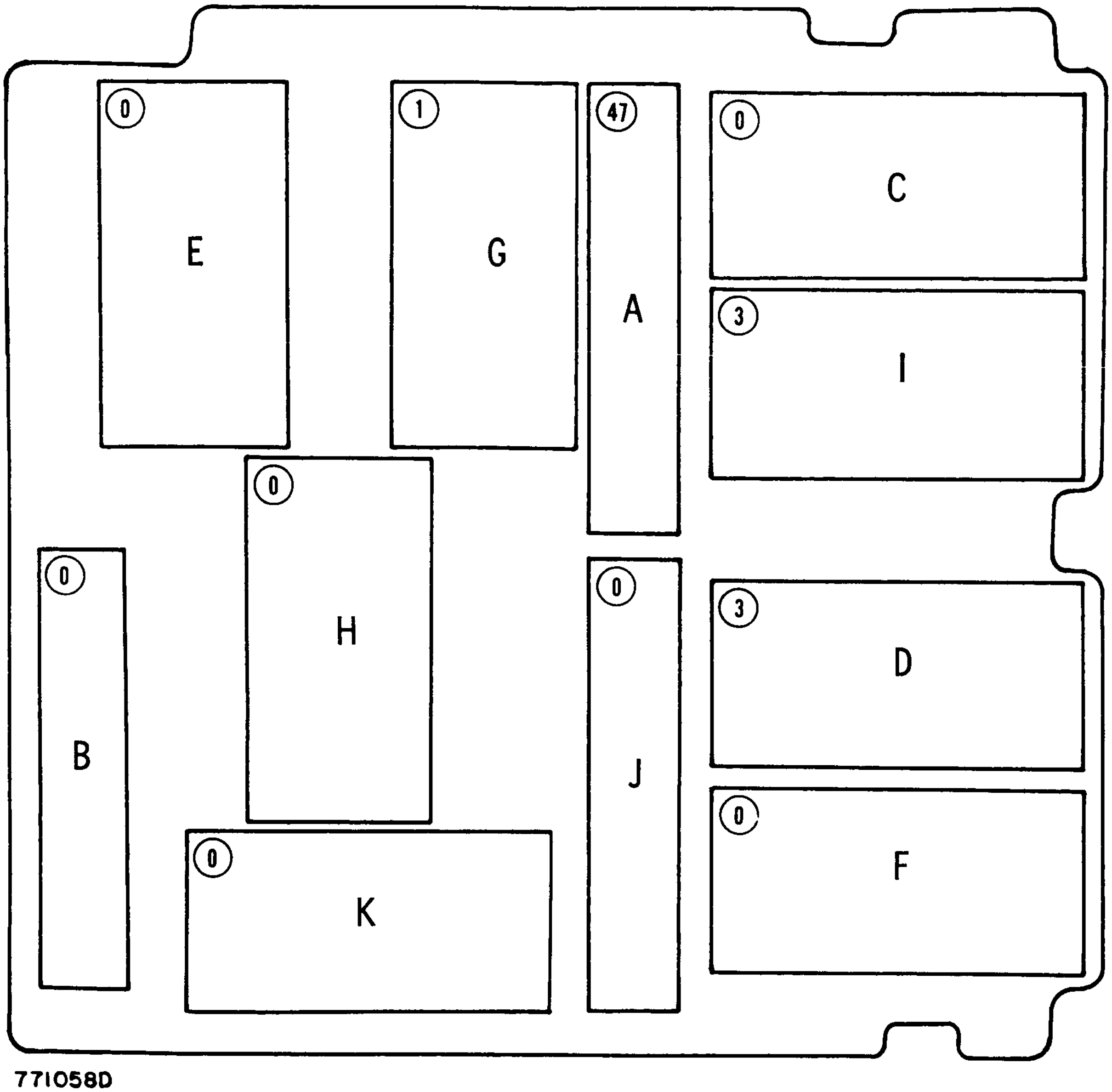

The circled number in each coupon indicates the number of viable particles per coupon

Figure 10. BIOLOGICAL BURDEN LEVEL FOUND ON BIO-ASSY COUPONS A TO K DURING THE CONSTRUCTION OF BOARD NO. 4

Table III lists the biological burdens found on coupons A to K on Boards 2 and 4.

Table IV lists the surface areas and burden of coupons and components on Board 2.

Table V lists surface areas and burdens of coupons and components on Board 4.

Tables VI, VII, VIII, and IX list the biological burden levels for intramural air, assemblers clothing, the areas to the immediate left and right of the assembler, and on the assemblers work table.

Tables X and XI list the biological burden found on the surfaces of the CMTM by swabbing and then culturing on TSA at 32°C.

Figures 11 to 16 illustrate the structures of the CMTM and their areas assayed for biological burden.

TABLE III

BIOLOGICAL BURDEN LEVEL FOR BIO-ASSAY COUPONS USED WHILE ASSEMBLING P.C. BOARDS 2 AND 4

	Board 2	Board 4
Coupon A	0 V.P.*	47 V.P.*
Coupon B	Dropped through EASL Floor Grating	0 V.P.*
Coupon C	1 V.P.*	0 V.P.*
Coupon D	750 (A) V.P.*	3 V.P.*
Coupon E	3 V.P.*	0 V.P.*
Coupon F	0 V.P.*	0 V.P.*
Coupon G	0 V.P.*	1 V.P.*
Coupon H	630 (A) V.P.*	0 V.P.*
Coupon I	0 V.P.*	3 V.P.*
Coupon J	0 V.P.*	0 V.P.*
Coupon K	0 V.P.*	0 V P.*

*V.P. = Viable Particle

(A) = See discussion for explanation of these values.

TABLE IV

SURFACE AREAS AND BURDENS OF COUPONS AND COMPONENTS ON BOARD NO. 2

1. Surface areas of coupons on Board 2

   6 coupons, 2- x 1-inch = 12 in^2

   2 coupons, 2- x 1-inch = cantaminated

   2 coupons, 2- x 1/2-inch = 2.5 in^2

   Total surface area for coupons = 14.5 in^2

2. Biological burden for 14.5 in^2 of coupon was 4 V.P.*

3. Biological burden per in^2 of coupon = $\boxed{\text{0.3 V.P.*}}$

4. Total surface area of components and surfaces on Board 2

6 capacitors	0.6 in^2	= 3.60
6 diodes	0.14 in^2	= 0.84
6 modules	2.25 in^2	= 13.50
3 relays	2.56 in^2	= 7.68
6 resistors	0.08 in^2	= 0.48
6 transistors	0.2 in^2	= 1.20

Total surface area of components = 27.30 in^2
2 surfaces (top and bottom) 36.8 in^2 = 73.60 in^2

Total surface area for components and board = 100.90^2

5. Biological burdens on components of board 2 = 3 V.P.*

6. Biological burden per in^2 of components = $\boxed{\text{0.11 V.P.*}}$

7. Biological burden per in^2 of components and top and bottom surfaces of board = $\boxed{\text{0.0275 V.P.*}}$

V.P.* = Viable particles

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TABLE V

SURFACE AREAS AND BURDENS OF COUPONS AND COMPONENTS ON BOARD 4

1. Surface areas of coupons on Board 4

8 coupons, 2- x 1-inch, = 16 in^2

3 coupons, 2-1/2- x 1/2-inch, = 3.75 in^2

Total surface area for coupons = 19.75 in^2

2. Biological burden for 19.75 in^2 = 54 V.P.*

3. Biological burden per in^2 of coupon = $\boxed{2.73 \text{ V.P.*}}$

4. Total surface area of components and surfaces of Board 4.

6 capacitors	0.6 in^2	=	3.60
6 diodes	0.14 in^2	=	0.84
6 modules	2.25 in^2	=	13.54
3 relays	2.56 in^2	=	7.68
6 resistors	0.08 in^2	=	0.48
6 transistors	0.2 in^2	=	1.20

Total surface area of components = 27.30 in^2

2 Surfaces (top and bottom) 36.8 in^2 = 73.60 in^2

Total surface area of components and board = 100.90 in^2

5. Biological burden on components of Board 4 = 194 V.P.*

6. Biological burden per in^2 of components = $\boxed{6.6 \text{ V.P.*}}$

7. Biological burden per in^2 of components and top and bottom surface of board = $\boxed{1.77 \text{ V.P.*}}$

*V.P. = Viable particles.

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TABLE VI

BIOLOGICAL BURDEN FOUND DURING THE ASSEMBLY OF BOARD NO. 1

Assembler: A. G.
Assembler: Right handed
Clothing: Street clothes plus sterile gown, hood, mask and gloves

Date Assembled: 6 December 1966
Date Assayed: 12 December 1966
Assayers: A. G., J. B., F. S.

Intramural Air (Reynier Sampler)

Aerobic Mesophilic Viable Particles/ft^3

Time	Site 3	Site 10	Site 13
One-half hour before start	0	0	0
First hour	0	0.067	0
Second hour	0	0.067	0.016
Third hour	0	0	0
Fourth hour, end of assembly	0	0.100	0.003

Clothing Biological Burden (Rodac Plates)

Aerobic Mesophilic Viable Particles/ft^2

	Left Side of Assembler						Right Side of Assembler					
Time	Smock Sleeve	Trouser Cuff	Smock Chest	Smock Hem	Smock Shoulder	Gloves	Smock Sleeve	Trouser Cuff	Smock Chest	Smock Hem	Smock Shoulder	Gloves
Start	0	0	0	0	0	---	0	0	0	0	0	---
End First hour	108	0	38	0	0	---	76	0	76	0	0	---
Fourth hour, end of assembly	506	76	0	506	0	0	1102	38	152	647	0	684

Biological Burden Beside Assemble (Stainless Steel Strips)

Aerobic Mesophilic Viable Particles/ft^2 (counts average of three strips)

	Left Side of Assembler			Right Side of Assembler		
Time	Spores	Vegetative Cells	Total Count	Spores	Vegetative Cells	Total Count
Controls	0	0	0	0	0	0
Start	0	38	38	0	0	0
First hour	0	0	0	0	0	0
Third hour	0	0	0	48	48	96
Fourth hour, end of assembly	0	0	0	0	0	0

Biological Burden Surface Work Table (Rodac Plates)

Aerobic Mesophilic Viable Particles/ft^2 (Count Average of 5 Rodac Plates)

	Left Side of Assembler		Right Side of Assembler	
Time	Site 1	Site 2	Site 3	Site 4
Controls	0	0	0	0
Start	0	0	0	0
First hour	7	0	0	7
Third hour	0	7	4	14
Fourth hour, end of assembly	7	14	14	0

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TABLE VII

BIOLOGICAL BURDEN FOUND DURING THE ASSEMBLY OF BOARD NO. 2

Assembler: A. G.
Assembler: right handed
Clothing: street clothes plus sterile gown, hood, mask, and gloves

Date Assembled: 8 December 1966
Date Assayed: 9 December 1966
Assayers: A. G., J. B., E. S.

Intramural Air (Reynier Sampler)

Aerobic Mesophilic Viable Particles/ft^3

Time	Site 3	Site 10	Site 13
One-half hour before start	0.016	0	0
First hour	0	0.05	0
Second hour	0	0.03	0.016
Third hour	0	0	0.016
Fourth hour after start	0	0	0

Clothing Biological Burden (Rodac Plates)

Aerobic Mesophilic Viable Particles/ft^2

	Left Side of Assembler						Right Side of Assembler					
Time	Smock Sleeve	Trouser Cuff	Smock Chest	Smock Hem	Smock Shoulder	Glove	Smock Sleeve	Trouser Cuff	Smock Chest	Smock Hem	Smock Shoulder	Glove
Start	0	0	0	0	0	---	0	0	0	0	0	---
End first hour	0	0	76	0	0	38	228	76	0	38	38	0
End fourth hour	912	72	38	266	38	0	2052	38	76	646	38	38

Biological Burden Beside Assembler (Stainless Steel Strips)

Aeorbic Mesophilic Viable Particles/ft^2 (counts average three strips)

	Left Side of Assembler			Right Side of Assembler		
Time	Spores	Vegetative Cells	Total Count	Spores	Vegetative Cells	Total Count
Controls	0	0	0	0	0	0
Start	0	0	0	0	86	86
First hour	0	0	0	0	0	0
Third hour	0	0	0	0	0	0
End of Assembly	0	0	0	0	0	0

Biological Burden Surface Work Table (Rodac Plates)

Aerobic Mesophilic Viable Particles/ft^2 (counts average of five Rodac Plates)

	Left Side of Assembler		Right Side of Assembler	
Time	Site 1	Site 2	Site 3	Site 4
Controls	0	0	0	0
Start	0	0	0	0
First hour	0	0	0	0
Third hour	28	21	7	7
Fourth hour, end of assembly	4	7	7	0

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TABLE VIII

BIOLOGICAL BURDEN FOUND DURING THE ASSEMBLY OF BOARD NO. 3

Assembler: G. C. M.
Assembler: right handed
Clothing: street clothes

Date Assembled: 12 December 1966
Date Assayed: 13 December 1966
Assayers: G. C. M. J. B., E. S.

Intramural Air (Reynier Sampler)

Aerobic Mesophilic Viable Particles/ft^3

Time	Site 3	Site 10	Site 13
One-half hour before start	0	0	0
First hour	0	0.03	0
Second hour	0	0	0
Third hour	0	0	0
Fourth hour after start	0	0	0

Clothing Biological Burden (Rodac Plates)

Aerobic Mesophilic Viable Particles/ft^2

Left Side of Assember							Right Side of Assembler					
Time	Sleeve	Shirt Cuff	Chest	Trouser Seat	Shoulder	Trouser Cuff	Sleeve	Shirt Cuff	Chest	Trouser Seat	Shoulder	Trouser Cuff
Start	---	---	---	---	---	---	---	---	---	---	---	---
End of first hour	342	570	646	1634	913	3800	988	836	988	1, 673	1, 482	2, 014
End of fourth hour	266	152	114	722	646	1443	228	380	304	913	342	1, 294

Biological Burden Beside Assembler (Stainless Steel Strips)

Left Side of Assembler				Right Side of Assembler		
Aerobic Mesophilic Viable Particles/ft^2 (count average of three strips)						
Time	Spores	Vegetative Cells	Total Count	Spores	Vegetative Cells	Total Count
Controls	0	0	0	0	0	0
Start	960	1440	2.4×10^3	0	1.4×10^5	1.4×10^5
First hour	144	179, 856	1.8×10^5	0	9.6×10^3	9.6×10^3
Third hour	394	69, 606	7.0×10^4	0	1.7×10^4	1.7×10^4
End of assembly	0	0	0	48	9.6×10^1	1.4×10^2

Biological Burden Surface Work Table (Rodac Plates)

Aerobic Mesophilic Viable Particles/ft^2 (count an average of five Rodac plates)				
Time	Site 1	Site 2	Site 3	Site 4
Controls	0	0	0	0
Start	0	0	0	0
First hour	7	7	0	0
Third hour	0	0	7.0	0
Fourth hour end of assembly	14.0	14	14	0

TABLE IX

BIOLOGICAL BURDEN FOUND DURING THE ASSEMBLY OF BOARD NO. 4

Assembler: G. C. M.
Assembler: Right handed
Clothing: Street clothes

Date Assembled 14 December 1966
Date Assayed: 15 December 1966
Assayers: G. C. M., J. B., E. S

Intramural Air (Reynier Sampler)
Aerobic Mesophilic Viable Particles/ft^3

Time	Site 3	Site 10	Site 13
One-half hour before start	0	0	0
First hour	0	0	0.016
Second hour	0	0	0.016
Third hour	0	0	0
Fourth hour after start	0	0.03	0.03

Clothing Biological Burden (Rodac Plates)
Aerobic Mesophilic Viable Particles/ft^2

	Left Side of Assembler						Right Side of Assembler					
Time	Sleeve	Shirt Cuff	Chest	Trouser Seat	Shoulder	Trouser Cuff	Sleeve	Shirt Cuff	Chest	Trouser Seat	Shoulder	Trouser Cuff
Start	---	---	---	---	---	---	---	---	---	---	---	---
End of First hour	228	456	114	912	152	3800	114	342	190	1370	304	3496
End of fourth hour	114	266	304	494	342	2660	684	342	608	494	418	1900

Biological Burden Beside Assembler (Stainless Steel Strips)
Aerobic Mesophilic Viable Particles/ft^2 (count average of three strips)

	Left Side of Assembler			Right Side of Assembler		
Time	Spores	Vegetative Cells	Total Count	Spores	Vegetative Cells	Total Count
Controls	0	0	0	0	0	0
Start	0	8×10^4	8×10^4	0	2.4×10^2	2.4×10^2
First hour	0	4.8×10^2	4.8×10^2	48	1.75×10^4	1.75×10^4
Third hour	0	1.4×10^4	1.4×10^4	0	8.0×10^3	8.0×10^3
End of assembly	288	2.9×10^5	3×10^5	0	4.0×10^2	4.0×10^2

Biological Burden Surface Work Table (Rodac Plates)
Aerobic Mesophilic Viable Particles/ft^2 (count an average of 5 Rodac Plates)

	Left Side of Assembler		Right Side of Assembler	
Time	Site 1	Site 2	Site 3	Site 4
Controls	0	0	0	0
Start	0	0	0	0
First hour	0	0	0	0
Third hour	0	0	0	0
Fourth hour end of assembly	0	0	0	0

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TABLE X

Assayer: J. B.

AEROBIC MESOPHILIC MICROORGANISMS SURFACE BURDEN ON CMTM HARDWARE AS DETERMINED BY SWABBING AND CULTURING ON TSA AT 32°C

Cultured on TSA at 32°C

Hardware	Viable Particles/ft^2		Total Surface Area of Hardware (ft^2)	Total Viable Particles for Hardware	
	Veg.* and Spores	Spores		Veg.* and Spores	Spores
Impact Limiter					
Site Assay A	630	90	11.05	6961	994
Site Assay B	90	0	11.05	994	0
Site Assay C	90	0	11.05	994	0
Antenna Relay Canister					
Site Assay A	36	0	1.969	71	0
Site Assay B	108	0	7.619	823	0
Site Assay C	1728	0	1.774	3065	0
Parachute Canister					
Site Assay A	360	90	1.227	442	110
Site Assay B	0	0	10.630	0	0
Site Assay C	90	90	1.670	150	150
Payload Structure					
Site Assay A	306	0	28.913	8847	0
Site Assay B	720	0	0.876	631	0
Site Assay C	972	270	18.333	17,820	4949
Site Assay D	792	90	7.861	6226	707

*Veg -- Vegetative cells

TABLE XI

AEROBIC MESOPHILIC MICROORGANISMS SURFACE BURDEN ON CMTM HARDWARE
AS DETERMINED BY SWABBING AND CULTURING ON TSA AT 32°C

Assayer: J. B.

Cultured on TSA at 32°C

Hardware	Viable Particles/ft^2		Total Surface Area of Hardware (Ft^2)	Total Viable Particles for Hardware	
Electron Chassis	Veg.* and Spores	Spores		Veg. and Spores	Spores
Site assay A	1638	1260	0.283	476	357
Site assay B	720	0	0.239	172	0
Site assay C	180	90	0.239	43	22
Site assay D	36	0	2.444	88	0
Site assay E	1836	180	0.375	688	68
Sterilization Canister					
Site assay A and B (ave)	90	0	98	8820	0
Site assay B	72	0	98	7056	0
Site assay C	1404	0	171.4	240,645	0
Site assay D	0	0	171.4	0	0
Site assay E	0	0	196.1	0	0
De-orbit Motor					
Site assay A	216	0	1.812	391	0
Site assay B	6660	360	1.812	2068	
Site assay C	270	0	5.80	6523	0
Site assay D	0	0	3.172	0	0
Site assay E	0	0	3.172	0	0

*Veg. -- Vegetative cells.

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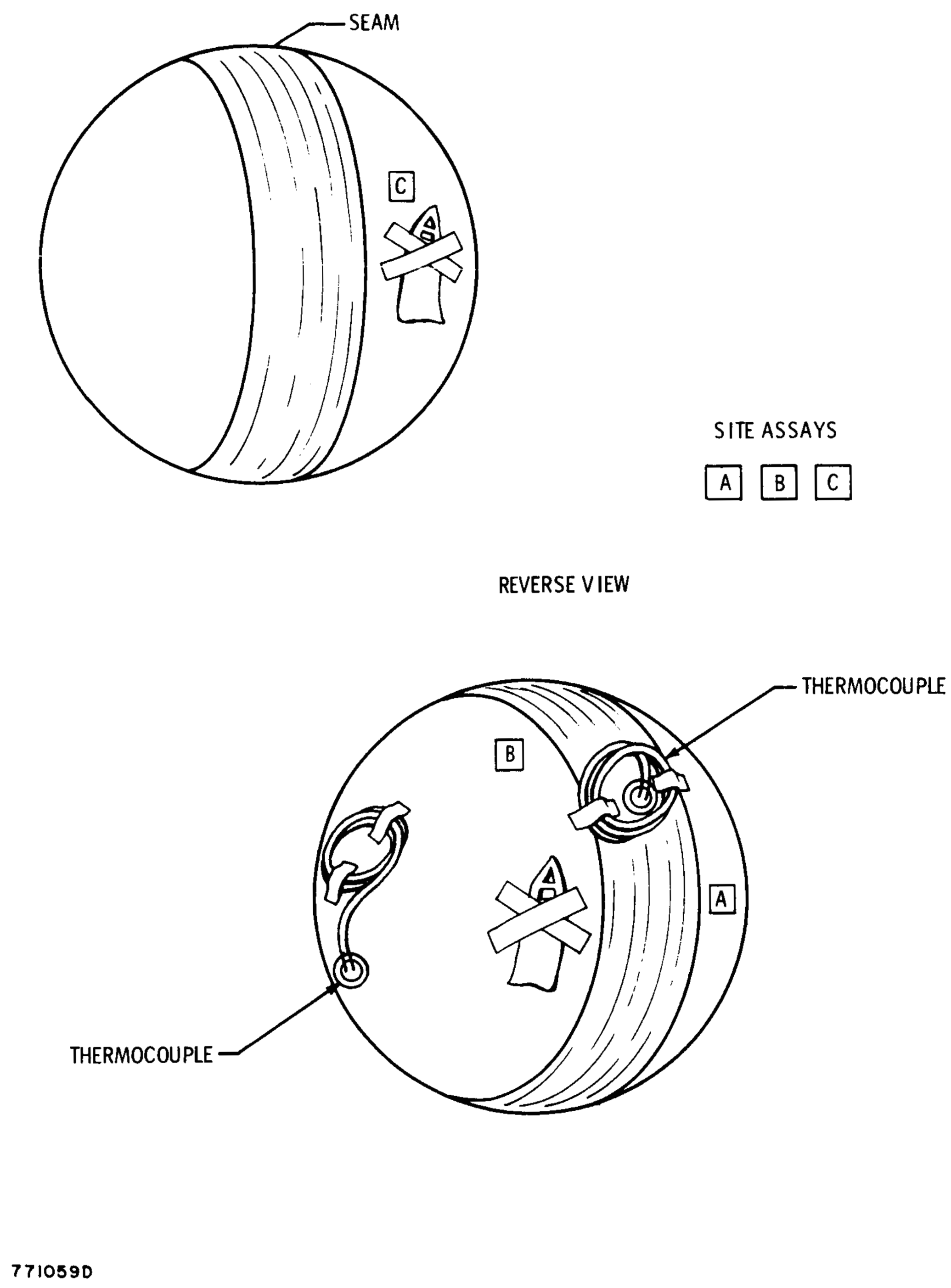
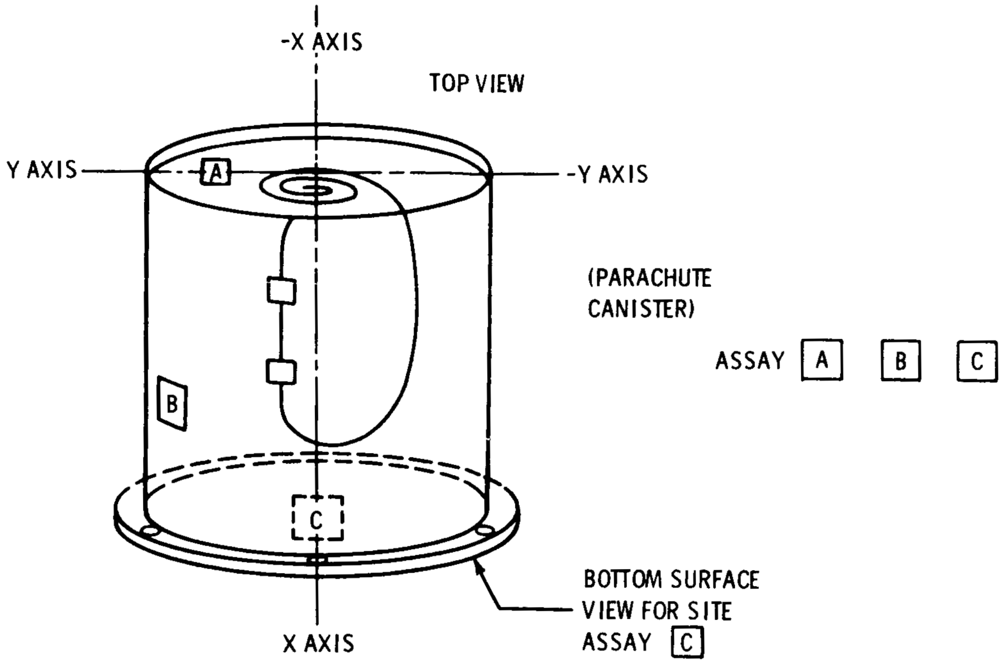


Figure 11. AREAS ASSAYED ON THE IMPACT LIMITER



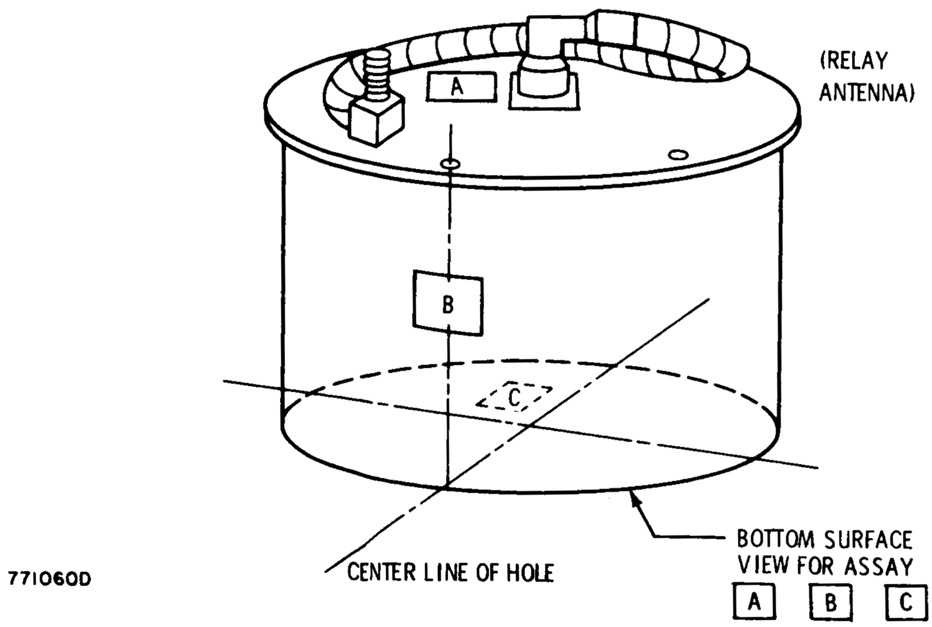


Figure 12. AREAS ASSAYED ON THE PARACHUTE CAN AND RELAY ANTENNA

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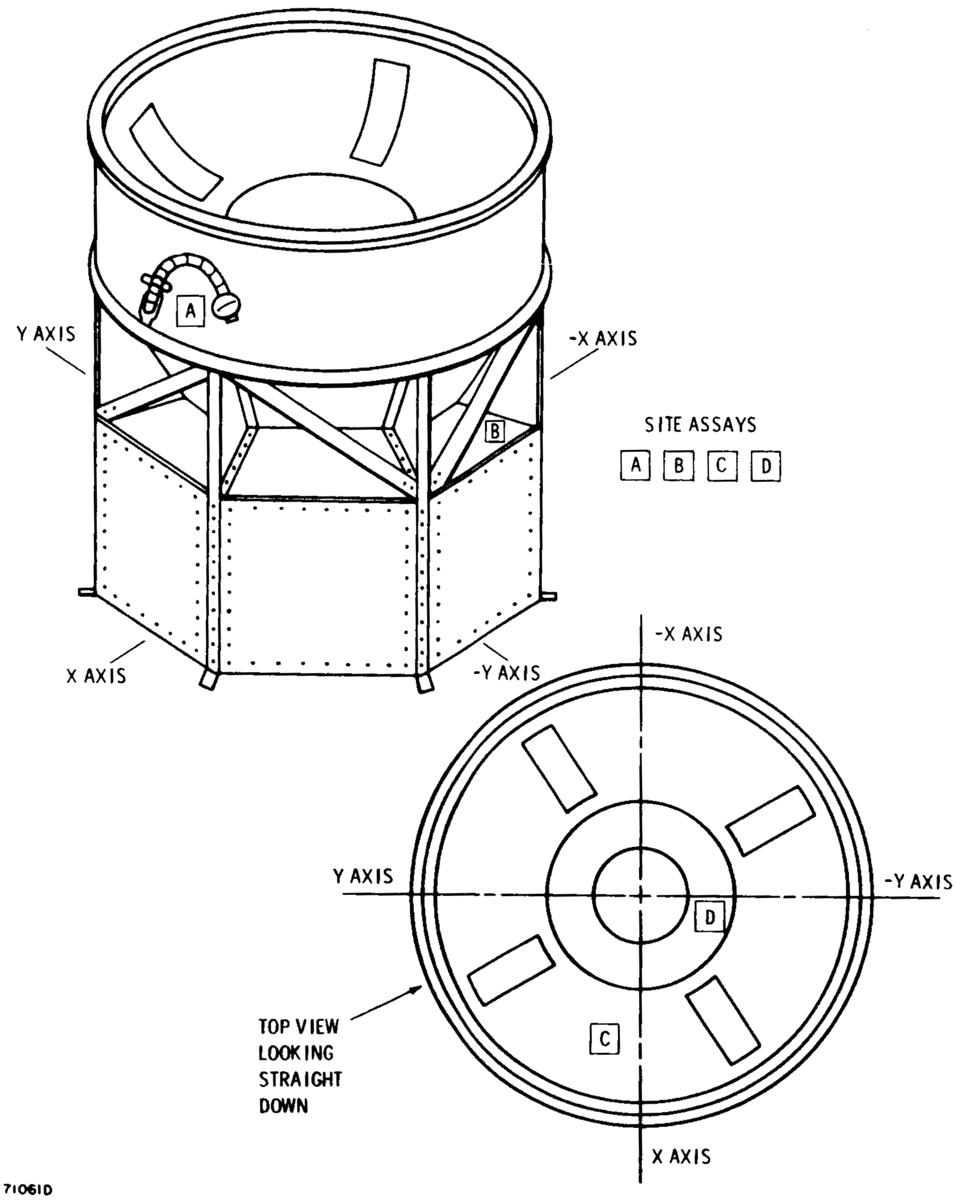

Figure 13. AREAS ASSAYED ON THE PAYLOAD STRUCTURE

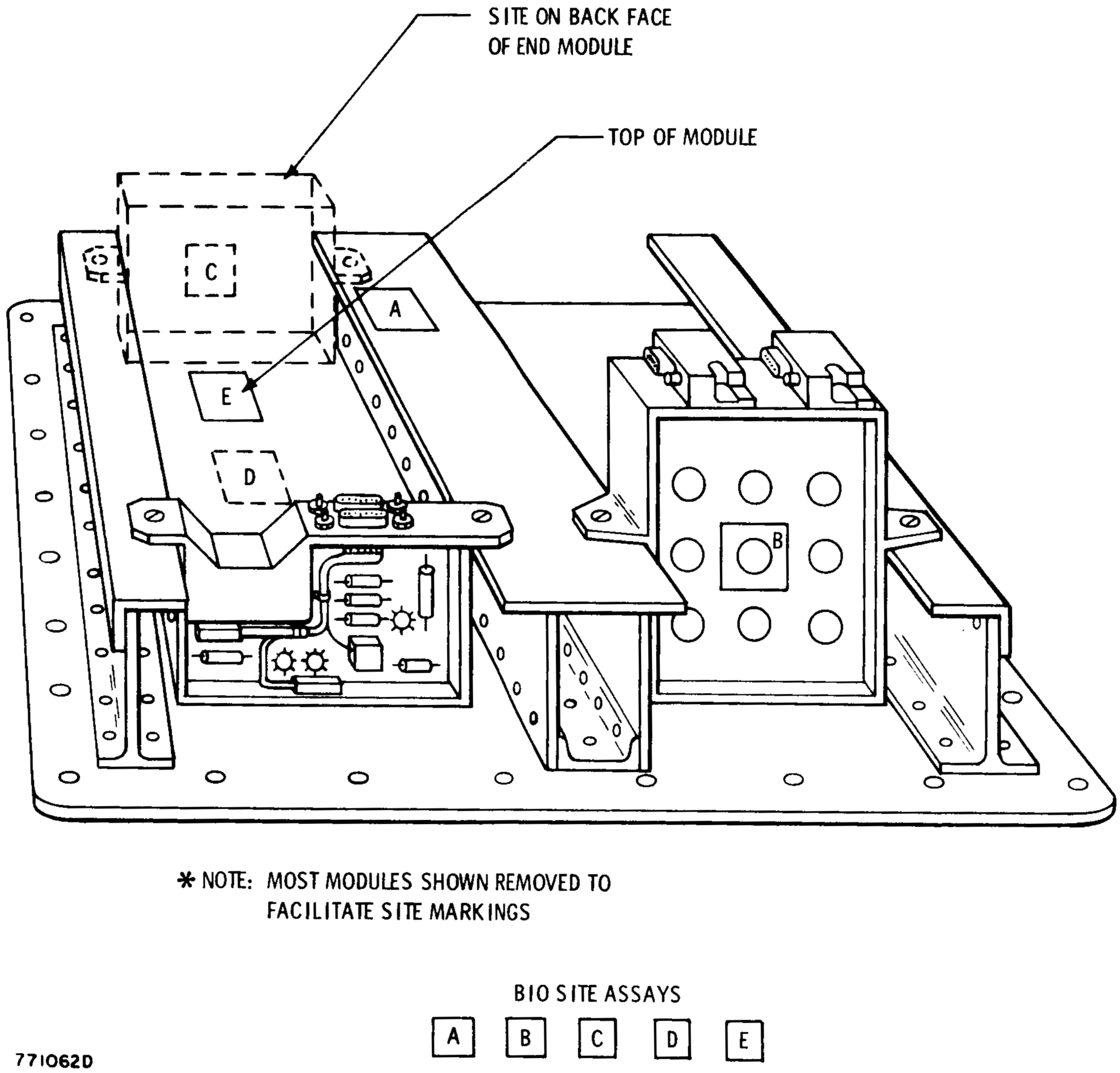

Figure 14. AREAS ASSAYED ON CHASSIS NO. 6

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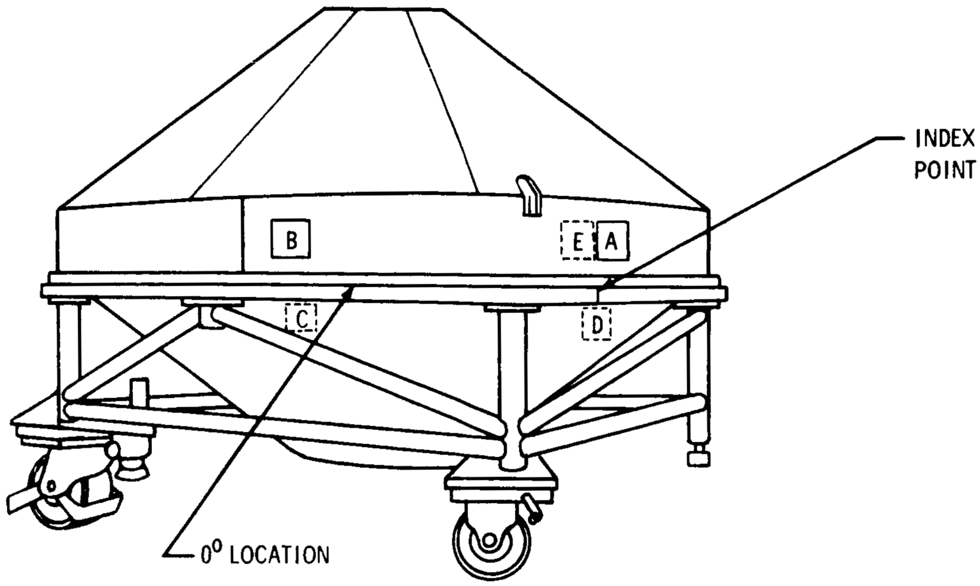

SITE ASSAYS

NOTE: DOTTED LINES INDICATE SITE INSIDE OF CANISTER

A B C D E

771063D

Figure 15. AREAS ASSAYED ON THE STERILIZATION CANISTER AND DOLLY

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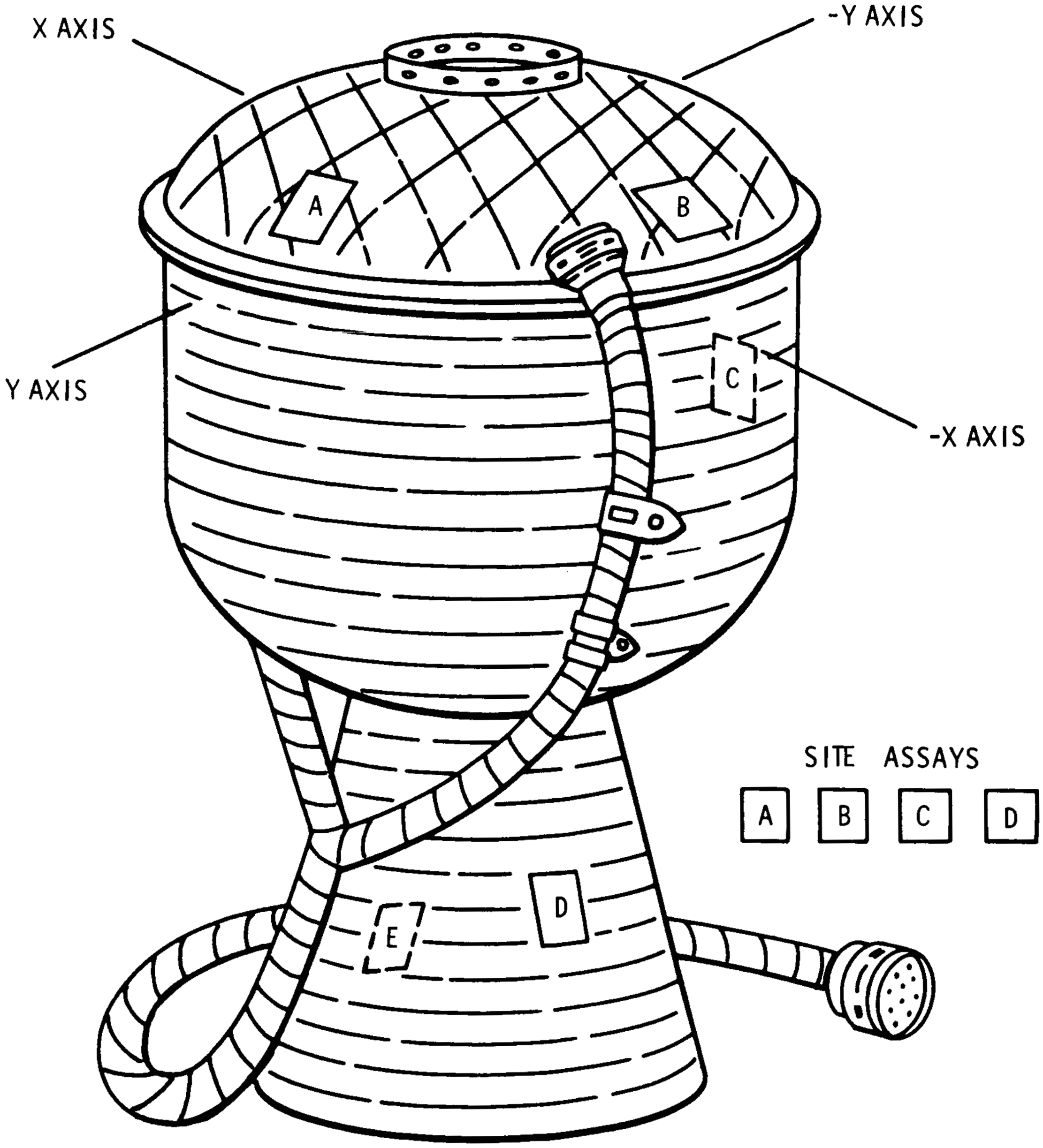

* NOTE: DOTTED LINES INDICATE
SITES INSIDE OF MOTOR

771064D

Figure 16. AREAS ASSAYED ON THE DE-ORBIT MOTOR

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IV. DISCUSSION

The assembly of Boards 1 and 2 in EASL was performed by the workers,dressed in sterile protective clothing. A low biological burden (0 to 3 viable particles) was generally obtained from each of the components and bio-assay coupons However, on Board 1 capacitor 3 (48 viable particles), capacitor 5 (330 viable particles). and relay No. 3 (360 viable particles) had what appeared to be an excessive biological burden. The bio-assay coupons H (630 viable particles) and D(750 viable particles) on Board 2 also had what appeared to be an excessive biological burden.

Before attempting to correlate biological burden on piece parts with that on the bio-assay coupons, the Quality Assurance Log was examined to determine if the burden on capacitors 3 and 5, relay 3, and biocoupons H and D were due to abnormal situations such as laboratory accident or a nonrepresentative violations of aseptic procedure. The Quality Assurance log revealed that while Board 1 was being built, a violation of technique occurred. It was noted that the assembler s glove top was not covering his cuff as prescribed in the approved dressing technique. This meant that the assembler's bare wrist was exposed to the environment. Further examination of the Quality Assurance log revealed that the assembler's wrist had touched the area where the capacitors were being mounted. It was therefore assumed that the assembler's bare wrist contaminated capacitors 3 and 5.

In the case of relay 3, the Quality Assurance log noted that there was considerable difficulty in removing it for bio-assay from Board 2. Relay 3 was bolted in place and during the process of removal, trouble was encountered while removing the bolts. An excessive amount of manipulation was employed. It was therefore assumed that high burden on relay 3 was due to the excessive handling in the biology laboratory. The Quality Assurance log also revealed that the burden on biocoupons H and D was due to an assembly accident. Coupon D was accidentally jarred loose from its proper position and was on top of coupons H and J. Coupon D was placed in its proper position by the assembler,who used a pair of forceps which were not sterile. It was therefore assumed that the burdens on the two coupons were due to the contaminated forceps.

Comparisons were made of biological burden on the coupons of Boards 2 and 4 with the burdens from components on Boards 2 and 4. A relationship does appear to exist between the burden on coupons and that on components. For example, coupons which usually had biological burden were removed from areas which had biological burden; and conversely, coupons which usually yield zero burden were removed from areas whose components usually had zero burden.

The coupons generally delineate areas of burden versus areas of non-burden. For purposes of burden location, the coupons, therefore, could be considered satisfactory.

When comparing quantitative determinations of burden with the coupon technique with that of individual piece part assay, the coupon technique was found to be variable in response. For example, on Board 2 the biological burden per square inch of coupon was 0.3 viable particles, while the burden for a square inch of component was 0.11 viable particles. (See Table IV.) If the same comparison were made for 2 square inches of component and board surface (top and bottom), the value would be 0.027 viable particles. (See Table IV.) It should be noted that the burden level per square inch for coupons was almost three times greater than that for components, and almost 10 times greater for that of components and board surface. The results obtained from the Board 4 study also revealed a variation of response. The biological burden per square inch of coupon was 2.73 viable particles, while the burden per square inch of components was 6.6 viable particles. (See Table V.) If the same comparison were made for a square inch of component and board surface (top and bottom), the value would be 1.77 viable particles. (See Table V.) It should be noted that the burden per square inch for coupons was approximately 1/3 that of the component. Also, when a comparison was made of burden per square inch of coupon against that of components and board surfaces (in^2), the coupons had approximately 1-1/2 times more burden than the components and board surfaces.

In the process of evaluating the data for Boards 1 to 4, it was decided not to include the data from Board 1 with that of Board 2, and the data of Board 3 with that of Board 4. Boards 1 and 3 were built without coupons It was felt that there would be sufficient difference in biological burden between the boards, that averaging the values of Boards 1 with 2 and Board 3 with 4 would not be valid.

Since the result obtained with the coupon technique was variable, it was decided to assay the parts of the CMTM for biological burden with a swabbing technique It should be noted that the assays were performed on the CMTM when it was exposed to an uncontrolled environment.

The surface biological burden on the different parts of the CMTM hardware varied from a low of 0 recovered Aerobic Mesophilic organisms/ft^2 to 6,600 Aerobic Mesophilic organisms/ft^2. Strict interpretation of the levels obtained cannot be made, since we do not have an adequate number of biological assays to indicate that the biological burden levels obtained were realistic. In addition, we do not have an exact measurement of total surface area.

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V. CONCLUSIONS

1. Areas of biological burden can be determined by the use of biocoupons.

2. The results of the quantitative determination of biological burden, using the bio-assay coupon technique, was found to be variable based on the information evolved from this study.

3. Additional studies on the efficacy of the bio-assay coupons as a means of quantitative biological burden determinations are required.

VI. RECOMMENDATIONS

1. Additional study on the bio-assay coupon technique is recommended

2. Additional study on the determination of the biological burden of the CMTM is recommended.

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VII. REFERENCES

1. NASA, Preliminary Standard Procedures for the Microbiological Examination of Space Hardware, Washington, D. C., June 1966.

2. Jet Propulsion Laboratory, General Specification Certification Requirements for the Experimental Assembly and Sterilization Laboratory, XOV-50543-GEN., 25 October 1965.