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Issue 1

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MONTHLY LETTER REPORT

23 June to 23 July 1966

Investigation of the Reliability of Sterile Insertion Techniques for Spacecraft

July 1966

Contract NASW1407

Prepared by
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Approved by

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FOREWORD

This informal monthly letter report is the first of three to be issued in accordance with the requirements specified in NASA Contract NASW1407.

CONTENTS

I. Test Fixtures

Two isolator configurations - one for sterile insertions and one for determining allowable hole sizes in isolators through which external contamination may enter, were designed. The sterile insertion isolator configuration consists of a contaminated room isolator and two internal isolators which will be used as sterile areas. The use of two sterile isolators allows sterile lock and split seam insertions to be made concurrently instead of consecutively as originally proposed by Martin. The sterile insertion isolator is being fabricated by Snyder Manufacturing, New Philadelphia, Ohio, and is scheduled for delivery to Martin on 5 August 1966.

The isolator configuration for determining permissible gross hole sizes in thin film isolators that permit contamination to start is being built by Martin and will be independent of the sterile insertion isolator system. This test can therefore proceed in parallel with the sterile insertion tests rather than as a terminal phase using the sterile insertion isolators.

Support equipment, such as regulators, shutoff valves, bio assaying devices, nebulizers, etc. have been ordered and delivery is anticipated in time for the test set-up which is scheduled to start on 8 August 1966.

II. Test Planning

Test Planning documents for both the sterile insertion tests and the leakage tests have been prepared and issued to cognizant test personnel. During checkout of the test fixtures, which includes some dry runs to verify procedures, the test documentation may be slightly modified to make them consistent with the results obtained. The intent of these

planning documents is to establish procedural controls throughout the duration of the test program. Copies of the planning documents are attached to this report.

III. Reports

A Gnotobiotic Survey Report (VOY-CR-66-5) was compiled and issued in compliance with contractual requirements. This report consists of a concise summary of the development history of gnotobiotic techniques, and a selected list of abstracts relating to gnotobiotic technology, sterilization, and microbiology and space.

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ATTACHMENT #1

TEST PLAN

INVESTIGATION OF STERILE INSERTION TECHNIQUES FOR SPACECRAFT APPLICATION

JUNE 1966

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TABLE OF CONTENTS

FOREWORD

This test plan is issued to satisfy the requirements of Task 2 of Contract NAS W 1407, Investigation of the Reliability of Sterile Insertion Techniques for Spacecraft Application.

I. PURPOSE

Sterile insertion techniques must be developed to permit the replacement or addition of sterile missile or spacecraft components on a sterile vehicle without violating overall sterility or resorting to a dry heat sterilization cycle to re-establish overall sterility after the replacement or addition has been made.

The purpose of this test plan is set forth in the requirements for demonstrating the feasibility on a Go-No-Go basis of making sterile insertions by two different techniques . . . the sterile lock method and the split seam method.

II. TEST OBJECTIVES

The test has the following objectives:

A. Primary

1) Demonstrate the feasibility, by making 20 insertions, of using the sterile lock method to violate without compromising a sterile barrier.

2) Demonstrate the feasibility, by making 20 insertions, of using the split seam method to violate without compromising a sterile barrier.

B. Secondary

1) To establish sterile insertion techniques that are worthy of further development.

2) Demonstrate the feasibility of making electrical and mechanical connections of the sterilely inserted component after it has been inserted into the primary sterile environment.

III. TEST DESCRIPTION

A. Test Fixture

The test fixture is shown in Fig. 1. It consists of a large pressure tight transparent plastic film bag, henceforth known as a room isolator. The room isolator is supported by external structure to maintain its expanded shape. A nebulizer injects a spore cloud of Bacillus subtilis var. niger into the room isolator. An oscillating fan is used to distribute the spore cloud throughout the room isolator.

Located within the contaminated room isolator are two sterile bio-isolators. These pressure-tight isolators are also fabricated from flexible transparent plastic film. Their shape is maintained by applying a pressure of about ½ in. of water with sterile air. Both isolators contain a captive suit with entry from below.

B. Test Specimen

The test specimen, that is to be sterilely inserted, is shown in Figure 2. It consists of a piece of 16 gage type 301 stainless steel to which is brazed a six inch long pair of electrical leads containing an electrical connector. The piece of stainless steel contains four bolt holes. This type of specimen was chosen becuase it is easily assayed by immersion techniques in the laboratory. The electrical connector was chosen because of its heavy knurl which will provide a more severe test for the gloves which are part of the two sterile isolators.

Inside each of the sterile isolators is a mounting truss shown in Fig. 3. The cycle of a sterile insertion consists of inserting a test specimen through a contaminated environment into a sterile one, and

mechanically and electrically mounting it to the truss (Fig. 4) after removing the specimen that was installed on the previous cycle. Biological monitoring of the contaminated environment and sterile isolators will be done during the insertion cycle.

C. Test Set-Up Procedure

The following procedure is to be used to install and make ready the test set-up shown in Fig. 1. Refer to Fig. 5 for the flow of events.

1. Set plywood base of room isolator on two desks or table tops.
2. Support room isolator bag with external supports.
3. Insert thru split seam of the room isolator both bio isolators. Tape captive suits to base of room isolator.
4. Set up Nebulizer, Air Inlet and Air Outlet equipment and plumbing.
5. Install fan.
6. Run plumbing lines and electrical cords thru appropriate nipples in room isolator. Tape lines and cord to room isolator to make tight joint.
7. Attach plumbing lines to appropriate nipples on both bio isolators.
8. Test motors on fan, nebulizer compressor and air inlet pump.
9. Install transfer sleeves
   2 on room isolator
   1 on sterile lock isolator
10. Complete the plumbing to air, N_2, and ETO supplies and ETO/N_2 vent.
11. Install bio isolator air outlet assemblies and flow meters.
12. Pressurize both bio isolators with ETO/Freon and check for leaks.
13. Vent and purge bio isolators.
14. Pressurize bio isolators with air and check flow meter assemblies.

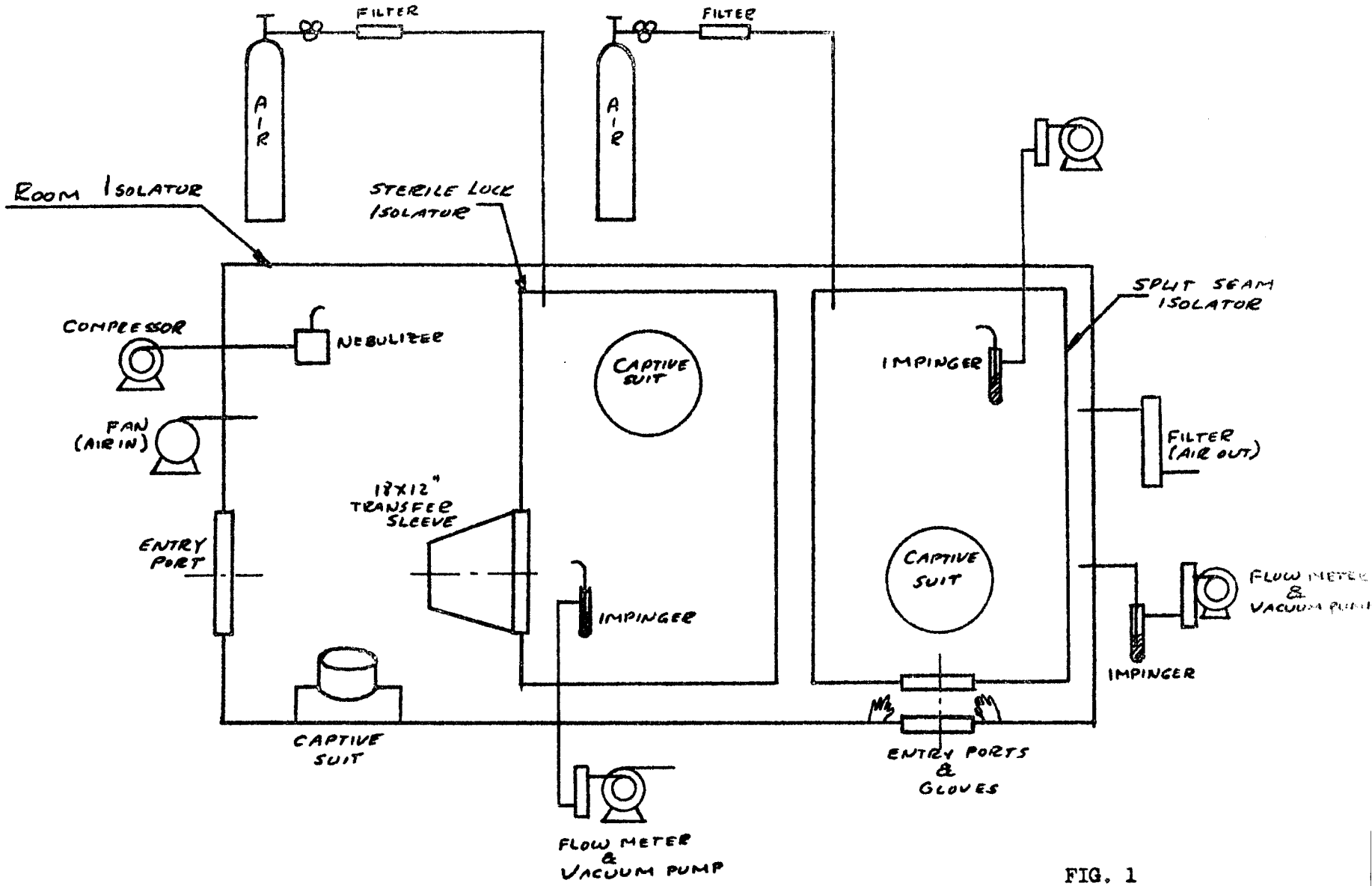
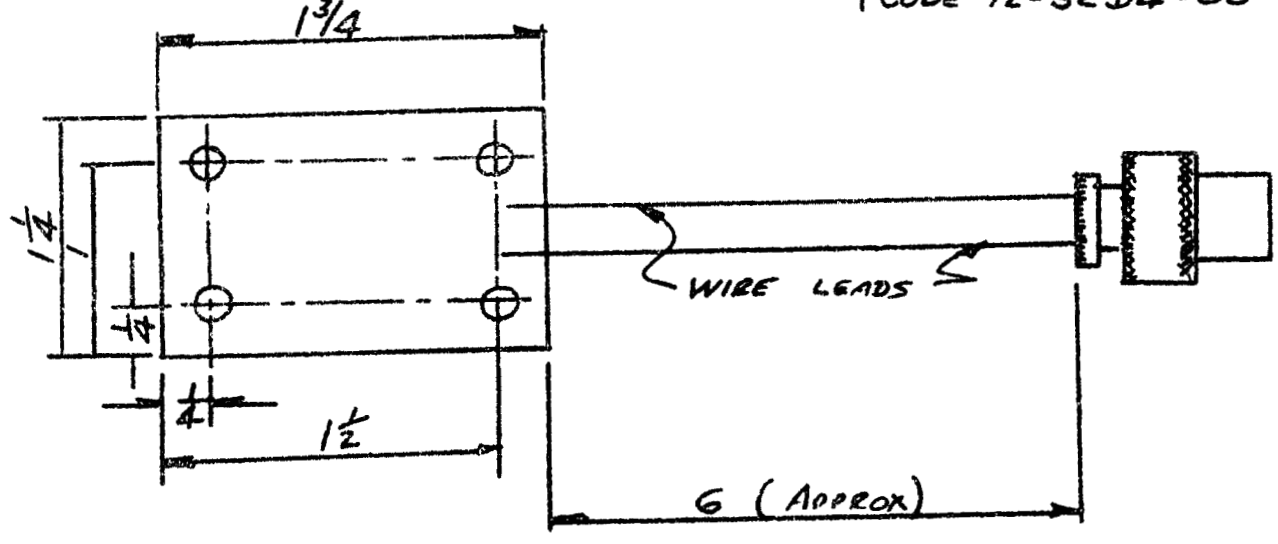


FIG. 1

SCHEMATIC OF TEST FIXTURE

MATERIAL 301 SS SHEET (16 GAGE)
(CODE 12-32D4-00-SH)

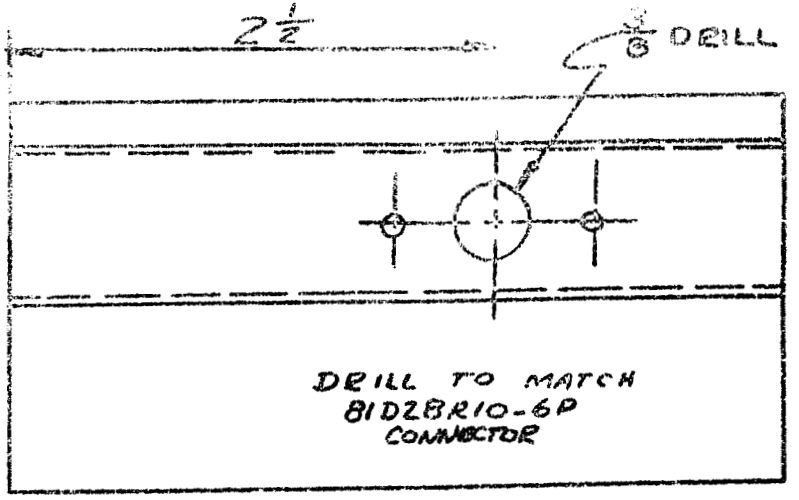
81D33PE10-GS
CONNECTOR



12 REQUIRED

TEST SPECIMEN

FIG 2



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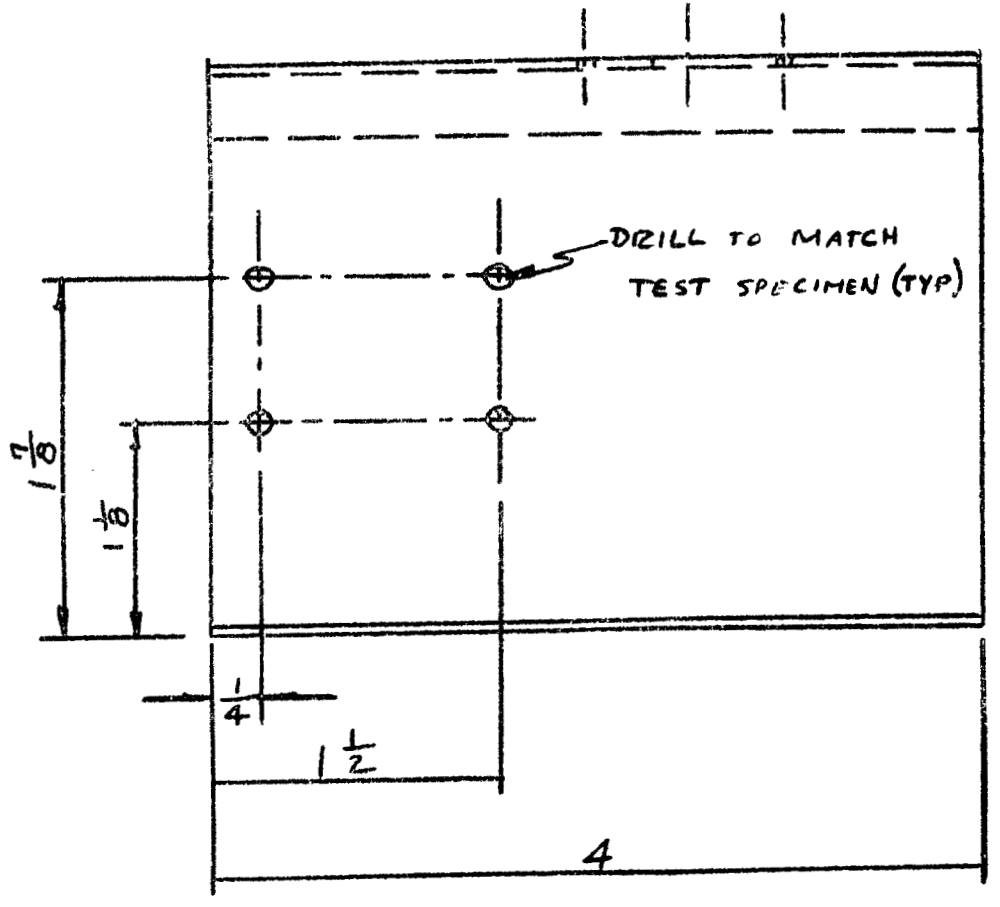
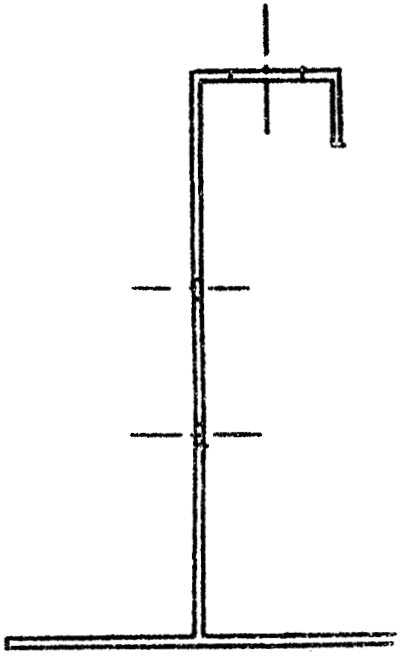
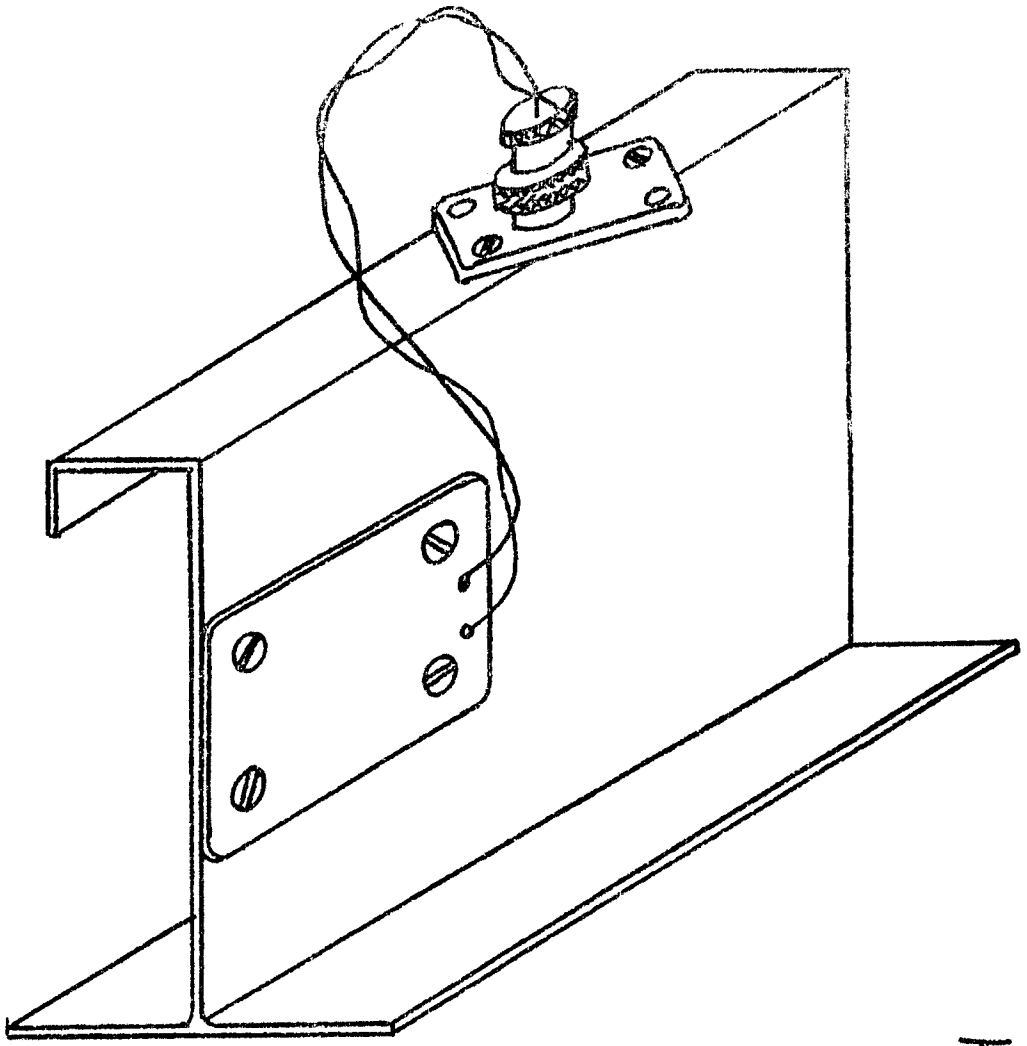
1 REQUIRED





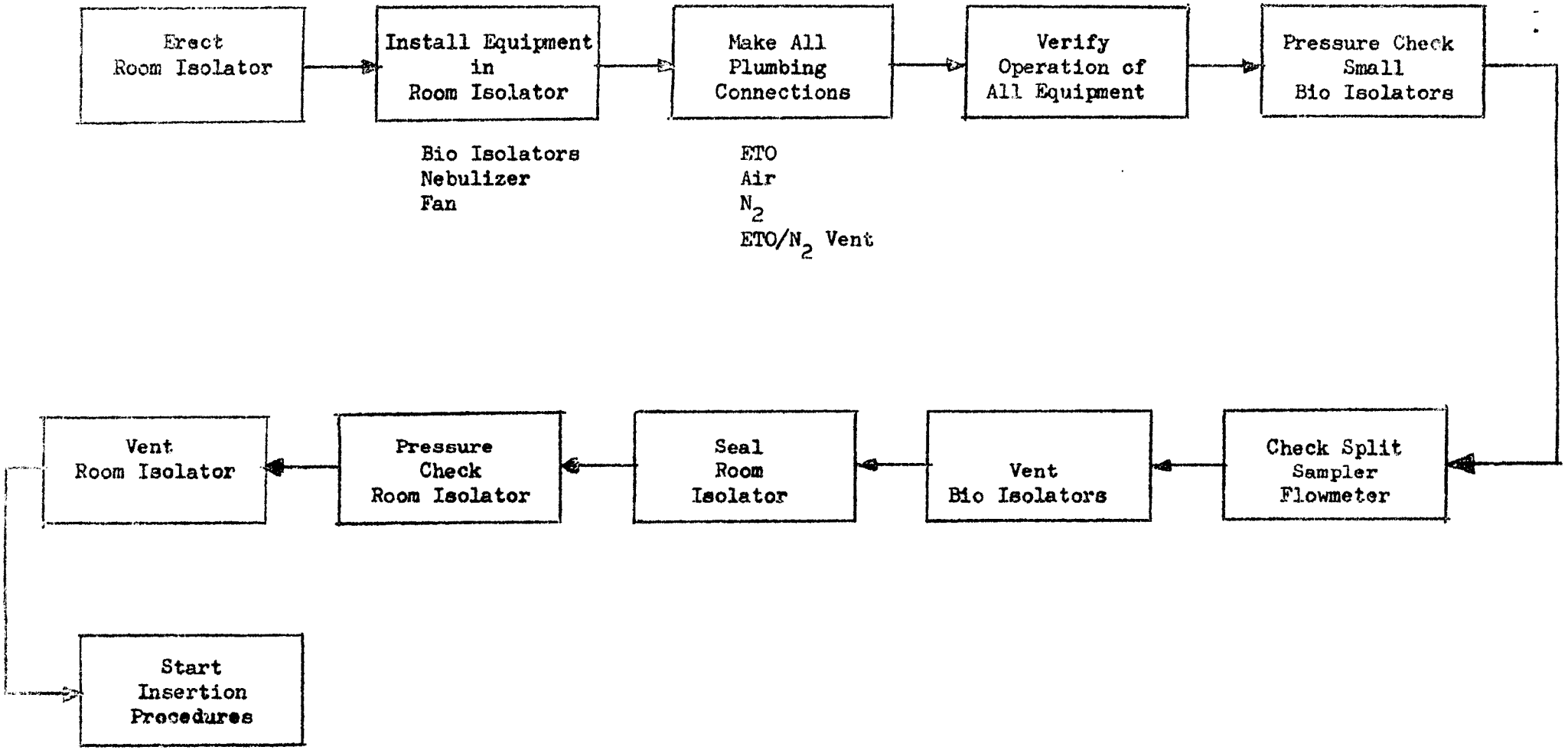
MOUNTING BRACKET
FOR TEST SPECIMEN

FIG 3



TEST SPECIMEN MOUNTED
ON BRACKET

FIG 4



TEST SET-UP PROCEDURE

FIG. 5

15. Seal split seam of the room isolator.

16. Cover all entry and access ports in the room isolator.

17. Pressurize room isolator with freon and check for leaks.

18. After system is vented, add spores to nebulizer.

19. System is now ready for test operation.

D. Sterile Lock Insertion Technique

Twenty insertions and test specimen replacements shall be made with the sterile lock technique. This method has been developed for the science of Gnotobiology and is dependent upon the transfer of sterile items through a contaminated environment via a sterile lock, the volume of which is decontaminated prior to admitting the item to its destined sterile environment.

The step-by-step procedure outlined below and shown in Fig. 6 shall be used to make sterile lock insertions. Color 16 mm motion pictures shall be taken of the general test set-up and one sterile insertion cycle.

1. Place an impinger, 20 field monitors, syringe, tubing, tube clamp, 500 cc of Peptone water, chemical stand and clamp, test specimen, 2 sq. in. template, hand tools, an empty stoppered 500 cc flask and mounting truss in a transfer drum. (May require 2 drums)

2. Autoclave the above for 2 hrs at 140°C.

3. Attach sterile drum to transfer sleeve.

4. Decontaminate bio isolator and transfer sleeve per Appendix A.

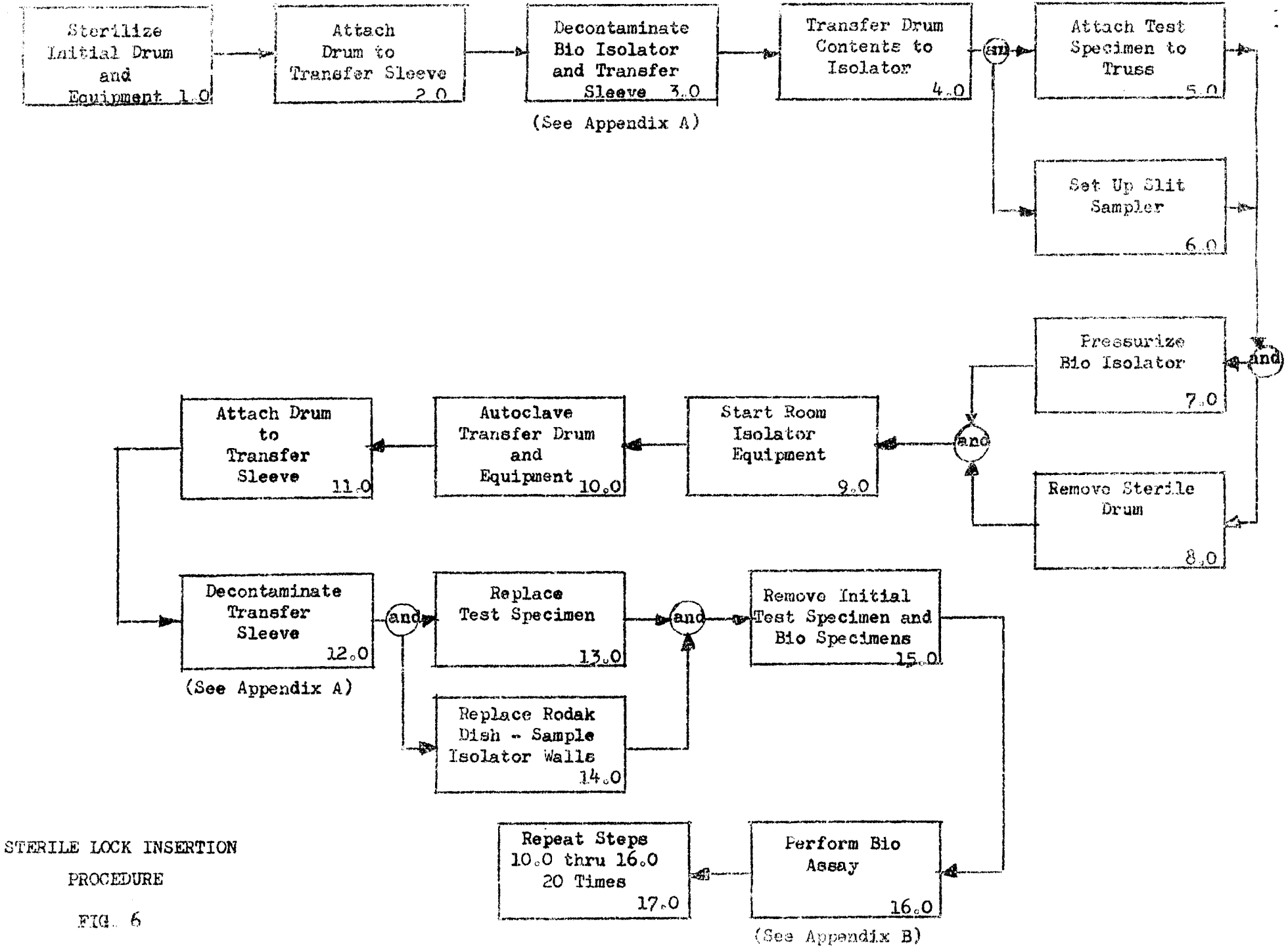
5. Using captive suit break sterile drum transfer sleeve interface.

6. Remove contents of sterile drum.

7. Set up the truss and mount the test specimen.

8. Set up impinger on chemical stand with ring clamp.

9. Hook up impinger to vacuum pump.



STERILE LOCK INSERTION

PROCEDURE

FIG. 6

10. Cap inner side of entry port of the bio isolator.

11. Pressurize isolator.

12. Remove the sterile drum.

13. Cap the inner side of the entry port of the room isolator.

14. Start room isolator pump.

15. Start nebulizer compressor.

16. Turn on fan.

17. Autoclave transfer drum containing 5 sealable plastic bags, test specimen and hand tools.

18. Place sterile drum and 3 swube tubes in entry port of room isolator.

19. Cap outer opening of entry port.

20. Remove inner cap and attach drum to transfer sleeve. Insert swube tubes in transfer sleeve.

21. Decontaminate transfer sleeve per Appendix A.

22. Pour 15 cc of Peptone water into impinger.

23. Start vacuum pump and monitor flow meter for flow rate of 12½ liters per minute.

24. Remove inner cap from isolator entry port.

25. Transfer contents of drum and sleeve to isolator.

26. Replace test specimen on truss.

27. Seal replaced test specimen in plastic bag.

28. Using template and swube tubes with 15 cc of Peptone water, swab 2 sq. in. area of each inner surface of the isolator. Use one swube tube on two surfaces.

29. Identify swube tubes as to surfaces swabed.

30. Seal swube tubes in plastic bag.

31. Place plastic bags and specimen back in transfer drum.

32. Turn off vacuum pump.

33. Using syring draw Peptone water in impinger through monitor.

34. Discard Peptone water into empty flask which will be used for all discarded water.

35. Place monitor in plastic bag.

36. Place bagged monitor in drum.

37. Cap inner side of bio-isolator entry port.

38. Insert drum in entry port of room isolator.

39. Cap inner side of entry port of room isolator.

40. Decontaminate entry port.

41. Remove drum from entry port.

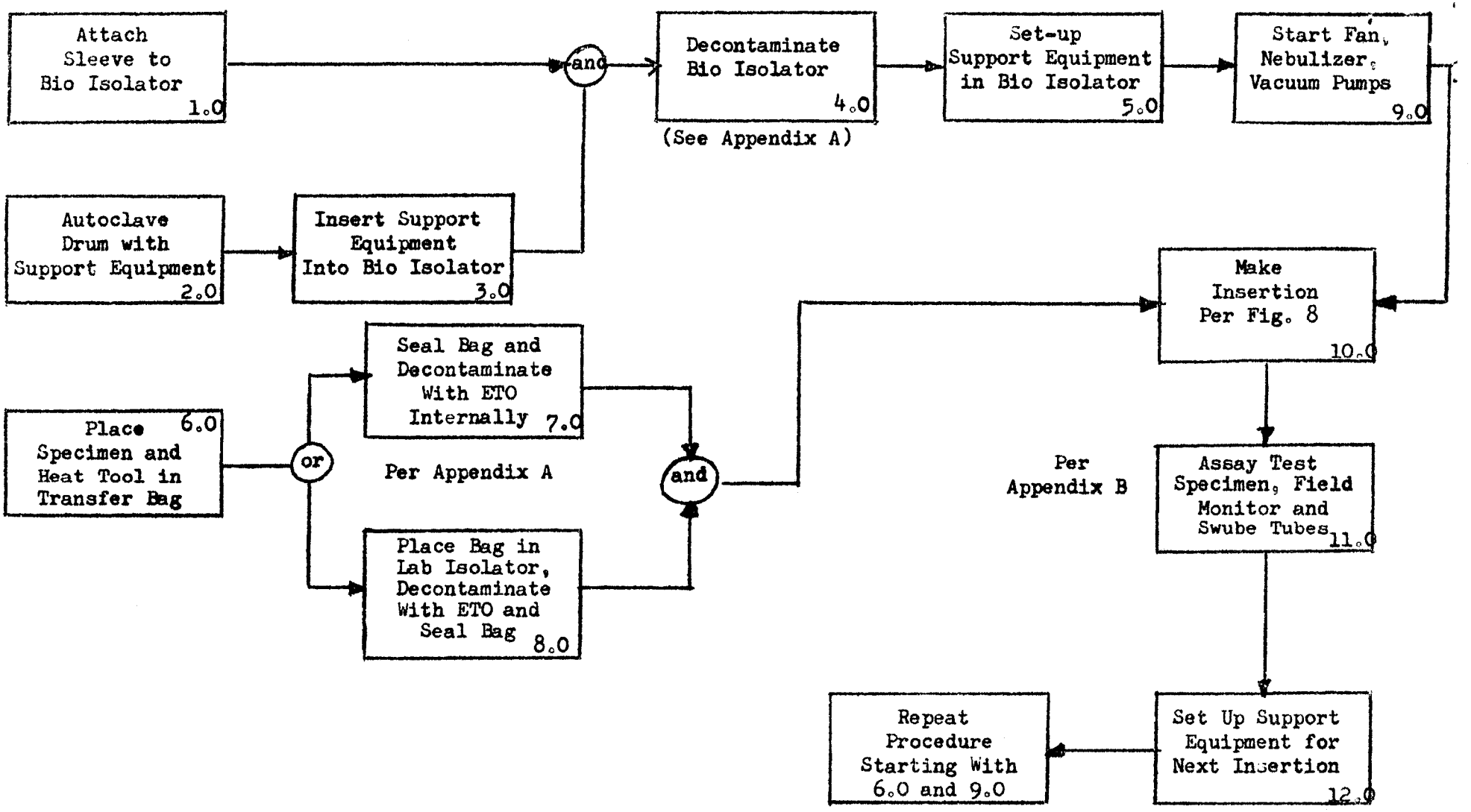
42. Assay monitor, test specimen and swube tubes per Appendix B.

43. Repeat Steps 17 thru 42.

E. Split Seam Insertion Techniques

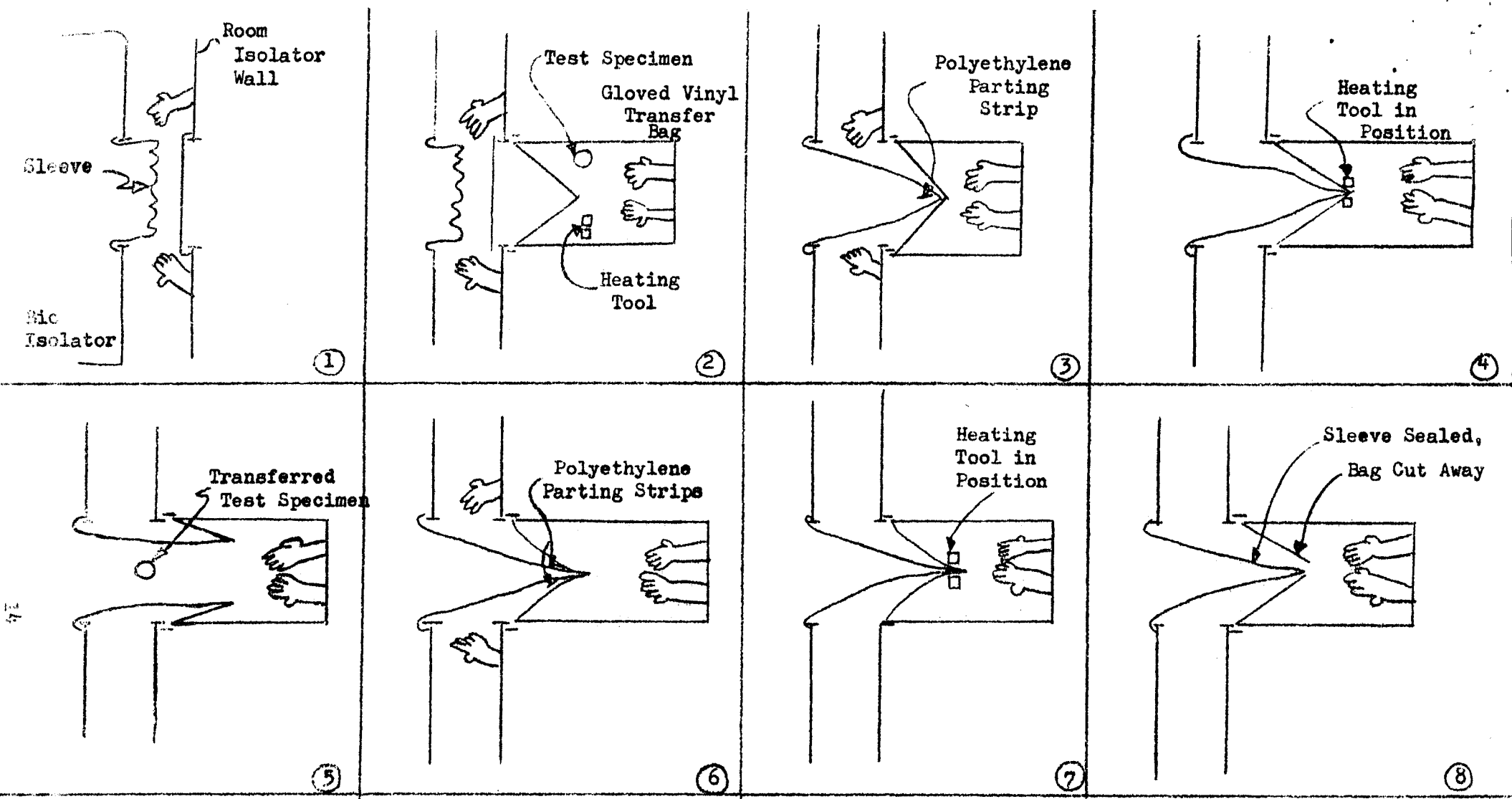
Twenty insertions and test specimen replacements shall be made with the split seam technique. This method is developmental in nature and requires that a flexible plastic bag, containing a sterile replacement part be attached to the contaminated exterior surface of a sterile isolator in such a way that the common bonded surfaces of the bag and isolator can be slit and the part transferred into the isolator without compromising the sterility of the isolator or the replacement part. Resealing the isolator and removing the bag must also be done.

The step-by-step procedure outlined below and shown in Fig. 7 shall be used to make split seam sterile insertions. Fig. 8 shows the steps required to actually make and break a split seal. Color 16 mm motion pictures shall be taken of the general test set-up and of 1 sterile insertion cycle.



SPLIT SEAM INSERTION PROCEDURE

FIG. 7



SPLIT SEAM INSERTION

SEQUENCE

FIG. 8

1. Attach the plastic sleeve (and parting tool) which serves as the attachment point during insertion to the inside flange of the entry port located in front of the bio isolator.

2. Place an impinger, 20 field monitors, a syringe, tubing, tube clamp, a 2 square inch template, a chemical stand and clamp, 500 cc of Peptone Water, an empty 500 cc flask, 60 Swube Tubes, the test specimen and hand tools in a transfer drum. (Two drums may be needed)

3. Autoclave the drum and its contents for two hours at 140°C.

4. Take an open transfer bag and insert a test specimen, 5 sealable plastic bags and a heat sealing tool.

5. Seal the insertion bag and decontaminate the internal surface per Appendix A. (Bag can also be open in an other bio isolator which is decontaminated per Appendix A, after which the bag can be sealed and removed from the isolator).

6. Insert the sterile drum thru an 18 inch access port in the room isolator and attach it to the 12 inch entry port of the bio-isolator.

7. Decontaminate the inner surface of the bio isolator per Appendix A.

8. Remove the contents of the drum into the bio isolator.

9. Cap the inner side of the 12 inch access port.

10. Pressurize the bio-isolator.

11. Remove the drum from the room isolator and cap the 18" port.

12. Set-up the impinger on the chemical stand.

13. A ttach the vacuum pump to the impinger.

14. Insure that nebulizer is charged.

15. Start room isolator pump.

16. Start nebulizer compressor.

17. Turn on fan.

18. Pour 15 cc of Peptone Water into impinger.

19. Turn on vacuum pump

20. Attach insertion bag (steps 4 and 5) to 12" entry port on room isolator.

21. With gloves in room isolator remove the inner cap of the 12" entry port.

22. With gloves in room isolator and gloves in transfer bag, work the attachment sleeve (step 1) into the transfer bag. Insure that the parting tool is in its proper position and that the surfaces of the adjacent bags are as free of wrinkles as possible.

23. Position the heat sealing tool over the entire length of the juncture of the sleeve and bag.

24. Holding the heat sealing tool with one hand, work out remaining wrinkles.

25. Apply heat of sufficient temperature and time duration to fuse the sleeve and bag together and to cut through them both.

26. Transfer sterile test specimen into bio isolator along with 5 sealable plastic bags.

27. Place "old" Test specimen in sealable plastic bag and place in transfer bag.

28. Use 3 swube tubes and 2 square inch template to swab inner surfaces of bio-isolator. Use one swube tube per 2 isolator surfaces.

29. Identify swube tubes with surfaces swabbed.

30. Place the 3 swube tubes in individual sealable plastic bags.

31. Place swube tubes in transfer bag.

32. Attach a field monitor to the impinger.

33. Turn off vacuum pump.

34. With syringe draw Peptone Water in impinger through the field monitor.

35. Discard Peptone Water in flask provided.

36. Disconnect Monitor from impinger and place in sealable plastic bag.

37. Place monitor in transfer bag.

38. Place two parting tools in place in preparation to reseal the attachment sleeve.

39. Position the heat sealing tool over the sealed sleeve and transfer bag surfaces.

40. After all wrinkles are removed, apply heat to reseal sleeve and cut the bag away from the sleeve.

41. Puncture bag and spray with a germacide. (This protects laboratory environment.)

42. Cap the inner side of the entry port in the room isolator.

43. Remove the transfer bag from the room isolator entry port.

44. Assay field monitor, test specimen and swube tubes per Appendix A.

45. Repeat Steps 4, 5 and 19 through 46.

IV. TEST FACILITY AND EQUIPMENT

A. Facility

The test set-up shown in Fig. 1 will be located in the Sterilization Laboratory on the 2nd floor of the EDL Building.

B. Test Equipment

The following equipment must be available prior to beginning the insertion program.

2	Transfer Drums
12	Test Specimens
1	Truss
1	Autoclave
1	ETO K Bottle, Regulator and Hose
1	K Bottle Dry Filtered Nitrogen, Regulator and Hose

B. Continued

1	K Bottle Dry Filtered Air
1	Nebulizer
1	Oscillating Fan
1	Incubator
1	Culture Media
10	Gal Sterile Distilled Water
3000	cc Peptone Water
2	Vacuum Pumps
2	Impingers and Flow Meters
40	Field Monitors
2	Syringes
4	500 cc Flasks
120	Swube Tubes

V. DATA REQUIREMENTS

A. Biological

Biological counts of test specimens, field monitors and inner isolator surfaces. Spore density in the room isolator.

B. Photographic

Color motion pictures (16 mm) of test set-up, decontamination, and insertion cycles which includes biological operations.

Still picture (8 x 10) shall be made of test set-up, insertion cycles and biological operations.

VI. TEST LOG AND DATA SHEETS

A test log, Fig. 9 and a Biological Data Sheet, Fig. 10 shall be used during the test.

VII. SCHEDULE AND MANPOWER

Fig. 11 shows the schedule of events for the insertion program and the manpower allocated.

VIII. REPORT REQUIREMENTS

A final report shall be prepared and submitted two weeks after completion of the insertion program. Status meetings (3) shall be held with the customer at Denver on a day during the 4th, 9th and 14th weeks of the program.

TEST LOG

DATE	TIME	EVENT

SAMPLE TEST LOG

FIG. 9

STERILIZATION DATA SHEET

DATE _____

Insertion Technique (Sterile Lock, Split Seam)

Room Isolator Spore Density _____

Incubation Time _____ Incubation Temp _____

No of Colonies Counted

Test Specimen _____

Swube Tube #1 (Isolator Floor / Isolator Ceiling) _____

Swube Tube #2 (Isolator Back Wall / Isolator Front Wall) _____

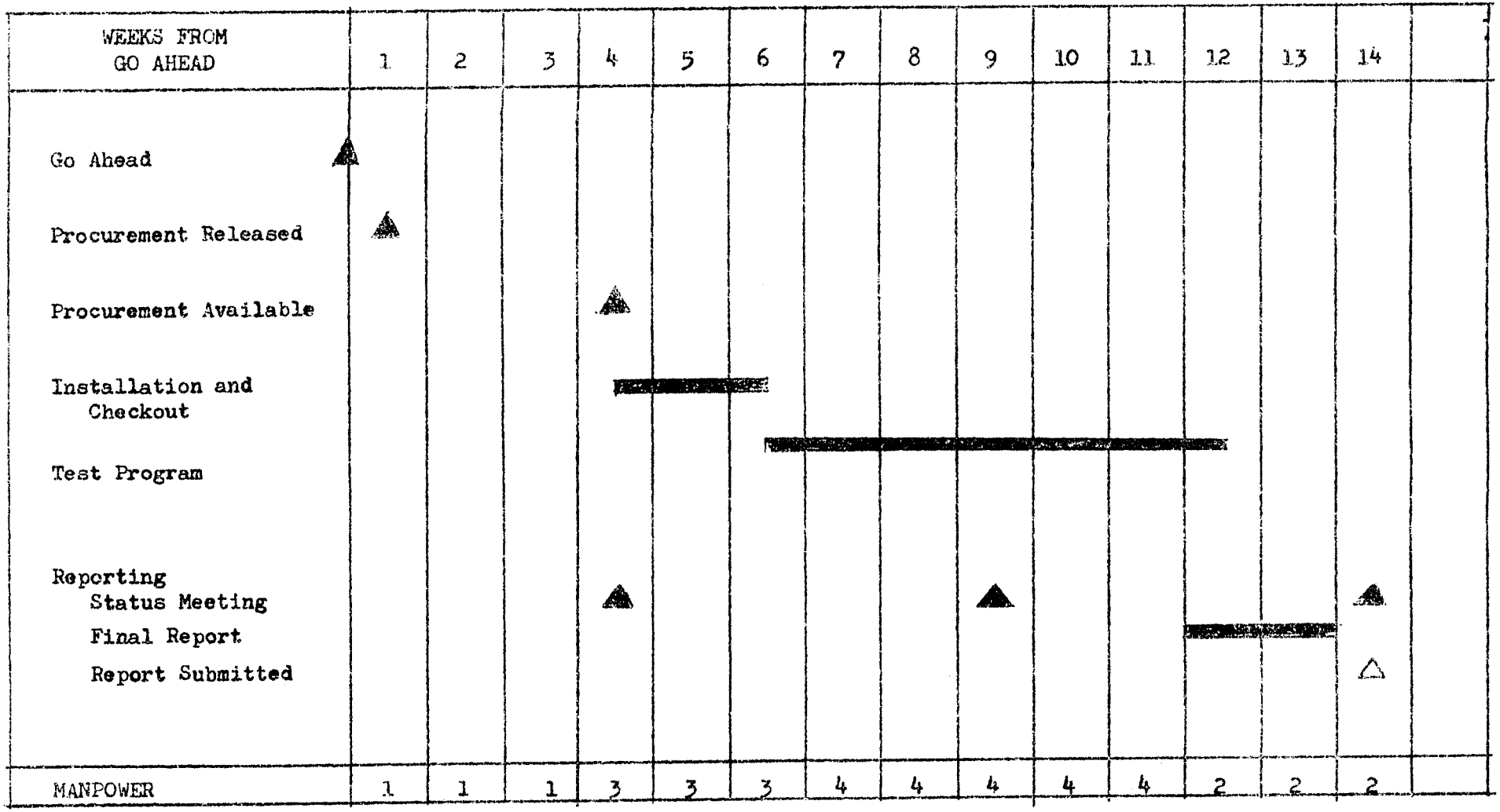
Swube Tube #3 (Isolator Left Wall / Isolator Right Wall) _____

Field Monitor _____

NOTES:

SAMPLE DATA SHEET

FIG. 10



MANPOWER AND SCHEDULE

FIG. 11

APPENDIX A

DECONTAMINATION PROCEDURES

Now Part of Lab Test

Procedure 40449205

APPENDIX B

BIO ASSAY TECHNIQUES

Now Part of Lab Test
Procedure 40449205

ATTACHMENT #2

TEST PLAN

MICROBIOLOGICAL CONTAMINATION OF STERILE ENVIRONMENTS BY BACKFLOW THROUGH SMALL CIRCULAR HOLES IN HORIZONTAL AND VERTICAL THIN WALLS

JUNE 1966

PREPARED BY:

L. SULLIVAN

APPROVED BY:

A. A. Rothstein/LS

A. A. ROTHSTEIN
PROGRAM DIRECTOR
STERILE INSERTION PROGRAM

MARTIN-DENVER

FOREWORD

This Test Plan is issued to satisfy, in part, the requirements of Task 3 of Contract NAS W-1407, Investigation of the Reliability of Sterile Insertion Technique for Spacecraft Application.

I. PURPOSE

The purpose of this Test Plan is to set forth the requirements and test methodology to determine the size of circular holes in bio isolators that will permit backflow microbiological contamination to enter the isolators from a contaminated area.

II. TEST OBJECTIVES

The test has the following objectives:

1) Determine the upper limit of circular holes in isolator walls that do not permit backflow microbiological contamination.
2) Determine if the location of the hole (i.e. in horizontal or vertical walls) has any effect on the upper limit of the hole size.

III. TEST DESCRIPTION

A. Test Fixture

The Test Fixture consists of a cubical isolator. The isolator will rest upon a work bench. The four corners of the isolator adjacent to the bench top will each contain a smaller cubical plastic isolator. These smaller isolators will be decontaminated with ETO and serve as independent sterile environments. The remaining volume of the larger cubical isolator will contain a known concentration of B. subtilus var. niger which will be injected into the large isolator's atmosphere via a nebulizer and agitated by a small oscillating fan. Each of the small sterile isolators will contain a glove and have a different size hole in one of their two vertical walls. The diameter of the four holes will be

.02 inches
.08 inches
.15 inches
.20 inches

The above hole sizes were selected within the constraints of isolator pressure capability and the desire of having laminar gas flow, when the small isolators have a positive pressure of ½ inch of water. A schematic of the Test Set-up is shown in Fig. 1.

B. Test Operations

After the Test Fixture is verified as being ready, the four small isolators will be pressurized with filtered nitrogen gas so that a ΔP of ½ inch of H_2O exists between the small isolators and the contaminated volume of the larger isolator. The nebulizer will inject biota into the contaminated volume. An impinger, with Peptone water, attached to each small isolator will monitor the nitrogen within its respective isolator. The test set-up will operate continuously. Prior to each days activities, the impingers will be replaced. A technician shall then insert his hand via the glove into each isolator in turn, tap each side of the isolator, and withdraw his hand. The purpose of inserting, moving and removing the glove is intended to simulate activity which may introduce turbulence which may be conducive to backflow contamination. After 5 volumes of gas have been collected from each small isolator the impingers will again be replaced. The contents of the two impingers from each small isolator will be assayed after withdrawing the Peptone water through a field monitor. The above procedure will

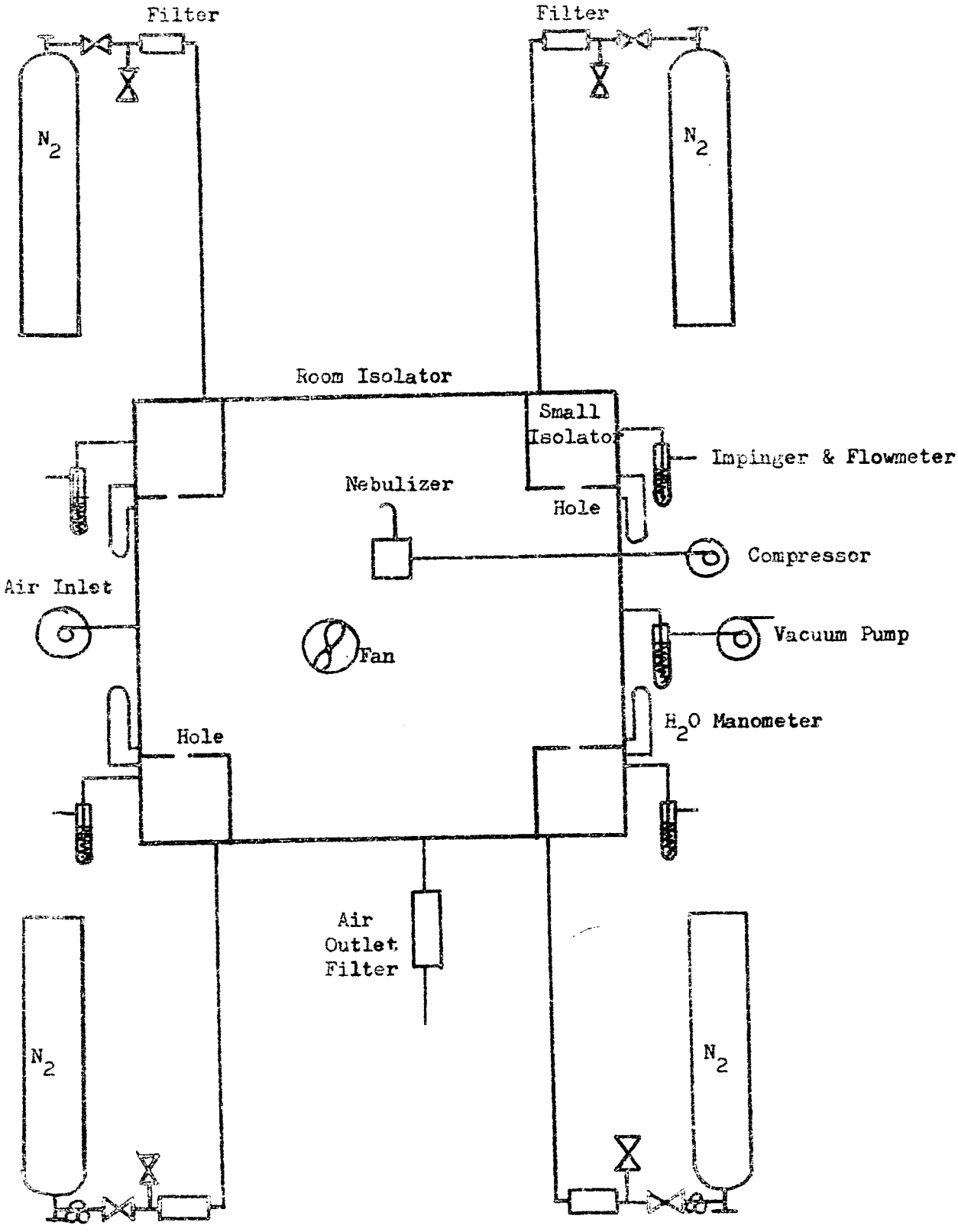


Fig. 1

TEST SET-UP

be repeated daily during the normal work week.

After half of the test span has elapsed (4 weeks), the test involving holes in vertical walls will be terminated. The holes in the small isolators will be taped shut, and identical hole sizes shall be made in the top wall of each isolator. The small isolators shall be decontaminated with ETO and the test repeated for holes in horizontal walls. This phase will also extend for the remaining half of the test span (4 weeks).

If contamination is discovered in any of the small isolators, the hole in that isolator shall be taped closed. The isolator will then be decontaminated with ETO and the test repeated to verify results. The test flow sequence is shown in Fig. 2.

IV. TEST FACILITIES AND EQUIPMENT

The test shall be conducted in the Sterilization Laboratory in the EDL Building. In addition to the permanent equipment in the laboratory, the following equipment is required:

1	Test fixture comprising a large isolator and four smaller integral isolators.
1	Kit containing germicidal cement and plastic sheeting similar to that of the isolator.
10	Impingers
1	Liter of Peptone water per week
1	Syringe
10	Field monitors and media ampoules per day
4	Water Manometers (¼" glass bent into U-tubes)
1	Vacuum Pump
5	Flow meters
1	Nebulizer and Compressor
1	Small oscillating fan
	Ethylene Oxide
	Nitrogen gas

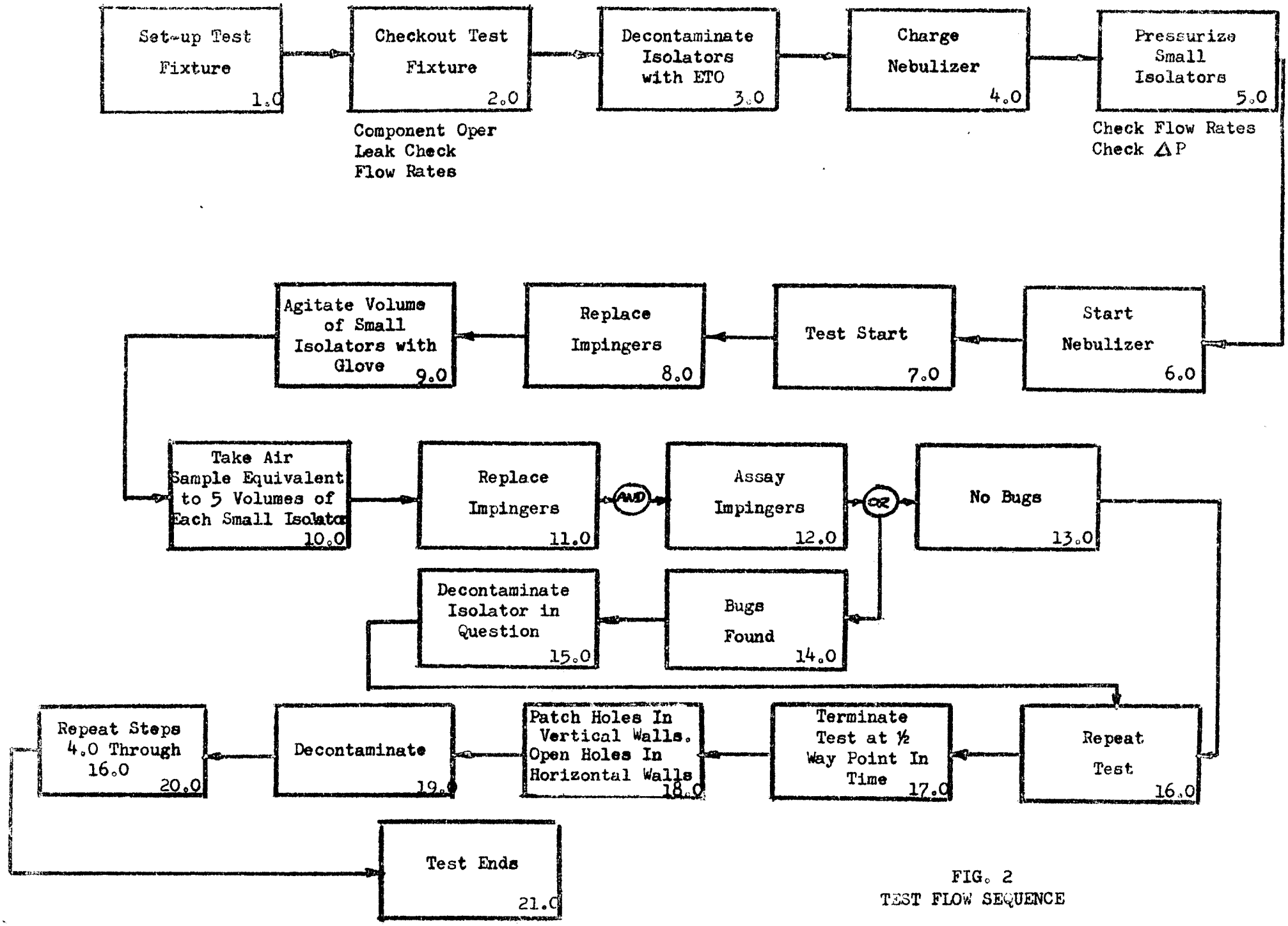


FIG. 2
TEST FLOW SEQUENCE

1 Kit containing 4 pressure regulators, tubing filters, and hand valves.

V. DATA REQUIREMENTS

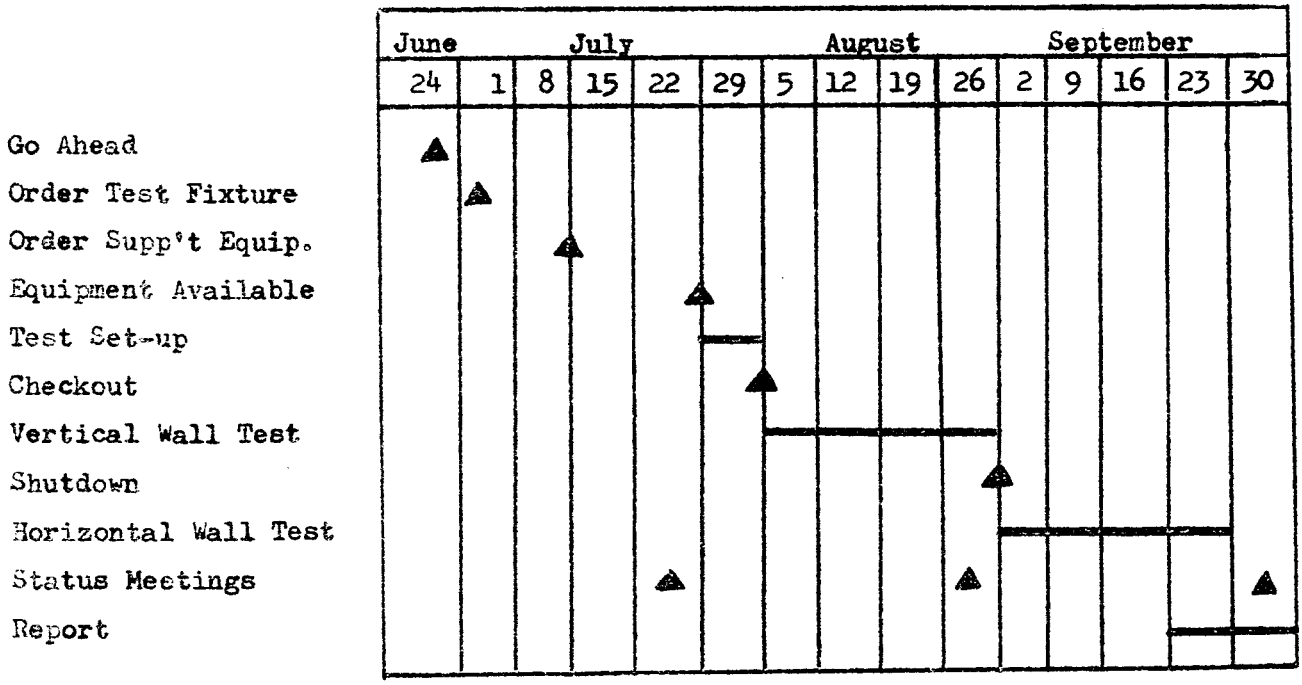
The data requirements will consist of biota colony counts. Counts will be made of the filtered residue on the field monitors. Counts will be made twice daily (before and after glove agitation). Photographs (8 x 10 black and white) will be taken of the test fixture and at significant events during the test sequence. Photographs will be taken at the descretion of the Test Conductor.

VI. TEST LOG AND DATA REQUIREMENTS

A log describing significant events during the test shall be maintained by test personnel. Fig. 3 shows a sample log sheet. A sample test data sheet is shown in Fig. 4.

VII. SCHEDULE

The schedule shown below shall apply:



TEST LOG

DATE	TIME	EVENT

FIG. 3

SAMPLE DATA LOG

TEST DATA SHEET

DATE _____ TIME _____

TYPE OF TEST (HORIZONTAL WALL, VERTICAL WALL)

ISOLATOR NO. (1, 2, 3, 4) HOLE DIA _____

FLOW RATE _____

VOLUME OF GAS SMAPLED _____

INCUBATION TIME _____ INCUBATION TEMP _____

COLONY COUNT _____

NOTES:

BY _____

FIG. 4

SAMPLE TEST DATA SHEET

VIII. REPORT REQUIREMENTS

A final report shall be issued as part of the overall Sterile Insertion Final Report two week after completion of the test (end of the 14th week). Status meetings shall be held with the customer at Martin Denver during the weeks of July 22, August 26, and September 30.