NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Technical Report No. 32-941

Experimental Assembly and Sterilization Laboratory (EASL) Operations: Phase I

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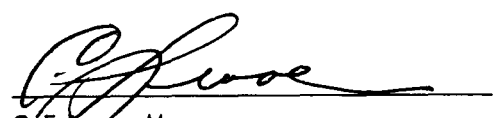

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April 15, 1966

Prepared Under Contract No. NAS 7-100
National Aeronautics & Space Administration

CONTENTS

TABLES

FIGURES

ABSTRACT

26287

This Report describes the Experimental Assembly and Sterilization Laboratory (EASL) effort initiated early in 1965 at the Jet Propulsion Laboratory. This document also includes a discussion of the EASL facility, the EASL operations, the supporting operations, the operational results, and the problems encountered. In addition, this document, a final report of Phase I of the EASL effort, presents conclusions and recommendations based on this Phase I study.

I. INTRODUCTION

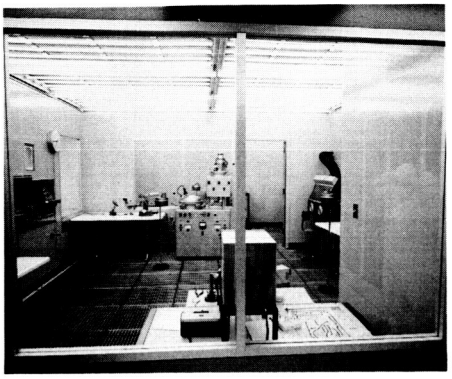
The Experimental Assembly and Sterilization Laboratory (EASL) effort was initiated early in 1965 as an experimental, full implementation of the requirements of the draft "Interim Requirements for Bioclean Facilities" (Ref. 1) being prepared by the Planetary Quarantine Officer, Office of Space Science and Applications, National Aeronautics and Space Administration, Washington, D. C.

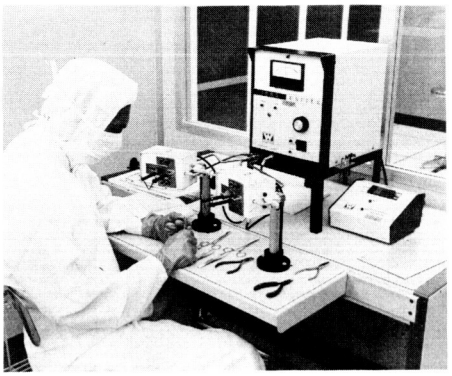
The function of the EASL effort was to construct and operate a facility based on (1) laminar downflow, (2) small positive-pressure gradient; (3) decontamination of all parts, tools, etc. entering the facility, and (4) rigorous personnel cleanliness techniques.

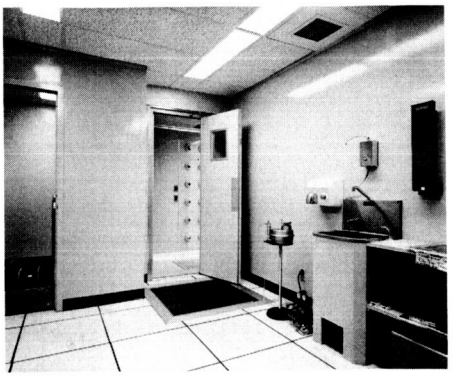
The objectives specified for the EASL effort were as follows:

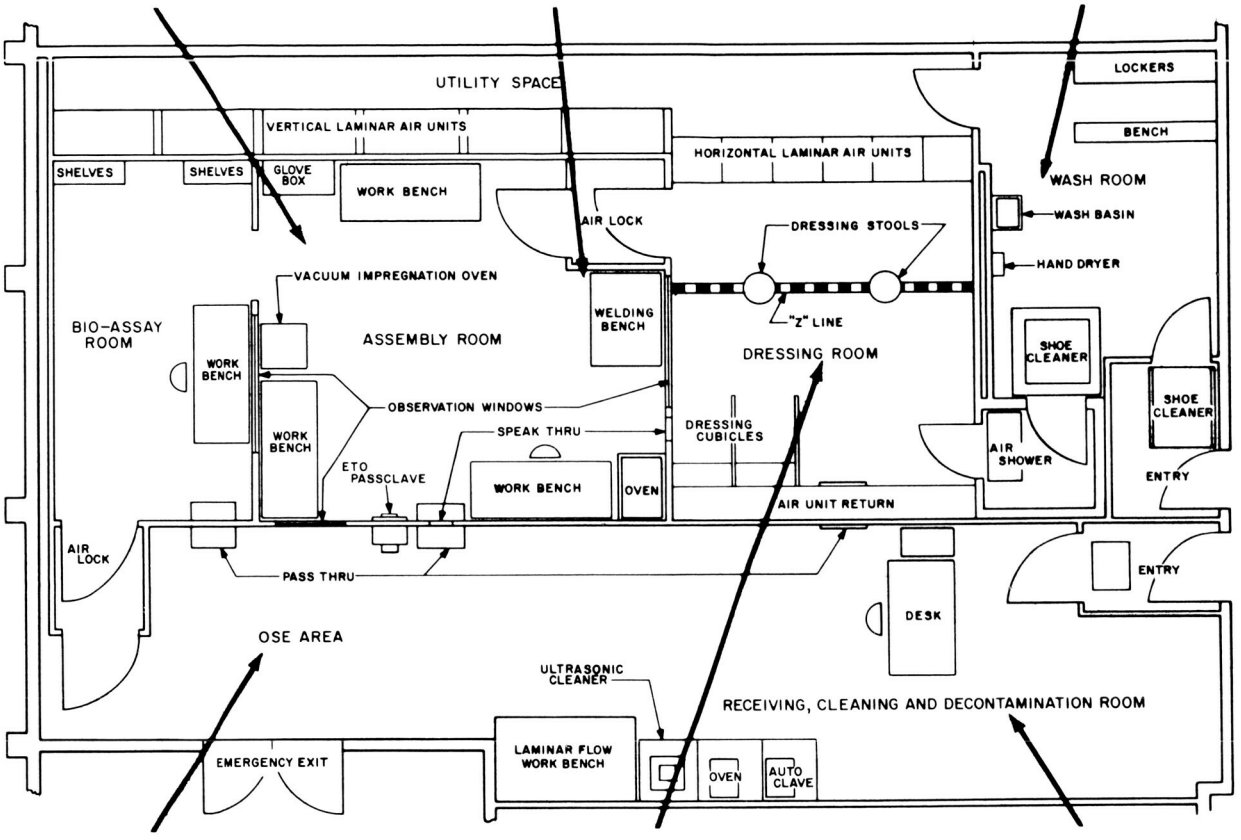
1. To evaluate the "Interim Requirements" provisions (as extended in EASL operations) in terms of:
   a. Resulting microbial contamination in finished assemblies.
   b. Quality and reliability of finished assemblies.
   c. Time, human-factor, and economic considerations.
2. To develop and obtain, for state-of-the-art and future facilities, the following information:
   a. Design and operational requirements.
   b. Microbiological monitoring requirements and techniques for fabrication, assembly, and test operations.
   c. Quality assurance requirements and procedures.

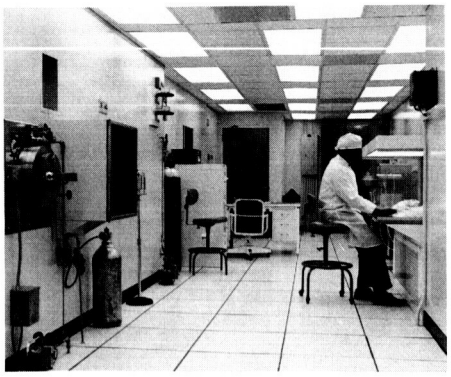
The facility was constructed and operations were commenced with Phase I. This phase was intended to provide familiarization and a general evaluation of facility, procedures, and the associated microbiological activities

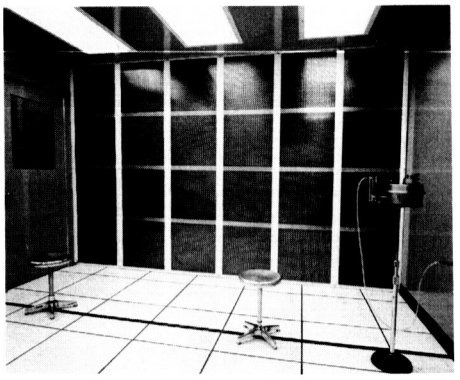












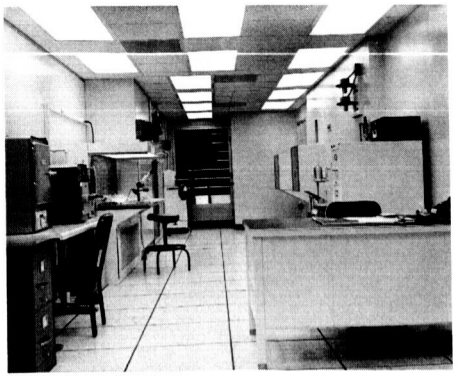


Fig. 1. Experimental Assembly and Sterilization Laboratory (EASL)

through the assembly of four welded cordwood modules, chosen as representative, simple and inexpensive samples of hardware expected to be built in such a facility. Phase II is expected to be an examination of the acceptable threshold level of cleanliness, based in part on Phase I results; subsequent phases will be determined by the results of prior phases.

This document is the final report of Phase I of the EASL effort.

II. EASL FACILITY

Layout and appearance of the facility are shown in Fig. 1. The facility consists of five rooms and a utility space. The washroom or locker room is isolated from outdoors by an outer room with a shoe cleaner and is not cleanly maintained microbiologically. The dressing room is isolated from the washroom by a shoe cleaner, air shower, and 0.15-in. water-gage (wg) positive air-pressure differential; it is divided by a "Z" line into "clean" and "dirty" sections. Laminar cross-flow air flows from the "clean" to "dirty" side, and both sections meet the requirements of Federal Standard 209 (Ref. 2), Class 100. The assembly room is isolated from the "clean" side of the dressing room by an airlock with a 0.10-in. wg positive-pressure differential, and utilizes the laminar downflow air principle. The bio-assay room opens off the assembly room and utilizes the same laminar downflow system; it is isolated from the Operational Support Equipment (OSE) area by maintaining an 0.25-in. wg pressure differential and an airlock. The OSE and receiving, cleaning, and decontamination room provides pass-throughs to the dressing, assembly, and bio-assay rooms (Fig. 2) and includes a laminar crossflow workbench; it is isolated

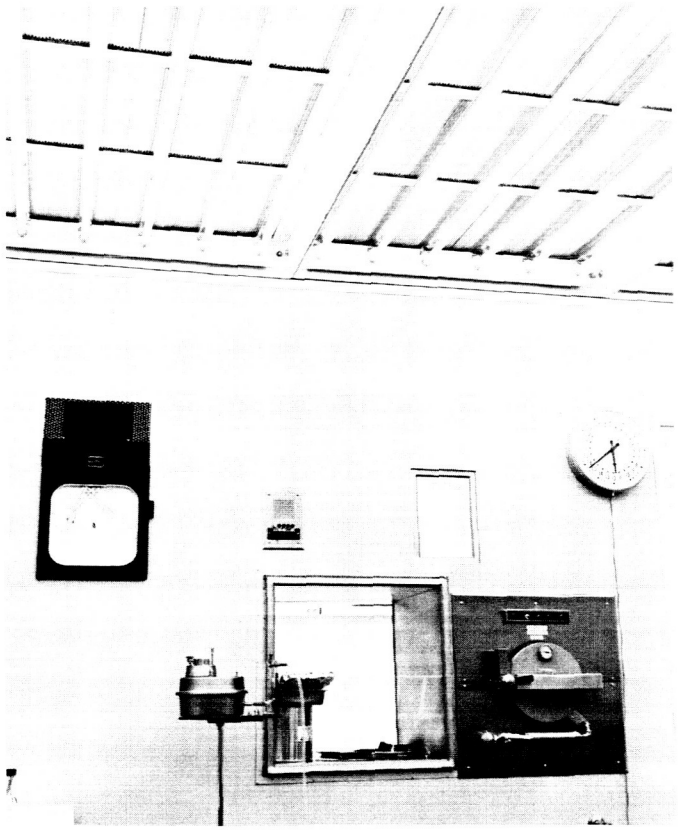


Fig. 2A. Laminar-flow room; view toward ceiling

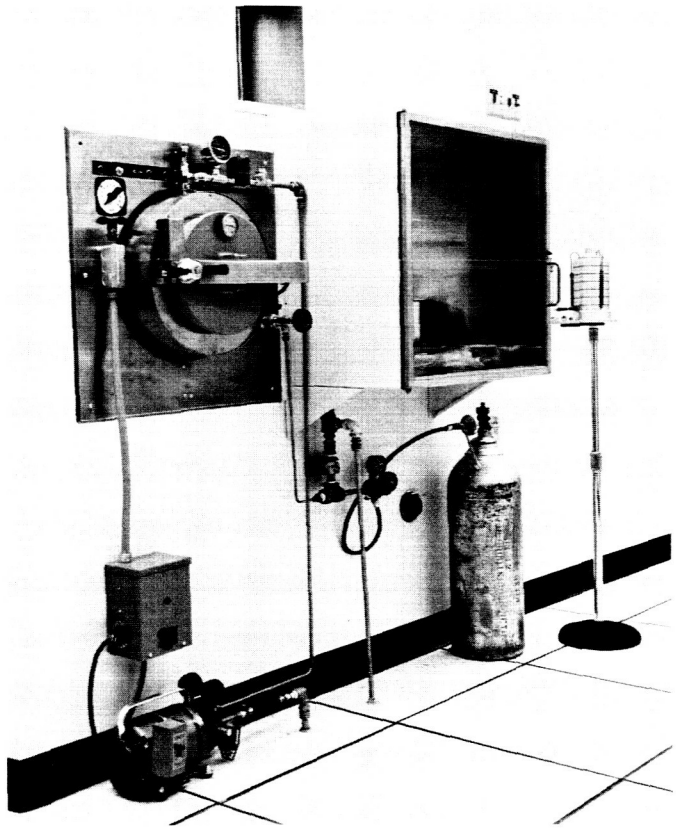


Fig. 2B. ETO pass clave and pass through OSE area

from outdoors by an outer room with a tacky mat. The utility space is part of the return air plenum, opening off the washroom.

Personnel traffic flows one way from the washroom to the dressing, assembly, and bio-assay rooms; exit is through the OSE area. Plastic diaphragm talk-throughs above eye level link the assembly room with the dressing and OSE areas, and an electrical intercom is located in the same three rooms.

Laminar air flow is maintained at a velocity of 75 ± 20 ft/min, at a temperature of 70° ± 10° F, and at a humidity of 45 ± 5%. Final air filtration at the wall or ceiling laminar inlet units is accomplished by absolute filters which remove 99.97% of particles, 0.3 μ or larger.

Facility certification is governed by JPL Specification.[1] For a complete description of the EASL facility, see Ref. 3.

[1] "General Specification: Certification Requirements for the Experimental Assembly and Sterilization Laboratory (EASL)," JPL Spec. No. XOY-50543-GEN, October 25, 1965.

III. EASL OPERATIONS

EASL operations consist of the controlled entry of parts, tools, equipment, and personnel to the assembly room, the assembly of the desired modules there, and the maintenance of the facility in bioclean condition. Supporting operations (for the purpose of this Report) are the microbiological and quality-assurance monitoring, review, and test functions.

A. Preassembly Activity

The first phase of operations in EASL consisted of getting the hardware and personnel into the assembly area in an acceptably clean condition.

1. Hardware

All items to be used in the assembly room were decontaminated by one of the following methods; where it was not possible, the item was placed on the waiver list (example: Petri dishes containing culture media).

a. Exposure to Ethylene oxide (ETO) (12%)–Freon 12 (88%)

b. Wiping with amphyl or 70% ethyl alcohol

c. Moist or dry-heat sterilization

For example, electronic components and certain tools were given ETO decontamination (Fig. 3); bagged clothing and mylar printed drawings were wiped with amphyl; and metal parts and other tools were heat sterilized (Fig. 4).

Heat and ETO decontamination prove to be time consuming, and the time was dependent on the item being cycled. The time consumed was:

a. *Heat Sterilization:*

Item preparation = 15 to 30 min

Cycle = 1 to 3 hr { Moist: 255° F / Dry: 275° F

b. *ETO:*

Item preparation = 1/4 to 1 hr

Cycle = 7 hr

In addition, in the ETO method the parts were placed in quarantine for 72 hr until the bio-assay results were available to show whether or not the cycle was successful. This was not the case for the heat cycle, as a piece of sterilization indicator tape was included with the load and it gave immediate indication after the cycle was complete.

To ensure that there would be no delay in the operations, spare kits of parts and tools to be used for replacements were decontaminated in the event that an item

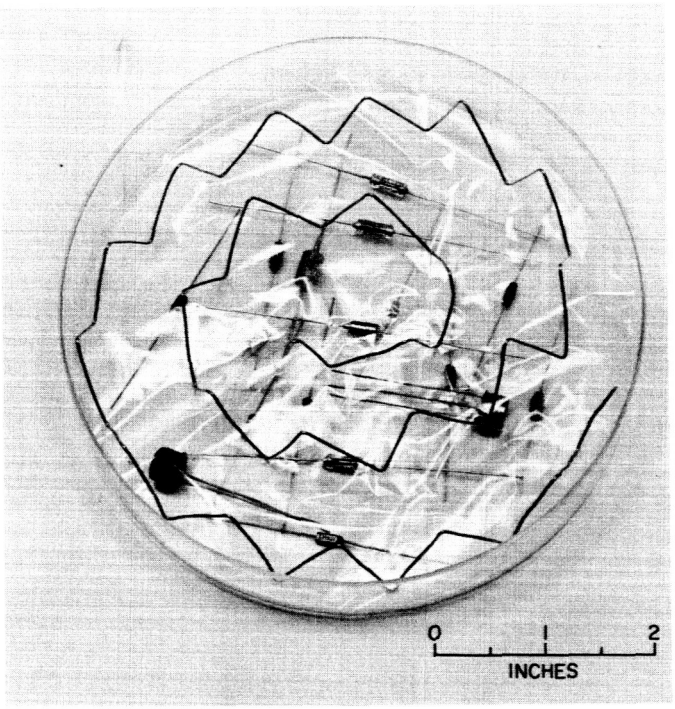


Fig. 3A. Packaging of components for ETO decontamination

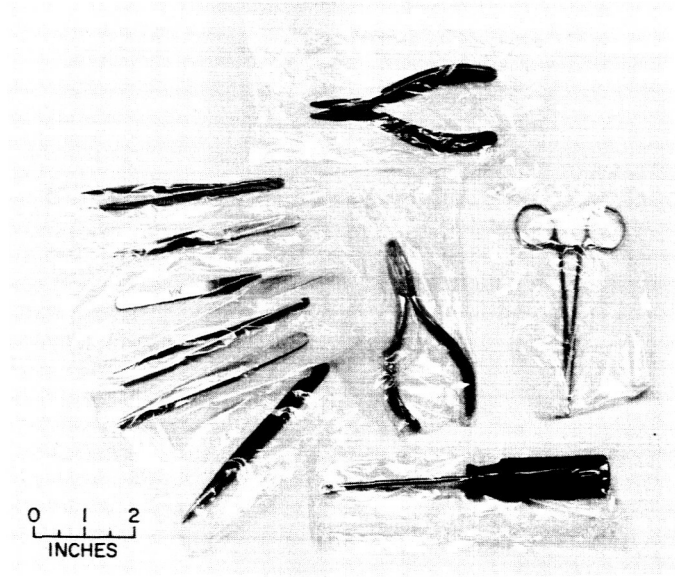


Fig. 3B. Packaging of tools for ETO decontamination

was dropped on the floor grating, which would invalidate the item's use on or in the module assembly. When this occurred, the dropped item was bio-assayed and re-decontaminated.

Fig. 4. Packaging of metal components for heat sterilization

These operations were conducted in the OSE receiving and decontamination area (Fig. 5) and, where necessary, in the JPL microbiological laboratory.

2. Personnel

Each person entering the facility to work in the laminar downflow assembly room (including inspectors and janitors) was given a health examination. The primary purpose of this examination was to screen out those persons emitting more than normal bodily fallout, such as sneezing, coughing due to colds, flaking skin due to sun burn or allergy, or unusual perspiration, etc. The examination technique was the visual assay and oral temperature methods, monitored by the facility operator and verified by Quality Assurance.

To eliminate excessive debris in the facility, three shoe cleaners, one at the entrance to the OSE area, the second at the entrance to the locker room, and the third at the entrance to the air shower, were employed. Cleanness of the shoes was assayed by the biologist and verified by Quality Assurance.

To ensure the minimum contamination to the facility and to the hardware, sterilized gloves were worn by all personnel working in the laminar downflow room. To control further possible contamination by bodily fallout or from the accidental tearing of a glove during assembly, a germicidal detergent was issued to each of the EASL personnel. This detergent was used for bathing and shampooing for two weeks prior to and during the

Fig. 5A. Sterilization equipment; OSE area

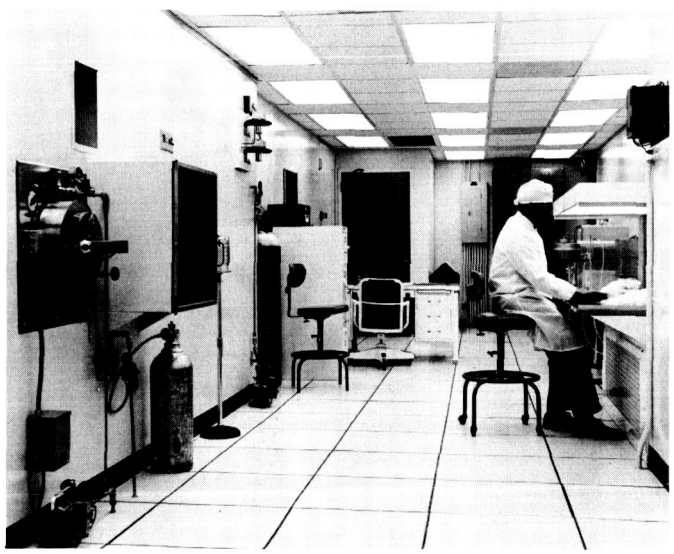


Fig. 5B. OSE area; view toward entrance

Phase I operations. In addition, before entering the laminar flow room, each person scrubbed his hands and wrists for 3 min with the germicidal detergent and a sterilized brush.

The clothing worn by the persons within the laminar downflow room consisted of ETO-treated dacron hood, coverall, and boots (Fig. 6). In addition, a sterilized surgeon's mask was worn by each person entering the laminar downflow room.

The shoe cleaning, scrub down, and donning of sterilized garments were repeated each time an individual

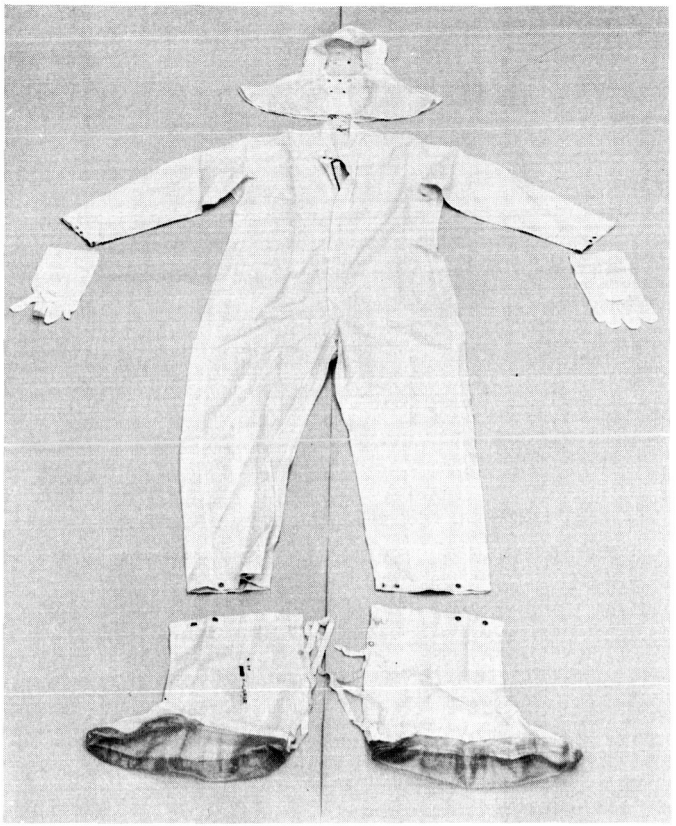


Fig. 6. EASL garments

entered the laminar downflow room. EASL was operated according to standard break and lunch schedules; and because traffic through the facility is "one-way", a minimum of four entries was made daily by operating personnel. In addition, the face masks were changed each hour, and the sterilized gloves were changed each time there was a procedural violation that might have caused increased microbial loading of either the facility or the hardware. These procedures of cleaning, scrubbing, dressing, changing, etc. reduced the available working time by 1 to 1-1/4 hr/day/man.

B. Assembly

Assembly operations consist of taking kits of electronic components, assembling them on mylar header sheets and assembly jigs, welding interconnect ribbons, inspecting and testing, and potting (two of the EASL assembled modules were left unpotted for bio-assay). The laminar downflow assembly room included one assembly station, one weld station, one microscopic inspection station, and a two-station potting station in which (1) the potting compound was mixed in a glove box vented to the outdoors and (2) the module was encapsulated in a vacuum impregnator.

A record of the assembly operations was obtained with a 16-mm motion-picture camera using color film. The camera operated at one frame every 4 sec and was used continuously during the time that flight components were handled and at special times, such as maintenance checks.

C. Maintenance

In the laminar downflow area, at the end of every working day, all horizontal upward-facing surfaces were vacuum cleaned and scrubbed with a germicide. Floors in the dressing room, washroom, and OSE receiving and decontamination areas were damp mopped with a germicide. The cleaning sequence was from the bio-assay room and assembly room to the dressing room, washroom, and OSE receiving area; this sequence was a reverse of the working traffic pattern. Four separate buckets of germicidal cleaning solution were used for the bio-assay room, assembly room, clean side of the dressing room, and all other areas.

Once each week, in the laminar downflow area, all vertical and downward-facing horizontal surfaces were vacuum cleaned and scrubbed with a germicide.

IV. SUPPORTING OPERATIONS

A. Microbiological Monitoring and Testing

During the course of EASL operations, the particulate and microbial profiles of the facility under normal working conditions were determined, assembled hardware was assayed, sterilization and decontamination activities were monitored, and some personnel studies were carried out.

1. Facility Monitoring

A brief recapitulation of the microbial and particulate requirements for the hardware assembly area is as follows.

a. Level of airborne microbial contamination. The concentration of viable particles in room air shall not exceed an average of two viable particles per ft^3 of air for any 10 successive samples (Ref. 1).

Volumetric air samples were collected with a Reyniers slit sampler (Fig. 7). Each sampler was calibrated to operate at an air-sampling velocity of 1 ft^3/min. Trypticase Soy agar was used for all volumetric air sampling.

b. Level of total particle contamination. For the Class 100 clean room, the particle count shall not exceed a total of 100 particles (0.5 μ and larger) per ft^3 (as specified in Sect. II of this Report).

A Royco particle counter (Model 202) was used to obtain the number of total particles per ft^3 of intramural air. All Royco air samples were collected at a velocity of 0.01 ft^3/min.

Fig. 7. Air-sampling apparatus

c. *Level of microbial contamination on surfaces.* The degree of microbial contamination accumulating on surfaces shall not exceed an average of 200 viable particles per ft^2 of surface (Ref. 1).

Stainless-steel strips, 1.0 × 3.0 × 0.06 in., were used as microbial fallout sampling surfaces. For use, the metal strips were washed with a nonionic detergent, rinsed with distilled water, re-rinsed with isopropyl alcohol, and then terminally rinsed with ethyl ether and allowed to drain dry. The clean stainless-steel strips were arranged as a monolayer on stainless-steel sheets and wrapped with an outer layer of aluminum foil (35 strips per tray). The wrapped trays of strips were sterilized by exposure to dry heat (175° C for 3.0 hr). Following sterilization and prior to use, five of the strips were removed from each tray and assayed as sterility-control check items.

An aluminum-foil-wrapped tray was placed at each of the microbial fallout sampling sites shown in Fig. 8. At each site, the aluminum foil was carefully removed from the tray, and if necessary, the sterile stainless-steel strips were rearranged as a monolayer with sterile forceps. Trays were placed at work height, i.e., on a level comparable to the height at which hardware was being assembled.

For each microbiological assay, five exposed strips were randomly selected from each of the microbial fallout sampling sites. Each strip to be assayed was placed into a separate sterile bottle. A sterile screw cap was replaced on each of the bottles.

To assay each set of five strips from their respective sampling sites, 25.0 ml of sterile 1.0% peptone water were added to each bottle containing an

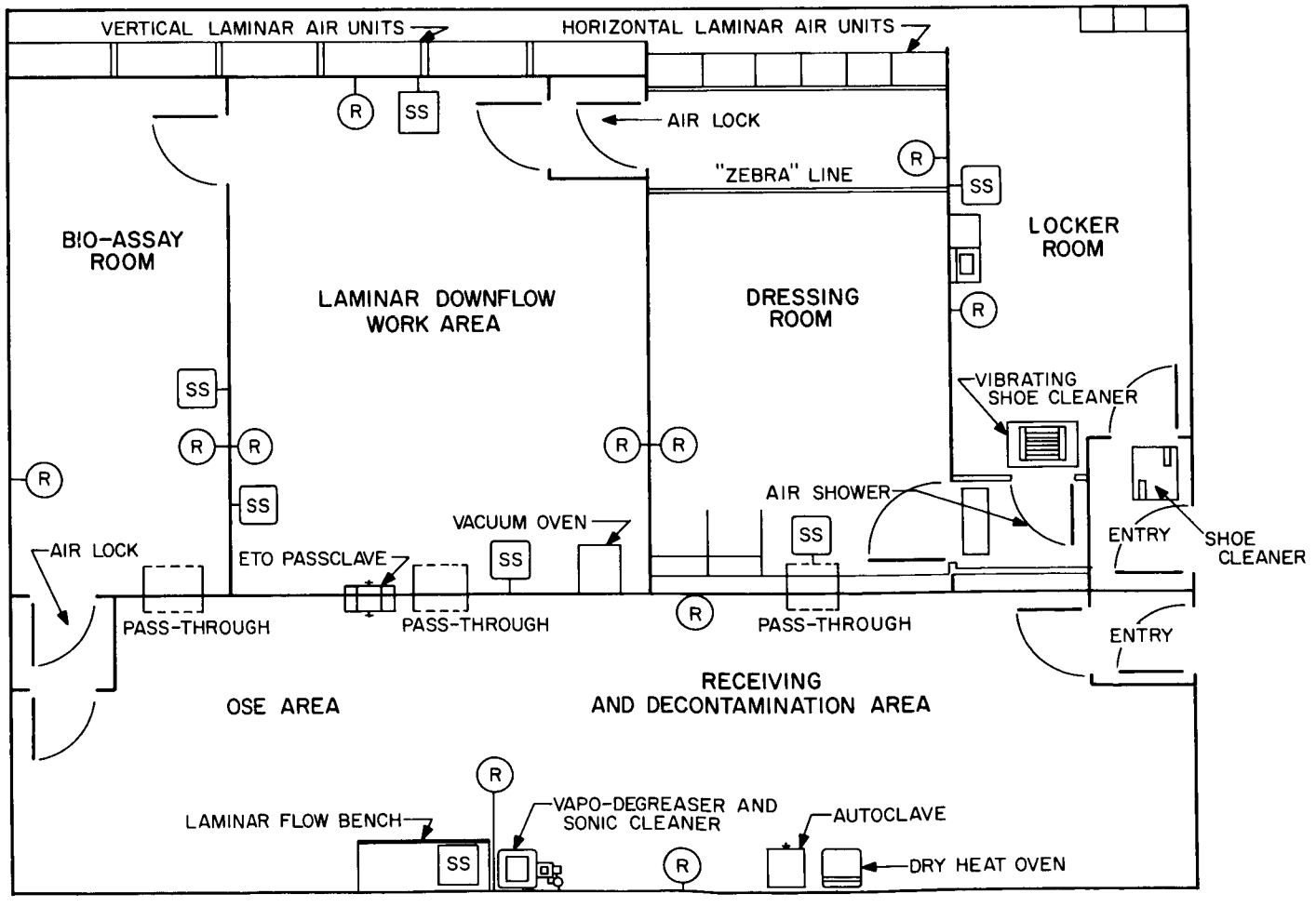


Fig. 8. Locations of microbiological sampling sites in the EASL; Reyniers slit samplers at location R, and trays containing microbial fallout sampling strips at location SS

environmentally exposed strip. Next, the bottle was mechanically shaken for 5 min. After shaking, the five samples in each series were processed as follows. First, the liquid contents of the bottle were aseptically transferred into a sterile 150-mm-D Petri plate; 25 ml of sterile double-strength Trypticase Soy agar were added to the strip rinse liquid, and the contents of the Petri plate were thoroughly mixed; following mixture, the plates were allowed to stand for 15 min at room temperature until the agar solidified. Secondly, all plates were incubated at 32° C for 72 hr; a preliminary colony count was made after 40 hr of incubation, and a final colony count was made after 72 hr of incubation at 32° C.

2. Assay of Assembled Hardware

For assay, each module was shaken in peptone water (30.0 ml), as described above in the section on microbial fallout sampling. However, after the module was shaken in peptone water, a 5.0-ml aliquot of this fluid was placed into each of three 150-mm-D Petri plates. Then, 250 ml of sterile, molten (50°C) Trypticase Soy agar were added to each plate. A 14-ml portion of this peptone water from each module was heat-shocked (80°C/15 min), and two 5-ml portions were plated in 250 ml of sterile, molten Trypticase Soy agar. The larger volumes of agar were used to dilute toxic substances that might have leached from the module during the peptone water rinsing. Finally, the entire module was plated in Trypticase Soy agar. All samples from each module were incubated at 32°C for a minimum of 10 days. After incubation, all negative culture plates were surface-inoculated with an aqueous suspension of *Bacillus subtilis var. niger* spores (*ca.* 100 spores per ml) to determine if bacteriostasis had occurred. Growth of the spore suspension indicated that the medium was capable of supporting microbial growth.

3. Monitoring of Sterilization and/or Decontamination Activities

Bacterial spore strips were inserted with each load to be sterilized by heat or decontaminated with ETO. The materials processed included tools, parts, and other items needed such as paper and cloth. After exposure to a given process, the spore strips were cultured in Trypticase Soy broth.

4. Personnel Studies

Finger-tip cultures were collected by having the subject place the tip of his thumb and tips of his fingers onto the surface of a Petri plate (150-mm-D) containing sterile Trypticase Soy agar. After sampling, the plates were incubated at 32°C for 72 hr and then the number of resultant colonies were counted.

5. Activities of the Visual-Assay Inspector

The visual-assay inspector was on duty inside the hardware assembly area during each period of work (Fig. 9). He documented the personnel hygiene, dressing routine, and operating activities of the people working in the EASL. However, his main function was to observe for breaks in technique (worker using punctured rubber glove, worker dropping tool or part, etc.) or microbial accidents (inadvertent touching of sterile item with contaminated object, sneezing, etc.). When situations such as those described above did occur, the visual-assay inspector provided technical advice and collected microbiological samples if needed. The role of visual-assay inspector was developed considerably during Phase I. This portion is a new approach to the sterilization effort and provides an interface between microbiology and quality assurance. The duties of this person overlap both

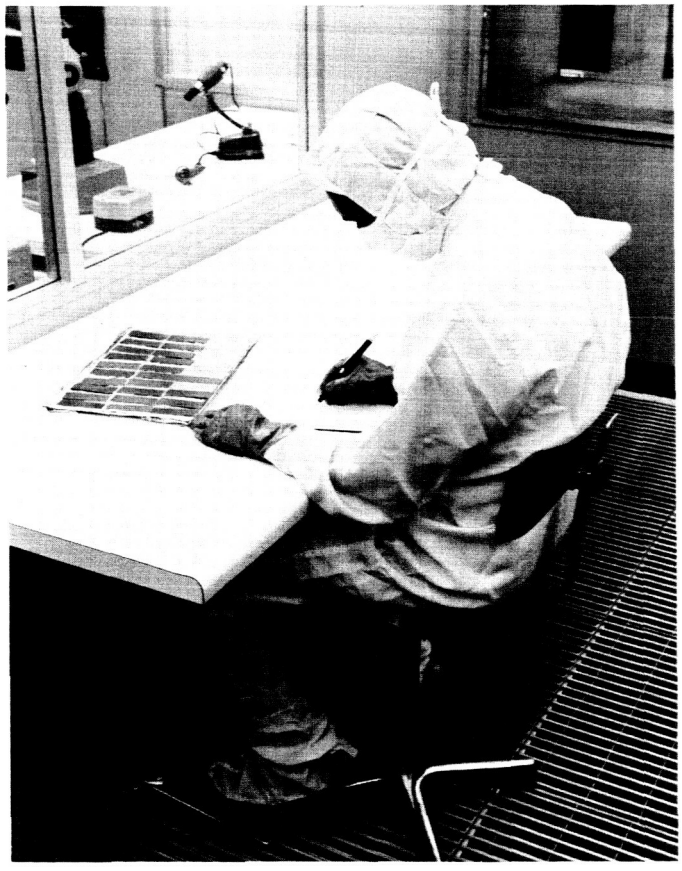


Fig. 9. Bio-assay station; bio-assay room

the disciplines listed above and as such are not clearly defined. The EASL exercise is providing a good working ground to develop fully this job of the visual-assay inspector.

B. Quality Assurance

The EASL Quality Assurance (QA) organization included a QA engineer with supervisory, system initiation, and documentation functions; an assembly-room inspector; and an OSE/decontamination area inspector. QA responsibilities included the receiving inspection, contamination-control monitoring (including decontamination monitoring, visual assay, facility-maintenance monitoring, parametric measurement, etc.), and assembly inspection and test monitoring.

The quality system, as established and implemented, included planning, procedures, and instruction preparation, documentation, and implementation. Drawings, specifications, and parts lists were reviewed; the quality plan, electronic-piecepart screening and mechanical inspection plans, and decontamination/sterilization plans were also reviewed and coordinated with the cognizant personnel.

1. Documentation

The QA specification chart is shown in Fig. 10. The documents used by QA to control the flow of the hardware consisted of the following:

a. Unit Traveler (UT), reflecting every operation from receiving to shipping. (A space was included for every operation for which QA acceptance was required.)

b. Inspection Report (IR), used by QA Mechanical Inspection as a permanent record of inspection of mechanical parts.

c. Part Quality-Assurance Report (PQAR), used by PQA as a permanent record of visual inspection on electronic piece parts.

d. Non-Conforming Material Report (NCMR), used by QA in EASL to document discrepancies and make disposition of discrepant items.

e. Problem/Failure Report (P/FR), used to document any event of a suspicious nature concerning hardware or software requirements or contamination control.

f. Facility Decontamination List (FDL), used as a permanent record of all decontamination/sterilization events.

g. Assay Report (AR), issued by the microbiologist as a permanent record of bio-assay.

h. Module Log, a permanent log maintained by QA per module, containing a copy of every document (items a through g) written for the module by serial number.

2. Implementation

Figure 11 is a typical flow plan of hardware assembled in the EASL. No provision is made in the plan for rejected hardware. All discrepant items were documented and disposition made with NCMR (d).[1] The disposition could be "to scrap," "to rework," or "to accept as is." If a particular item was accepted, the traveler was stamped by the QA inspector.

The electronic and mechanical piece parts were inspected by the QA support groups. Since they are not part of EASL, a PQAR (c) or IR (b) was supplied to EASL on accepted material.

The EASL receiving-inspection group verified that the initial inspection requirement had been met and performed inspection for shipping damage. If items were accepted, the inspector stamped the UT (a).

Sterilization and decontamination were witnessed by QA and, if accepted, the FDL (f) was stamped.

Material was bonded until the microbiologist issued AR (g). Then the UT (a) was stamped.

As required, the material was released to the assembly area where QA performed in-process inspection, stamping the UT (a) at every inspection point if accepted.

Electrical testing of the module was witnessed by QA.[2] If the test was accepted, UT (a) was stamped.

Module potting was witnessed by QA. If accepted, UT (a) was stamped.

[1] Lower-case letters in parentheses refer to listing of QA control documentation on this page.

[2] This section describes the intended EASL operations; however, testing was waived in this phase (see Sect. V-A).

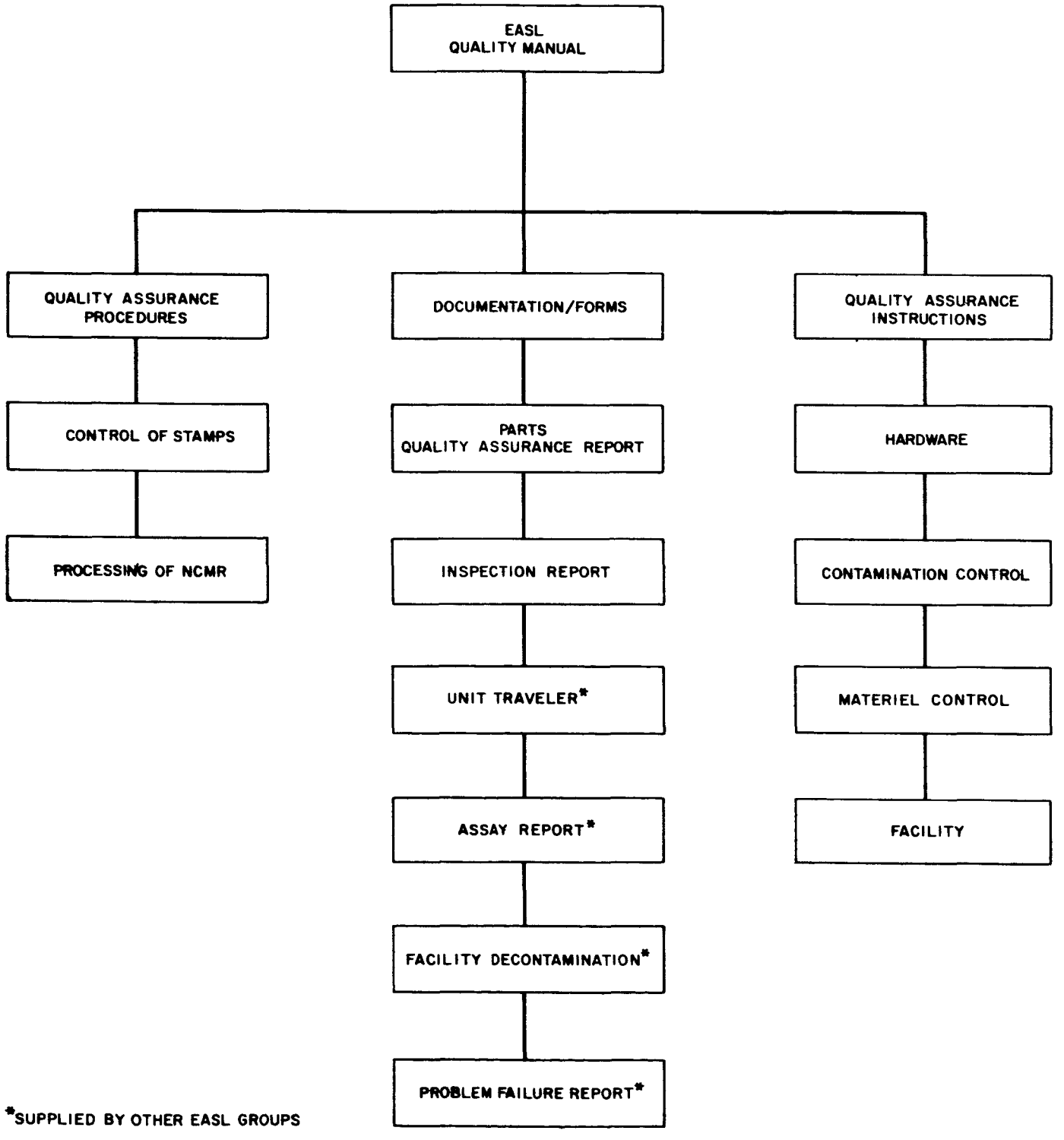


Fig. 10. Quality-assurance specification chart

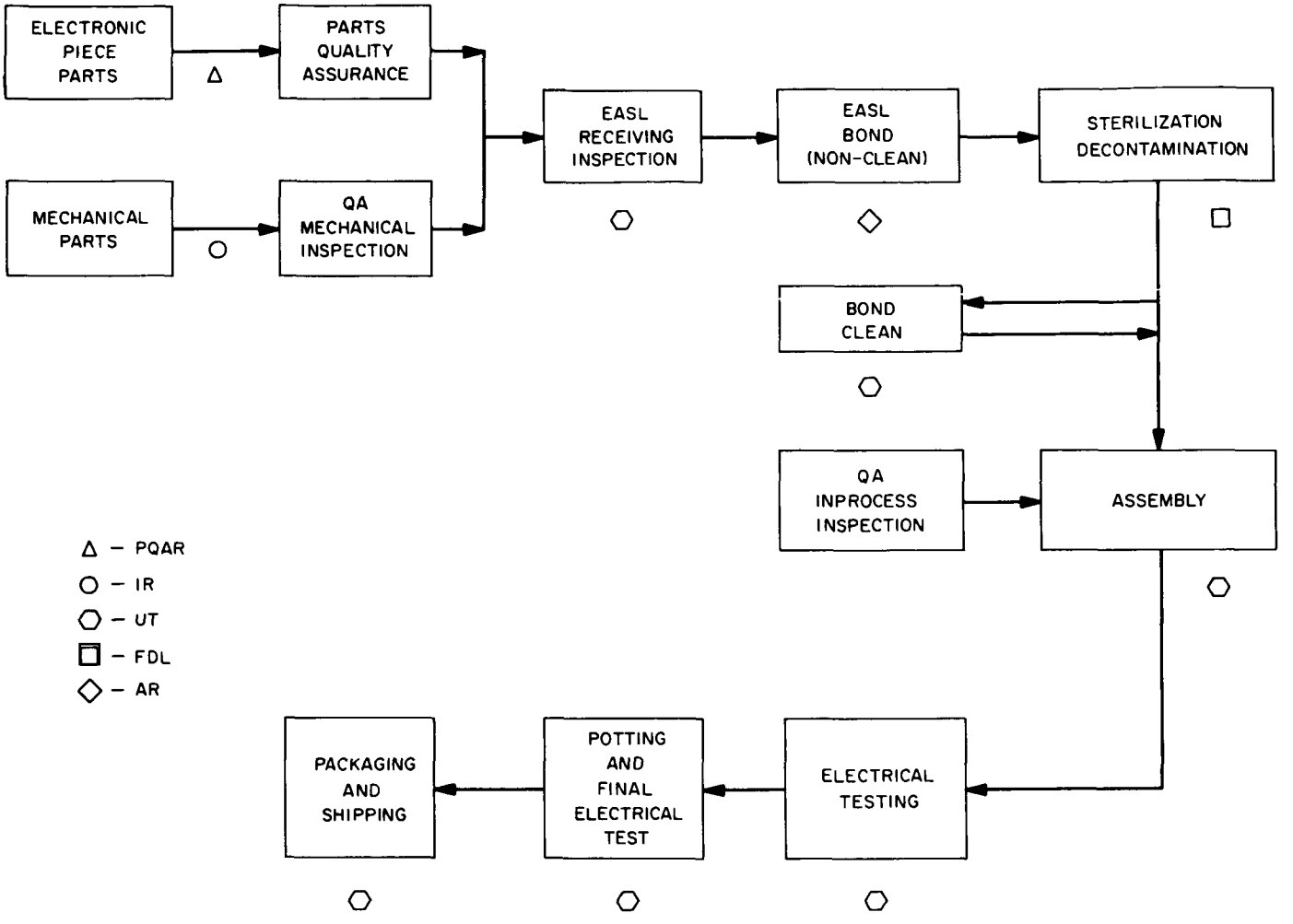


Fig. 11. Typical hardware flow-plan EASL project

The module was inserted into the biological barrier container and shipped to the biologist for assay. If the insertion procedure was accepted, QA stamped the UT (a).

3. Parametric Measurements

The temperature, pressure and relative humidity was measured independently by QA every hour during working day to verify that the facility remained within its designed parameters. The P/FR was used to document deviations of the parameters from the limits.

4. Visual Assay

Personnel procedures were monitored to ensure that the method for entering, working in, or leaving the laminar downflow room was followed. Violations were documented using the P/FR. This was a backup tool to the bio-assay.

5. Sterilization/Decontamination

QA monitored every decontamination/sterilization event as follows:

a. Preparation of items.

b. Setting of temperature pressure and humidity as specified.

c. Insertion of item and spore strips into chamber.

d. Checking to see that chamber did not deviate from setting.

e. Verifying the time as specified.

f. Removal of items and spore strips from chamber.

g. Stamping of the FDL if operation was accepted.

6. Traceability

Each sterilized article was listed by serial number on the FDL. When the articles were received into EASL, the QA inspector verified that the sterilization process was accepted as evidenced by the acceptance stamp by serial number on the FDL.

At the time the article was required in the laminar downflow room, the QA inspector again verified that the article was sterilized and the facility operator noted the date of entry in the laminar downflow room. When the article was removed, the date was noted and that portion of the FDL was closed out.

In certain cases where serialization was not possible, thermal indicating tape was used on the packaging. Stripes appeared if the tape reached the sterilization temperature, and if the process was accepted, the inspector stamped the tape.

This system allowed identification of the contamination status of all articles in the EASL.

V. OPERATIONAL RESULTS

Four welded cordwood modules were built in the EASL during Phase I operations. Four others were built under normal cleanroom conditions as a control. Half of each group was inspected and encapsulated, and the other half was inspected and stored, pending electrical testing and microbiological assay.

A. Hardware Testing

The in-process and final inspections showed the modules were typical of flight-quality hardware, and the modules were placed in quarantine while awaiting electrical testing. However, for administrative reasons and coupled with the need for microbiological data on the modules, the electrical tests were waived.

B. Microbiological Testing

Microbiological results were obtained in the areas of facility monitoring, assembled hardware assays, and personnel studies.

1. EASL Facility Monitoring

a. Microbiological sampling of air. During the Phase I operation in the EASL, sequential air samples were collected during a total of 40 days. This represents a total of some 1330 samples. Table 1 contains a set of typical results of microbiological sampling of the air in the EASL. These results indicate that the number of airborne viable particles in a vertical laminar-flow air

Table 1. Typical results of microbiological sampling of air within the EASL

Area sampled	Time sample collected (Aug. 31, 1965)	Volume of air sampled (ft^3)	Number of viable particles per volume of air sampled	
			Average per ft^3	Range per ft^3
Locker room	0905–1905	600	0.96	0.00–7.06
Dressing room	0910–1910	600	0.05	0.00–0.26
Laminar-flow work area:				
Site 1	0935–1835	540	0.00	0.00
Site 2	0935–1835	540	0.001	0.00–0.06
Site 3	0940–1840	540	0.001	0.00–0.06
Bio-assay station	0940–1840	540	0.00	0.00
Receiving/decontamination area	0905–1905	600	0.44	0.00–1.93

stream is extremely low (0.00 to 0.06 ft^3; average is 0.001 per ft^3 of air), considerably below the acceptable level of 2.0 per ft^3 (Ref. 1). Results similar to those shown in Table 1 were obtained during the other 39 days that volumetric air samples were collected.

Items, such as tables that stop the laminar flow of air in localized areas, cause turbulence to occur beneath the item stopping the air flow. To determine if microbial contamination was occurring or accumulating under work benches, four Reyniers samplers were placed beneath the table holding the arc welder. The samplers were approximately 24 in. from the floor and on a diagonal line across and beneath the long side of the table. A technician sat at this table and operated the arc welder for some 3 of the 5+ hr that constituted the sampling period. Results of this test are shown in Table 2. From the data contained in this table, it would appear that the air beneath the table was as uncontaminated as air throughout the vertical laminar air-flow room. However, since the operating technician was clothed in an ETO decontaminated bunny suit and had a sterile mask and sterile rubber gloves on, it is very likely that the dissemination of microorganisms from him was extremely low. Therefore, there probably was little contamination in the air beneath the bench because there was a very low source of microbial contamination to start with. Studies such as this will be expanded and repeated in the Phase II EASL operation.

b. Total particle contamination. Air samples collected with the Royco particle counter generally indicated that the EASL facility met the particulate level specified in Sect. II of this Report. However, the Royco counter has a very low sampling velocity (0.01 ft^3 of air per minute) and one wonders how representative a given sample may be.

The EASL has roughly 300 ft^2 of ceiling surface in the vertical laminar air-flow rooms. Thus, there is a column of air 300 ft^2 moving at a speed of about 85 ft per minute. Removal of a sample of this air stream through a tube having a diameter approximately that of a soda straw at a velocity of 0.01 ft^3 per minute hardly constitutes an adequate sample; yet the Royco counter is one of the more popular devices used to monitor levels of particulate contamination in clean rooms. The same sampling problems are encountered with the Reyniers samplers. Clearly, sampling devices or procedures must be up-dated for use in laminar air-flow clean rooms.

c. Level of microbial contamination on surfaces. A summary of the microbial fallout studies is presented in Fig. 12. A rise in the level of microbial contamination on surfaces occurred in the vertical laminar air-flow rooms on the 7th day of exposure of stainless-steel surfaces. During this period, heavy rains resulted in the flooding of the floor in Bldg. 233. Workmen entered the area to affect repairs. Several workmen were not clothed according to the EASL clothing regulations, and these improperly clothed people probably caused the transient rise in surface contamination. Other unexplained rises in contamination occurred in these rooms, but the level of contamination was around 40 microorganisms per ft^2 of surface, considerably below the acceptable level of 200 ft^2 (Ref. 1). Microbial contamination on surfaces

Table 2. Results of special microbiological sampling of air within the EASL

Area sampled	Time sample collected (Sept. 17, 1965)	Volume of air sampled (ft^3)	Number of viable particles per volume of air sampled	
			Average per ft^3	Range per ft^3
Locker room	0905–1405	300	0.55	0.00–1.73
Dressing room	0910–1510	360	0.00	0.00
Laminar-flow work area:*				
Site 1	0915–1430	300	0.004	0.00–0.06
Site 2	0915–1435	300	0.00	0.00
Site 3	1020–1430	240	0.005	0.00
Bio-assay station	1020–1430	240	0.00	0.00
Receiving/decontamination area	0900–1400	300	0.86	0.00–2.26

*Sites 1, 2, and 3 were located under arc welder's table at elevation of approximately 24 in. from the floor. Samplers were placed on a diagonal line across and beneath the length of the table.

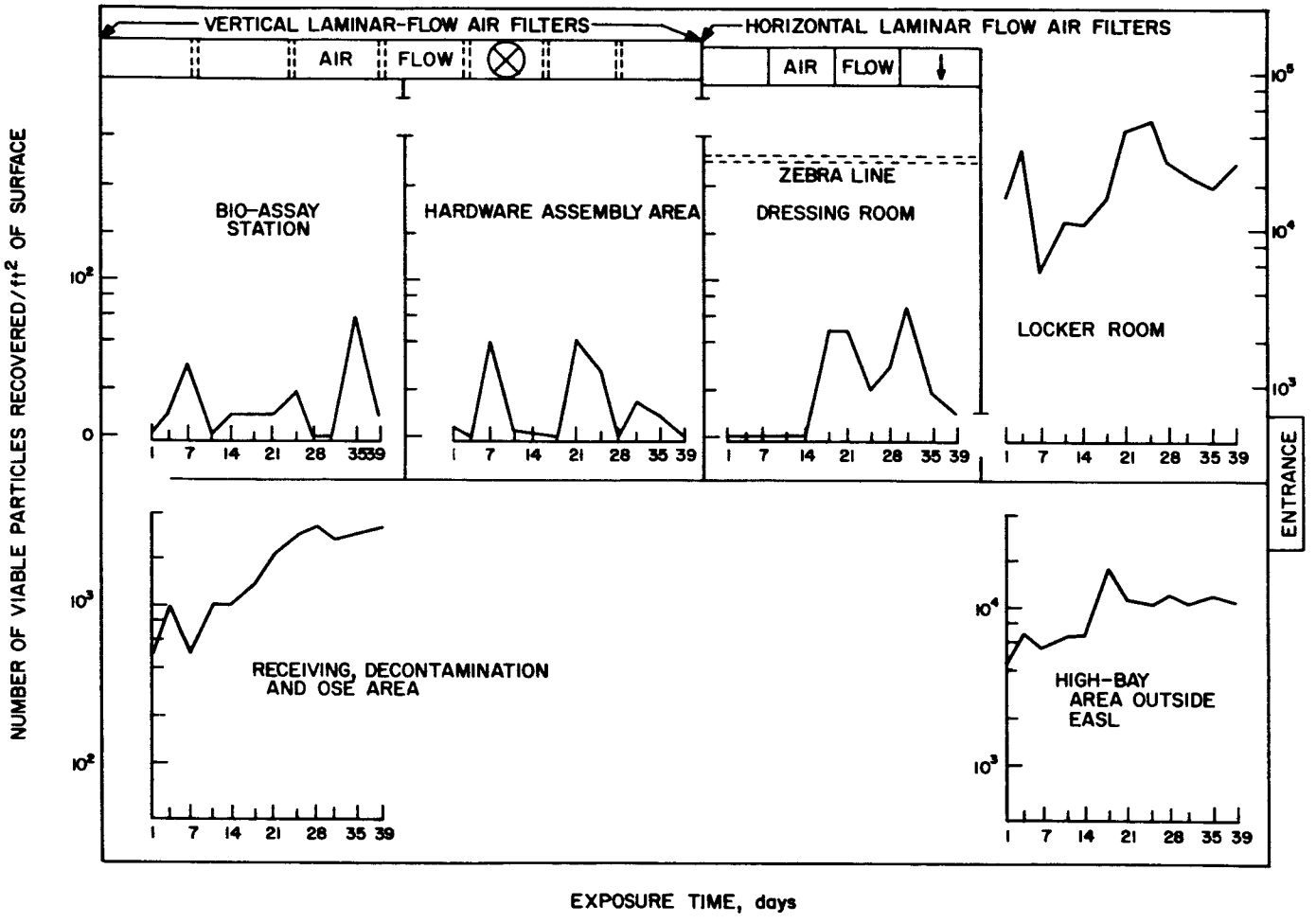


Fig. 12. Results of microbial fallout sampling studies conducted in the EASL

outside of the vertical laminar air-flow rooms was considerably higher (up to 50,000 microorganisms per ft^2 in the locker room), ranging from two to three orders of magnitude higher than levels within the bio-assay station and the hardware assembly area. These data indicate that the strip method of sampling microbial contamination on surfaces is reliable and fairly sensitive.

2. Microbiological Assays of Assembled Hardware

Table 3 contains the results of microbiological assays done on hardware assembled in JPL Bldg. 18 and also in the EASL. Modules, No. 1 and 2, were assembled in JPL Bldg. 18. Due to several conditions, these modules were not assayed immediately after assembly. However, the modules built in JPL Bldg. 18 were assayed some 8 to 12 weeks after assembly. No special precautions were taken to protect these modules from microbial contamination during this 8- to 12-week period. Modules, No. 3 and 4, were assembled in the EASL and assayed approximately 10 to 14 days after assembly. Precautions were taken to protect the EASL-assembled modules during this 10- to 14-day period. Delay in assay was necessary as the assembled units had to be tested electrically before the microbiological assays could be conducted. Results of these assays are most interesting. Viable microorganisms were recovered from the modules assembled in Bldg. 18, but no viable microorganisms were recoverable from the modules assembled in the EASL. The 10- to 14-day quarantine of EASL-assembled modules could have resulted in a considerable "die-away" of surface-exposed vegetative bacteria. Also, the method of assay may be relatively insensitive when the number of contaminating microorganisms is low. In any case, more data are necessary to evaluate more fully these results.

3. Monitoring of Sterilization or Decontamination Cycles

Some 200 spore-strip sterility test indicators for moist heat (15 lb steam (121°C) and ETO sterilization/decontamination processes were cultured. All sterility check items were sterile.

4. Personnel Studies

Some 30 to 40 finger-tip cultures were taken of the assembly technicians' gloves. These data showed that sterile gloves become contaminated during use. Such contamination appears to be at a low level. However, if the glove touches human skin or hair, such "microbiological accidents" usually resulted in a considerably higher level of contamination on the workers' gloves.

C. *Quality-Assurance Results*

1. Quality of the Hardware

a. Switch Logic #3 S/N 001 (4201416) had one rejection which was reworked and accepted.

b. Telemetry Buffer #4 S/N 001 (4201415) was scrapped for bad welds due to improper alignment of electrodes.

Table 3. Results of assays for microbial contamination on surfaces of welded cordwood modules built in Bldg. No. 18 and the EASL

Module assayed	Number of microorganisms (surface)				
	Peptone water aliquot			Plated module	Total per module
	Aerobes	Anaerobes	Heat-shocked		
No. 1 (Bldg. 18)	46	ND[a]	ND	19	65
No. 2 (Bldg. 18)	211	ND	ND	27	238
No. 3 (EASL)	0	0	0	0	0[b]
No. 4 (EASL)	0	0	0	0	0[b]

[a] ND: Not Done.
[b] Medium not bacteriostatic; confirmatory spore inoculum grew out on all plates.

c. Telemetry Buffer #4 S/N 003 (4201415) was accepted.

d. Power Switch S/A S/N 001 (4201408) was accepted.

e. Gyro Switch S/N 001 (4201419) was accepted.

2. Contamination Control Problems per Module*

a. Switch Logic #3 S/N 001

1. Personnel violations 15
2. Facility problems 9
3. Operational problems 9

b. Telemetry Buffer #4 S/N 303

1. Personnel violations 1
2. Facility problems 3
3. Operational problems 6

c. Power Switch S/A S/N 001

1. Personnel violations 5
2. Facility problems 2
3. Operational problems 2

d. Gyro Switch S/N 001

1. Personnel violations 3
2. Facility problems 3
3. Operational problems 9

*These modules were not fabricated in simple sequence; contamination control problems decreased with time.

VI. PROBLEMS ENCOUNTERED

In the Phase I operations, one of the main concerns was the identification of gross problems for future facilities. Many problems were encountered with the equipment, facility, and operation procedures. The major facility problem was the repeated failure of the air conditioning, which delayed operations and affected the microbiological experiments. All other problems found in the operations were considered minor and were resolved easily. The problems encountered are listed below.

A. Facility

The facility air-conditioning system failed 20 times in the first 30 days of operation, with a temperature rise to 80–90°F in 10–15 min and usually a down time of 2–3 hr. This not only delayed operation but permanently affected the long-term microbiological experiments. The failures were caused by two problems:

1. A malfunctioning relay in the EASL air-conditioning control circuitry.
2. Use of the Bldg. 233 chiller unit for the EASL air conditioning. This unit had sufficient excess capacity to meet the EASL requirements and offered a cost saving for the EASL facility construction. However, it was found to have poor operational reliability and a history of breakdowns.

De-ionized water bottles were located within the facility (in the OSE area) causing inconvenience in replacement and risk of water spillage in a working area.

Many doors lacked spring pneumatic closers, and could be left ajar and then slammed by air pressure when doors to high-pressure areas were opened. This caused damage to some doors.

Airlocks lacked windows or emergency lighting, and electrical interlocks failed in a mode locking both doors. A power failure would lock personnel in working areas, and, if in an airlock, these personnel would be in the dark and invisible from outside.

Emergency lights in the facility were judged insufficient.

Door and window moldings projected into the laminar downflow rooms, disrupting the airflow and accumulating dirt.

Three problems were noted with the pass-throughs:

1. Door latches failed through elongation of the locking slot in the aluminum channel door frame.

2. The door interlocks failed (permitting both doors to be opened at once) due to a design problem in the interlock push rod mechanism.
3. Only one pass-through was provided for two-way traffic between the OSE area and each "clean" room. (The interim solution was to divide each pass-through with a vertical stainless-steel separator.)

The plastic diaphragm speak-throughs and intercom speakers were located above eye level of most personnel, making their use difficult.

Noisy equipment, such as shoe cleaners and vacuum pumps, were located in working areas without adequate acoustic isolation.

The foot-pumped soap dispenser was not compatible with the viscous germicidal soap that was used.

B. Furniture

All stainless steel chairs with a friction height adjustment were used in the laminar downflow area. Because of the grille floor, they had to be lifted to be moved; on lifting, the height adjustment changed. They were also found to be cold and uncomfortable.

C. Maintenance

Free-standing, swinging-lid trash receptacles were used in the washroom, dressing room, and OSE area; instead of receptacles, the pass-throughs served for trash disposal from the laminar downflow area. Built-in, one-way, pass-through-type trash receptacles would be preferable for the laminar flow rooms.

The vacuum cleaner used for cleaning the laminar flow areas was operated through hose parts to the dressing, assembly, and bio-assay rooms, with the cleaner in the OSE area and a decontaminated hose used in the rooms being cleaned. This was an inconvenient procedure; a built-in vacuum-cleaning system would be preferable.

Access for maintenance to installed equipment was frequently difficult.

D. Operations and Personnel

The only real problem found in the assembly operation was that after starting work, the assembler would require that items which had been forgotten be placed in the tool and part kits. This occurred despite the fact that while work was being done in the conventional clean-room, a work item list was made to prevent this omission.

The first type of glove used was a close-knit dacron glove. These gloves were soon found to be slick and a change was made to a loose fitting, disposable plastic glove. The plastic glove was much better than the first type and was used for the balance of Phase I operations.

There were no mirrors either in the washroom or the dressing room. This caused minor inconveniences, particularly in checking correctness of dress for assembly room operations.

E. Surveillance

Observation windows in the laminar downflow area were judged to give insufficient coverage of the regions to be watched; work at the first assembly station could not be seen from the OSE area, for example.

Camera surveillance, as utilized by EASL, proved to be less than satisfactory. The frame speed (1/4 frame/sec) was not adequate to catch significant movement of personnel in the laminar-flow room. Also contamination violations could be missed when personnel were working with their backs to the camera.

Calibration of certain equipment was conducted for conditions not corresponding to their operating environment, and some errors were permitted. Specifically, the portable humidity recorders used in the laminar airflow areas had to be protected from the air flow to give correct readings.

F. Decontamination

In the dry heat cycle used for decontaminating some tools, the temper of some metal parts was ruined by the heat soak. In the moist heat cycles applied to some tools and some parts, corrosion was in evidence for metallic leads and some tools.

The heat-sterilizing oven in the assembly room, not a circulating air design, showed a 40°F differential within its cavity.

The vacuum pumps used in humidity cycles had to be left operating 5–10 min after close of the cycle to preclude their loading-up with water.

VII. CONCLUSIONS AND RECOMMENDATIONS

A. Phase I Evaluation

1. Facility and Operation

EASL Phase I operations demonstrated the feasibility of laminar downflow and absolute-filter environmental control for meeting the draft "Interim Requirements for Bioclean Facilities" (Ref. 1) on a small scale. The procedures implemented and modified in Phase I are considered to have been adequate to the Phase I task.

Further study is underway to define the sensitivity of cleanliness to operating and personnel procedures, and to find a threshold of cleanliness.

EASL Phase I cleanliness was found as follows:

a. The level of airborne viable contamination within the EASL hardware assembly area ranged from 0 to 0.06 (average 1 per 1,000 ft^3 air) viable particles per ft^3 of air.

b. The level of particulate contamination within the EASL is well below the acceptable level specified in Federal Standard 209.

c. The hardware assembled in the EASL appears to have a very low level of microbial contamination on its surfaces.

2. Microbiological Support

The stainless-steel strip method of microbial fallout sampling appears to be a reliable and sensitive indicator of the level of microbial contamination of surfaces. The level of surface contamination accumulating over a 39-day period reached a maximum value of some 40 microorganisms per ft^2 of surface.

The air-sampling techniques, both for non-viable (Royco) and viable (Reyniers particles), require updating. It would seem that routine sampling of the vertical laminar air-flow stream for viable particles is not practical. Periodic air samples should be collected, and more sequential sampling should be done under conditions where the laminar air-flow stream is interrupted.

Supplemental air samples were collected in the EASL air shower and at the air source of the personnel hand dryer. The bio-assay results indicated that these areas were not major sources of airborne contamination.

Sampling of ETO decontaminated clothing indicated that microorganisms could be recovered from clothing seams and folded areas. This could be due to a number of reasons, such as insufficient penetration of the gas, too low a gas concentration, ETO resistant microorganisms, too short an exposure period, etc. The vendor for the EASL was informed of this fact and the product quality improved; further spot monitoring failed to recover any microorganisms.

Microbiological Phase II support of the EASL operation is being designed to add to, and provide, confirmation data for the preliminary results obtained in the Phase I operation.

3. Quality-Assurance Support

Although the EASL was a small program, it proved that Quality Assurance (QA) could use its methodology developed for hardware programs modified to include the requirements of a contamination control program.

These QA activities included monitoring assay, sterilization, and decontamination events, and performing parametric measurements and certification of the facility. However, none of these functions for contamination control in any way required QA to make a judgment whether a given article was microbiologically contaminated. Their judgment was only whether a procedure was followed or a requirement was met.

Therefore, the EASL proved that QA can make a significant and necessary contribution to a contamination control program providing that the following ground rules are observed:

a. They (QA) do not establish the requirements for contamination control.

b. They do participate in the derivation of mechanisms to meet the requirements.

c. Their acceptance or rejection is based on specific and thorough procedures and requirements.

4. Cost

It had been anticipated that assembly costs in terms of man-hours would be greater in the EASL than in a conventional "cleanroom". However, it was found that the

lack of distractions and interruptions permitted comparable work speed in the EASL. Normal breaks and lunch periods were observed, and in no case did assemblers leave the facility except at these times. However, the ancillary operations, including personnel preparation, provision of decontaminated clothing, decontamination of parts, tools, and equipment, surveillance, maintenance, and biological monitoring, were costly.

Decontamination costs are hard to define because there was no previous, established, item-preparation procedure; and various methods were employed to try to optimize a procedure. As a result the labor cost varied considerably. The personnel procedures caused a loss in the total available working time, the loss amounting to 1 to 1¼ hr/man/day. Based on the usage of 100 suits and 50 wiping rags, the cost of laundering, sealing each piece in a plastic bag, and ETO-treating amounted to $423.00/wk.

The film-developing costs amounted to $135.00/wk (average), and three days elapsed before the film was returned. It was also found that the 16-mm lapse-time camera is not a standard item and has to be specially made.

Further study in the EASL program is expected to examine the prospects of reducing the complexity of the ancillary and support operations.

Manpower involved in the EASL daily operation totalled ten (including part-time personnel): the facility manager, test engineer, assembler, microbiologist (part-time), two biological technicians, quality-assurance engineer (part-time), two inspectors, and the janitor. The assembler, one biological technician, one inspector, and the janitor ordinarily worked in the laminar downflow area. A full day's work in this area involved four entry cycles (the janitor needed to enter only once).

B. Recommendations

The problems encountered in EASL Phase I were corrected or resolved to prevent future occurrence.

1. Short-Term

Solutions to certain specific problems are stated or implied in discussion of the problems in Section VI. Solution to, or accommodation of, other problematical conditions is implicit in earlier discussion.

The EASL air-conditioning problem has been solved by purchase of a separate chiller unit which will serve as the independent primary system; Bldg. 233 unit's extra capacity will serve as backup to this system.

The majority of facility problems and all operational problems have been corrected or are being corrected in the EASL by acquisition of new equipment, building work orders, and procedural changes.

2. Long-Term

During the design phase of a new facility such as EASL, all possible problem conditions, specifically including the EASL Phase I problems listed in Section VI, should be considered, evaluated, and precluded. Most of the problem solutions implemented in the EASL were working improvements rather than ideal design solutions.

Specifically, facility environment control should be both independent and reliable. Facility design should be such as to minimize cleaning and operational difficulties and facilitate monitoring and control of all operations. Ease of accomplishing the operational task and the necessary human engineering should be major factors in facility design. The microbiological and quality control and operational plans should be developed concurrently with the facility plan to ensure compatibility; and latitude should be allowed in the facility for reasonable changes in operation.

Personnel training is an important part of the development of an EASL-type capability; for a larger scale operation, the establishment of a formal training course should be considered. All personnel concerned in such operation require at least special familiarization and in most cases training in special techniques and conditions.

Surveillance of all operations should be provided for and facilitated. Observation windows, providing complete visibility from a minimum of observing stations, should be part of the facility. Mechanical surveillance devices, such as cameras or TV equipment, should give better coverage than the single 1 frame/ 5 sec camera used in the EASL Phase I.

One facility feature, which was found to be desirable in Phase I operations, is a bonded decontaminated-parts, tool, and equipment storage room. This would permit pre-operative decontamination of large quantities of material and equipment to be issued as needed from storage.

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ACKNOWLEDGMENT

The authors are indebted to C. D. Baker, M. R. Christensen, W. Paik, and C. Smith for their contributions to this Report, and to J. H. Wilson for his assistance in preparing it.