611-5

A FEASIBILITY STUDY OF LIQUID STERILE INSERTION

21 July 1969

D. M. Taylor
G. M. Renninger*
M. D. Wardle

*AVCO Corporation

JET PROPULSION LABORATORY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

A FEASIBILITY STUDY

OF

LIQUID STERILE INSERTION

CONTENTS

SECTION

FIGURES

CONTENTS (Contd)

FIGURES
(Contd)

SECTION I

INTRODUCTION

The sterile insertion of a liquid into a previously sterilized spacecraft provides a method that eliminates the need of sterilizing the liquid during the terminal sterilization of the spacecraft. This capability has the advantages of: 1) eliminating the need for designing the tank (reduction in weight) to withstand the pressure buildup during the sterilization cycle, 2) allowing the use of liquids which can not withstand the heat of sterilization due to degradation, and 3) possibly shortening the sterilization cycle.

This study included the development of a concept for liquid sterile insertion and a test program to evaluate the feasibility of such a system.

A. DEVELOPMENT OF A SYSTEM CONCEPT

The following rationale was used to develop a concept for liquid sterile insertion based on filtration as the process of sterilization. Any concept developed must meet two basic requirements: 1) that of having a very high degree of reliability or repeatability and 2) that the transferred liquid has a high probability of sterility in accordance with the planetary quarantine requirement. The planetary quarantine requirement states that the probability of the spacecraft not being sterile shall be less than 10^{-4}. With this probability assigned to the whole spacecraft, the probability of contamination in the liquid systems would be less, probably around 10^{-6}. This means if the conventional approach of just testing the filter is used, the sterile insertion system would have to be tested a great number of times (approaching 10^{6}) without failure, assuming an absolute method of verifying sterility. The above conditions indicate a near impossible task based on cost, time, and manpower required. Also, in order to verify the probability of sterility to the level of 10^{-6} a sample of the filtrate approaching 100 percent would be required. Therefore, the system concept developed had two basic criteria: 1) a method of reducing system reliability requirements by verifying the probability of sterility of the filtrate prior to actual insertion into the sterile spacecraft, and 2) a method for verifying the probability of sterility without assaying the filtrate.

System reliability requirement is reduced by including in the system a holding tank just prior to the penetration of the sterile barrier of the spacecraft canister. The holding tank allows the sterility of the filtrate to be verified before insertion. If the filter fails for some reason, the holding tank eliminates the possibility of contaminating the sterilized capsule which would require a costly and time consuming operation of resterilization. With the holding tank concept, if a failure in obtaining the probability of sterility occurs, the insertion system is disconnected and another is used, assuming a method or sterile environment is available for making an aseptic connection.

Based on data and experience of other work and the use of filters to sterilize liquid in other industries, it was assumed that if the filter did not degrade during sterilization or as the result of passage of the liquid, the resulting filtrate would be sterile. It was also assumed that the pore size distribution was within the vendor specification. Therefore, the probability of sterility of the filtrate could be determined and verified by any method or test that would measure the integrity of the filter following filtration, much as the time and temperature relationship is used to verify the probability of sterility in dry heat sterilization.

The above rationale and concepts resulted in a liquid transfer system (Figure 1-1) made up of the following subsystems and functions:

Storage tank - Reservoir in which liquid to be transferred is stored.
Roughing Filter System - This filter system removes the gross contamination from the liquid to increase the filtering life of the sterilizing filter system.
Sterilizing Filter System - This filter system, downstream of the roughing filter, functions to remove all bacteria.
Bubble Point Test System - This system is used to verify the pore size of the filters and to check for filter degradation during filtration.
Holding Tank - The holding tank has the function of holding the filtrate until a bubble point test is performed on the sterilizing filter system to check for degradation, thereby verifying the probability of sterility of filtrate.
Spacecraft Tank or Container - A receiving vessel with the spacecraft such as a fuel tank, media container, etc.

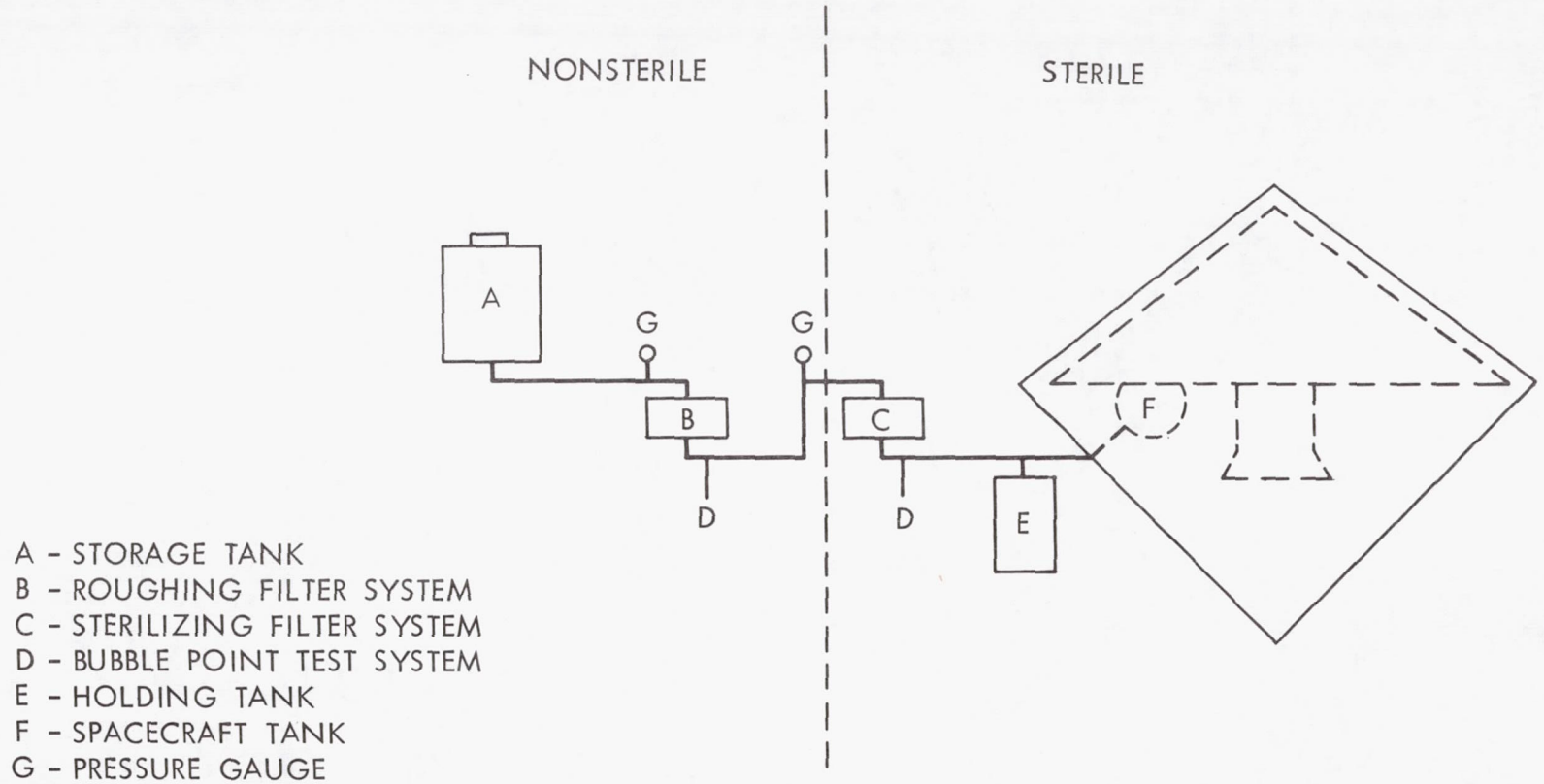

Figure 1-1. Schematic Diagram of Liquid Sterile Insertion System Concept

Pressure Gauge - The pressure gauges are located in the system to measure the pressure across the filters and the bubble point pressure.

B. TEST PROGRAM

The objective of this study was to determine the feasibility of the above system concept for the sterile insertion of a liquid. The scope was to determine the ability of a filter to sterilize liquid media, and to biologically determine the validity of measuring the integrity of the filter as a verification of the probability of sterility. The experimental setup used simulated the system by including all subsystems from the storage tank through the holding tank. The test system was designed to meet the following requirements:

1) The system shall employ two filters, one to remove gross particles and the other to serve as a sterilizing filter.
2) Filtration of the liquid shall be carried out with a pressure differential of one atmosphere across the sterilizing filter.
3) All subsystems downstream and including the sterilizing filter shall be sterilized as a sealed unit.
4) Physical tests of the filter integrity of both filter systems shall be performed before and after the transfer of liquid.
5) 100% of the filtrate shall be biologically assayed.

SECTION II

MATERIALS AND METHODS

A. MATERIALS

The following materials were selected to perform the stated objective:

1) 90 mm Millipore Solvinert filter

   a) 0.50 μ pore size
   b) 0.25 μ pore size

2) 90 mm filter holder
3) Bubble point apparatus
4) 1000 ml Erlenmeyer flasks (collection vessel)
5) Trypticase Soy Broth
6) Mixed population (bacterial isolates from spacecraft assembly environment)
7) Pseudomonas aeruginosa (FDA Strain 803)

The study did not call for an evaluation of either the filters or filter holders, therefore, filtration products from one vendor (Millipore Corporation) were utilized on the basis of the manufacturer's specifications for said products. Reasons for selecting the Millipore Solvinert filter were that the filter:

1) Withstands dry heat sterilization.
2) Is compatible with a wide variety of liquids.
3) Is available with 0.25 and 0.50 μ pore size.

The membranes of pore size 0.50 microns were used as a pre-(roughing) filter. The membranes of pore size 0.25 microns were used as the final or sterilizing filter.

B. METHODS

The bubble point test was selected as the physical method of verifying the probability of sterility, since it is a standard method of testing for maximum pore diameter (Federal Specification NNND-00370 DSA-DM). The theory behind bubble point testing of a membrane filter encompasses the following:

1) Wetting of the filter causes the pores of the filter to be filled with liquid.
2) The bubble point of a filter is a function of the surface tension of the liquid in the maximum pore size of the filter.
3) When gas under pressure is applied across a wetted filter, a pressure exists at which the gas will be forced through the pores, i.e., the bubble point.

Two groups of organisms were selected to challenge the filter system in this study. The first group of organisms was a mixed population found as the result of monitoring a Class 100 clean room (Room 140) in JPL's Sterilization Assembly Development Laboratory. These organisms were selected to challenge the filter system with a wide variety of bacteria types varying in size and having other morphological characteristics. The second group of organisms used was a pure strain a Pseudomonas aeruginosa, FDA strain 803*. This organism was selected because of its small size and its use (in a number of Laboratories) in the Organism Retention Test (NNND-00370 DSA-DM) instead of Vibrio percolans.

The challenge liquid was prepared by inoculating distilled water with both classes or organisms at a concentration not less than 2×10^6 organisms per 500 ml of challenge fluid. Samples of the inoculated liquid were taken to verify the required concentration.

The configuration for assembly of the filter holder is shown in Figure 2-1. The same configuration was used for the sterilizing and roughing filter

*The culture was supplied by Millipore Corporation.

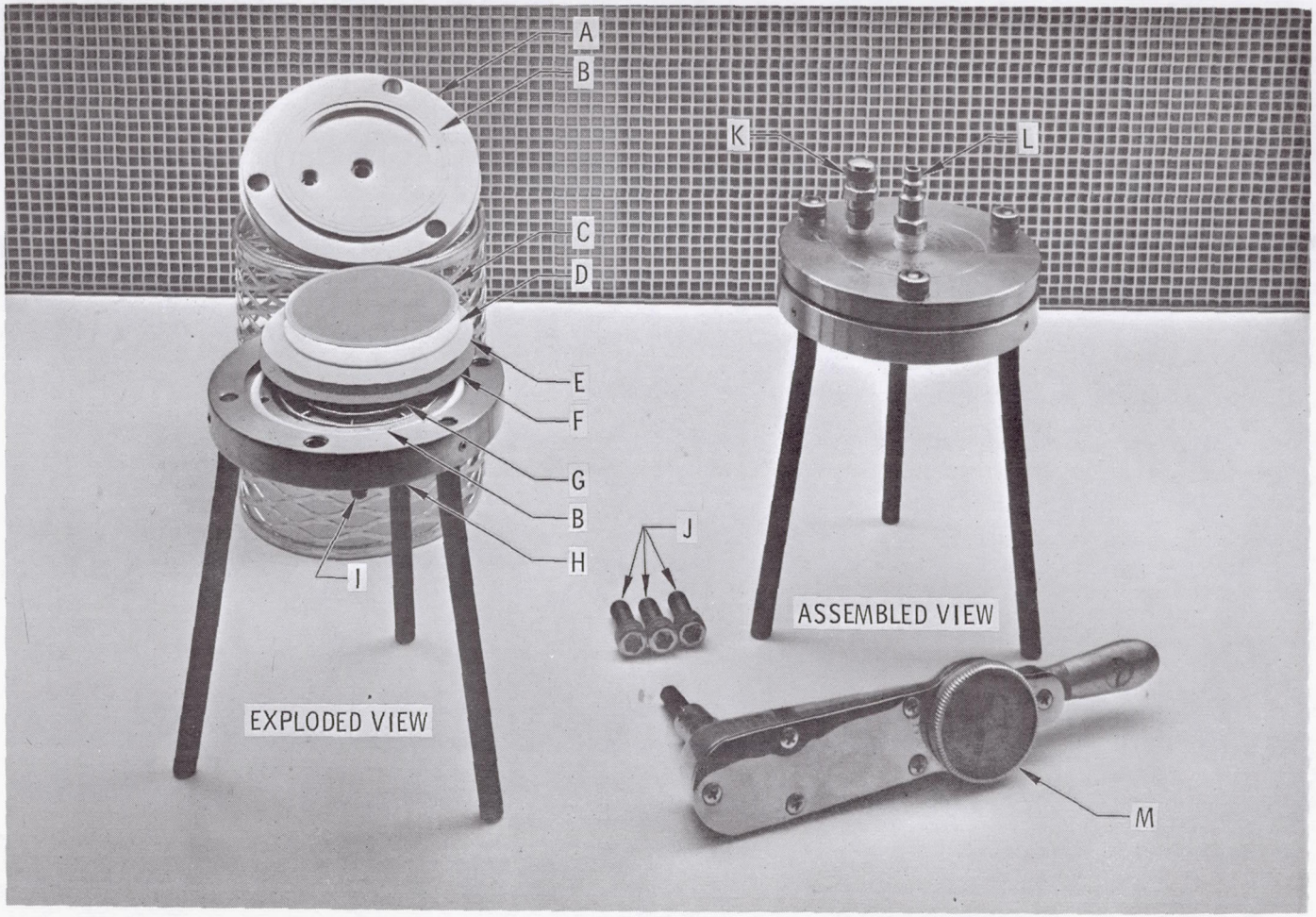

Figure 2-1. An exploded and assembled view of a 90 mm filter holder with its component parts: A) stainless steel inlet plate, B) Teflon O-ring, C) back pressure support screen, D) pre-filter, E) membrane filter, F) Teflon faced support screen, G) underdrain screen, H) stainless steel outlet plate, I) outlet hose connector, J) cap screws, K) air bleed valve, L) inlet hose connector, and M) torque wrench (in-lbs).

holders except that the filters for each were of different pore sizes. To prevent cracking of the membrane during autoclaving, the following precautions were invoked prior to autoclaving:

1) The filter holder and its components were thoroughly dried.
2) The filter holder top was screwed on (10 in -lbs) prior to autoclaving, but not torqued to 55 in -lbs (75 in -lbs in later tests) until after autoclaving and a sufficient cooling period on a laminar flow bench (approximately 20 minutes).

To prepare the system for steam sterilization, 18 gms of dehydrated Trypticase Soy Broth and 100 ml of distilled water were added to each of two collection vessels. These vessels were connected by a tubing system to the final filter holder containing a 0.25 μ filter. A bacterial trap and vent system were assembled by adding 700 ml of Amphyl disinfectant to a vessel. This vessel was equipped with two tubes capable of extending to the bottom of the vessel. A filtered vent was attached to one tube and the other tube was attached to the second collection vessel. Prior to sterilization, the vented tube was pulled up so the lower end of the tube was above the level of the disinfectant and the tube connected to the collection vessel was within one-half inch of the bottom of the vessel. This would allow expanding gas to escape the system and block possible back comtamination. All vessel tops and the filter holder intake adapter were wrapped with foil and steam sterilized for 40 minutes at 121°C and 15 psi as shown in Figure 2-2. Immediately after sterilization, the vented tube was pushed to the bottom of the vessel and the tube connected to the collection vessel was pulled above the level of disinfectant. This condition made it necessary for gases entering the system during the cooling period to pass through the filter vent and the disinfectant trap, thus affording a double safeguard against back contamination.

To complete the test apparatus, a pressure gauge was installed upstream of the final filter. Incorporated into the system at this point was a tube, with clamp, leading to a bubble test beaker. Upstream of the pressure gauge, a roughing filter was connected and this in turn was connected to a

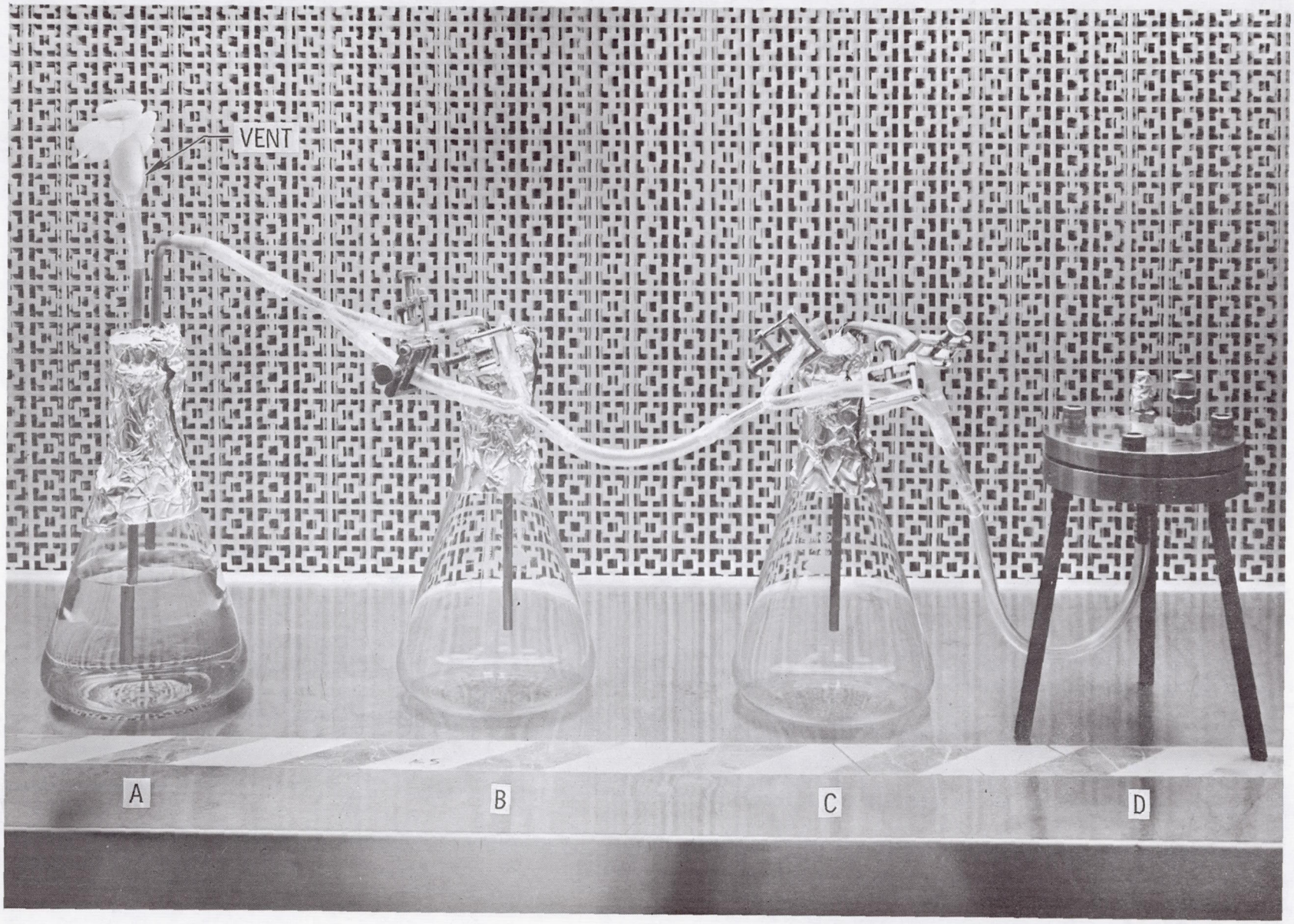

Figure 2-2. Sterilized portion of sterile filtration system: A) bacterial trap, B) collection vessel, C) collection vessel, and D) sterilizing filter.

pressure vessel equipped with a bubble point apparatus which allowed a selection of liquid or gas to pass through the system from the pressure vessel. Nitrogen was used to supply the pressure within the vessel. Clamps were placed on the tubing of the system to allow control of the flow direction, e.g., to flow into or by-pass a particular vessel. The completed laboratory system is shown in Figure 2-3.

To conduct the test, 10 liters of liquid containing test organisms were placed in the pressure vessel. Clamps were closed to prevent liquid flow into the sterilizing filter and pressure was increased to allow approximately 1 liter of liquid to pass through the roughing filter, and be collected in the bubble test beaker. At that time, liquid flow was stopped, the gas flow begun, and the pressure increased slowly until bubbles showed in the beaker of filtrate. This was recorded as the bubble point pressure of the roughing filter.

Pressure was then released, the line to the beaker clamped off, the line to collection vessel A opened, the line to collection vessel B clamped in by-pass position, and liquid was allowed to flow and pressure increased to collect 500 ml of filtrate in vessel A.

Pressure was then released, the line to vessel B opened, and the lines to vessel A clamped in by-pass position. Pressure was then increased to collect 500 ml of filtrate in vessel B.

Pressure was then released, the line to vessel B clamped in by-pass position, and the lines to vessel A opened. The liquid/gas line was opened to allow passage of gas and the pressure increased slowly until the appearance of bubbles in collection vessel A. This was recorded as the bubble point pressure of the sterilizing filter. *

---

*A change in procedure for bubble point test of sterilizing filter is discussed in Section III.

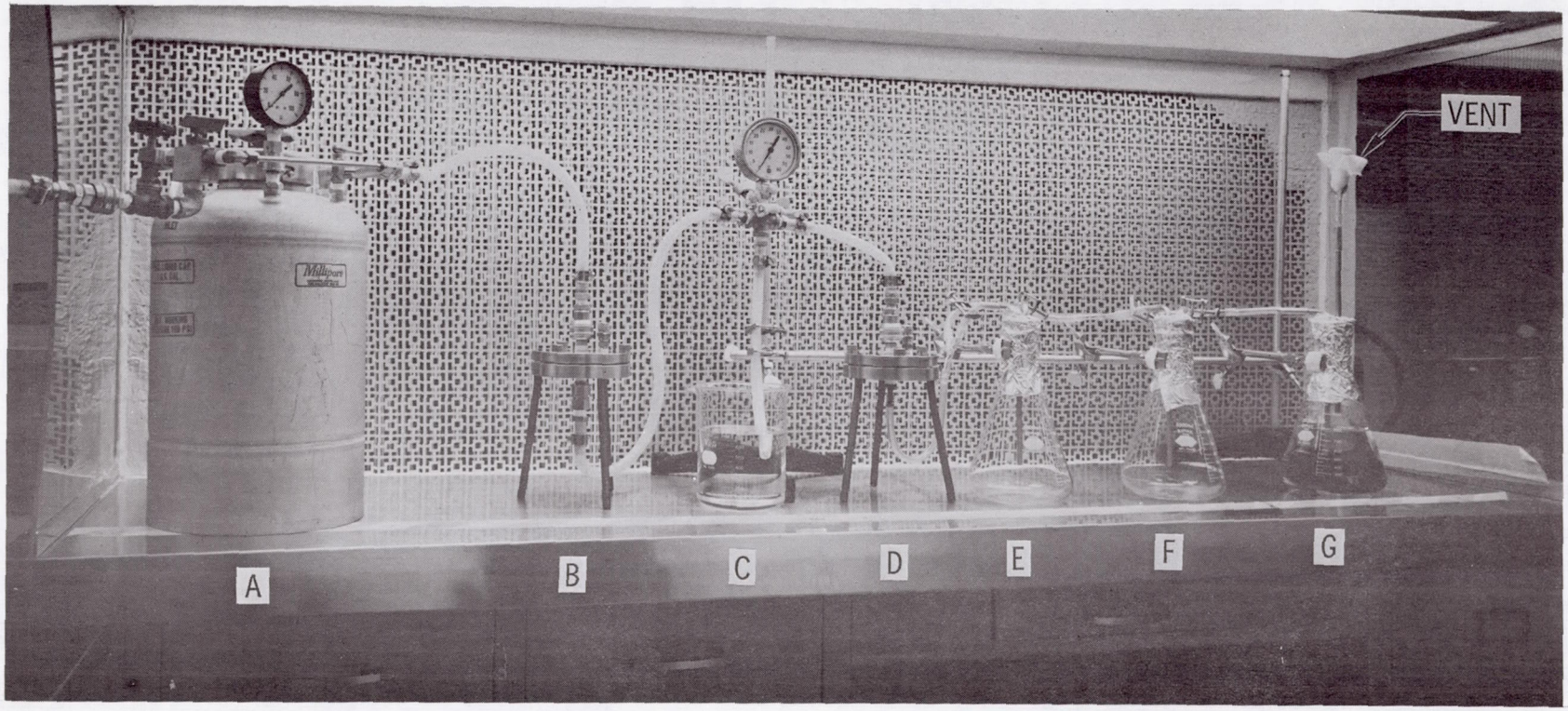

Figure 2-3. Complete laboratory apparatus used to study the feasibility of liquid sterile insertion: A) pressure vessel, B) roughing filter, C) bubble point test beaker, D) sterilizing filter, E) collection vessel A, F) collection vessel B and, G) bacterial trap.

SECTION III

RESULTS AND DISCUSSION

A. GENERAL

No mechanical difficulties were encountered in handling the filter systems. As stated in the Section II, special precautions were taken to completely clean and dry the components making up the filter systems before assembly and autoclaving. These precautions resulted in no observable membrane breaking from autoclaving and in the handling of the system.

A total of 54 transfers were made with the system. Two major changes in procedure were made during the course of this study. The first change in procedure was to eliminate the conducting of a bubble point test on the sterilizing filter system following transfer into vessel A and before transferring into vessel B. This change was initiated after the 16th transfer when it was observed that over 60% of the filtrates were contaminated. The change was made to check for the effect of the bubble point test on contamination. The procedure was changed so that the bubble point test would be conducted only after 500 ml were transferred to both vessels A and B and the bubble point observed only in vessel A. If the bubble point test were contributing to contamination, the contamination level of vessel A would have been higher than vessel B. As can be seen in Figure 3-1, the frequency of contamination was reduced as the result of this procedural change. However, the data indicated that the ratio of contamination in vessels A and B did not change indicating that the bubble point test probably was not contributing to filtrate contamination.

The second procedural change was made after the 27th transfer. This change involved increasing the torque of the filter holder screws from 55 to 75 in-lbs to decrease the chance of the filter holder allowing the liquid to pass around the filter membrane. The data (Figure 3-1) would indicate that this procedural change did reduce the frequency of filtrate contamination below the mean value for the complete test program.

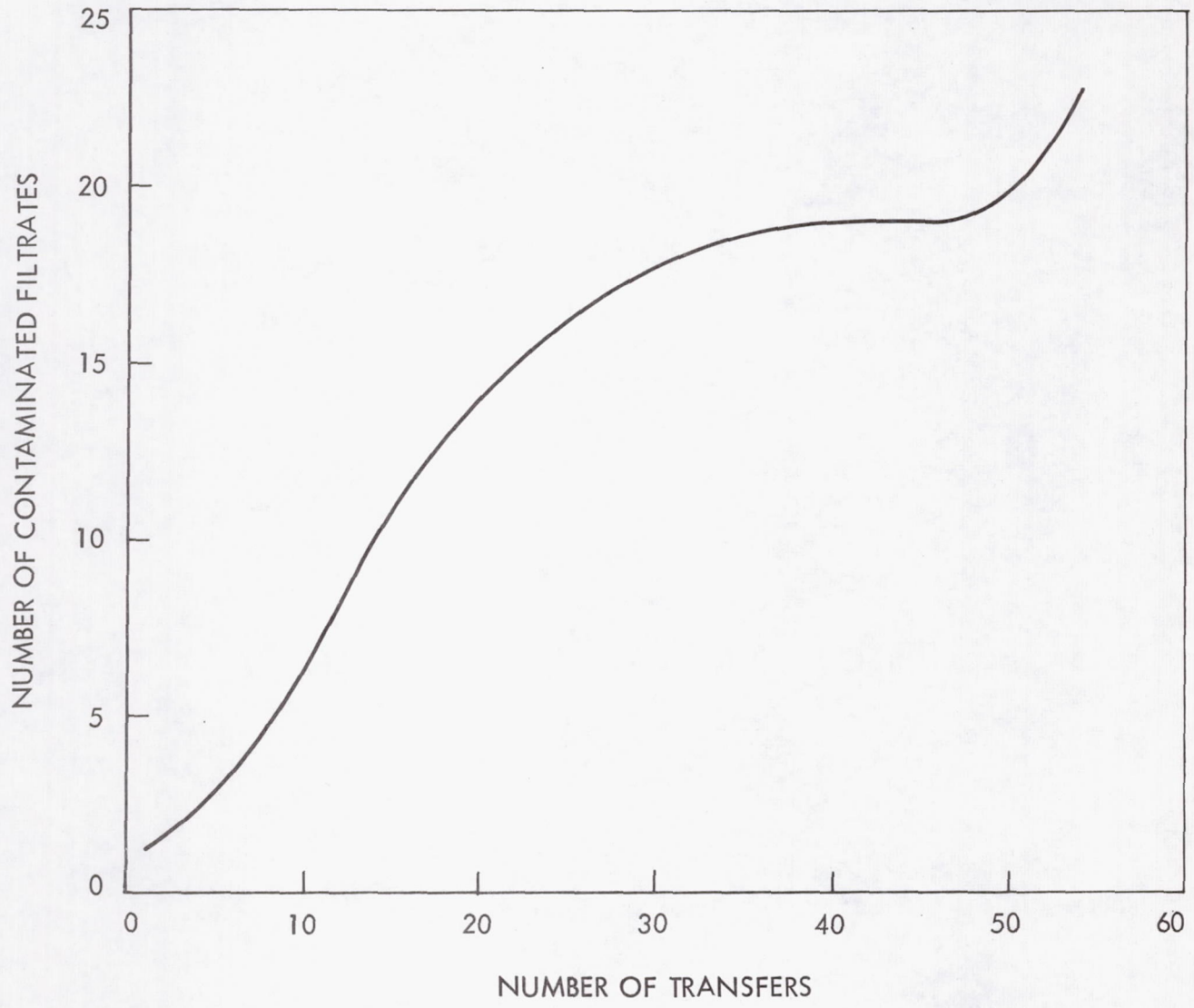

Figure 3-1. The Accumulative Distribution of Contaminated Filtrates with Increased Number of Transfers

B. NUTRIENT MEDIA TRANSFER

One of the original objectives of this study was to use Trypticase Soy Broth (TSB) as the liquid to determine the feasibility of the sterile insertion system. It was found that the filtration of this medium had a very slow flow rate. The vendor specifications indicate a flow rate of about 500 ml per minute of water with the sterilizing filter equipment selected; however, with TSB, the maximum flow rate was 100 ml per minute for the first 100-150 ml, after which the filter became clogged and the flow rate dropped to 2-3 ml per minute. A number of different techniques were tried to increase the flow rate and volume of TSB that could be filtered. These included autoclaving to increase the solubility of the different constituents, filtering at 55°C, and filtering the medium through the roughing filter system (0.5 μ pore size) three times before trying to pass it through the sterilizing filter. These pretreatments did increase the initial flow rate and volume before filter clogging; however, the resulting flow rates and volumes were considered unsatisfactory. The same problems resulted when the test was conducted with one-percent peptone water. These results indicate that the protein fraction of the media was the probable cause of low flow rate and clogging of the filter, and it is questionable if these types of liquids can be sterilized by filtration without altering the composition of the media.

As the result of this work, water was used as the transfer liquid and all data reported are based on filtering inoculated distilled water.

C. FILTRATE CONTAMINATION

Forty percent of all transfers made resulted in the filtrates in either or both vessels A and B being contaminated. Of the 40% of all transfers that resulted in contaminated filtrate(s), 71.6% of the time both vessels were contaminated and the remaining 28.4% not contaminated was evenly divided between vessels A and B. That is, the frequency of contamination of vessels A and B was the same.

The accumulative distribution of the contaminated filtrates versus number of transfers is shown in Figure 3-1. The distribution indicates that procedures and techniques used in operating the system were subject to a "learning curve," since the frequency of contamination became less after the 17th transfer. As stated above, the effect of the change in bubble point test on the 16th transfer would, in itself, not change the distribution because the data indicated this test did not contribute to filtrate contamination. The procedural change (27th transfer) of increasing the torque on the filter holder screws may have had an effect on the distribution, as stated above.

The contaminant(s) in the filtrates was found to exhibit similar growth patterns (rate of growth and formation of a pellicle) when incubated in the collection vessels. Trypticase Soy Agar (TSA) streak plates were made using inocula from the contaminated vessels. Colony morphology indicated that, in all cases examined (10% of the contaminated vessels), a single type of organism was responsible for contamination, and the colony morphology of the organism was the same as that of Pseudomonas aeruginosa when it was streaked on TSA and similarly incubated. A gram stain was made of the contaminant and only gram negative rods were observed. These results indicate that a single organism was the contaminant and that it was very likely the test organism.

The transfer of only the test organism would suggest that the cause of filtrate contamination was a function of particle size. Since the test organism was selected because of its small size, an attempt was made to determine the size distribution of the test organism using a scanning electron microscope. The results of the preliminary tests indicated the possibility that the size distribution of the organism could be smaller than the pore size (0.25 μ) of the sterilizing filter membrane.

D. BUBBLE POINT TEST

Part of the overall objective of the feasibility study was to evaluate the bubble test as a physical method of determining the probability of sterility of filtrates.

The bubble point test is based on the relationship between the surface tension of a liquid and the size of a capillary (pore) in which it is held.

The distribution of the bubble point pressure of the sterilizing filters through the test program is shown in Figure 3-2. As can be seen, the variation from one filter (0.25μ) to another is random and varies over a wide range. The range in bubble point pressure of the 0.25μ filter observed in this test was from 34 to 60 lbs/in^2 which would indicate, based on the bubble point test, that the pore size of the membrane filters had a wide variation.

The data would indicate that the procedures for conducting the bubble point test are not subject to a "learning curve" and that the variability in bubble point pressure of these membrane filters (0.25μ) occurred at random throughout the test program. As previously noted, the procedural change of increasing the torque (27th transfer) of the filter holder screws was coincident with a reduction in the incidence of filtrate contamination (Figure 3-1). Concomitant with this procedural change was a decrease in bubble point pressures (Figure 3-2). Therefore, it appears most unlikely that filter holder leakage was a source of system failure. Further evidence to support this was derived from observing the system for a gas pressure drop when the system was held at a constant pressure. No such pressure drop was recorded in any of the 54 transfers.

The relationship between bubble point pressure and filtrate sterility is shown in Figure 3-3. In this figure, the bubble point pressures (based on a range of plus or minus 2 lbs/in^2) are plotted against the total number of transfers and the number of nonsterilizing transfers within each range. It was found that contaminated transfers (nonsterilizing filtration) occurred in all bubble point ranges except the 58-60 lbs/in^2 range (only two filters tested were within this range), and the mean bubble point pressure for the nonsterilizing transfers was very close to the mean for all transfers (45 versus 44.3 lbs/in^2).

A series of statistical tests was performed to determine if there was any correlation between the bubble point pressure of a filter membrane and the sterility of the filtrate. Based on the Kolmogorov-Simirnov test, the distribution of the bubble point pressure of all filters was the same as those which resulted in contaminated filtrates. In addition, a plot of the cumulative percent frequencies of bubble point pressure on normal probability paper presented a

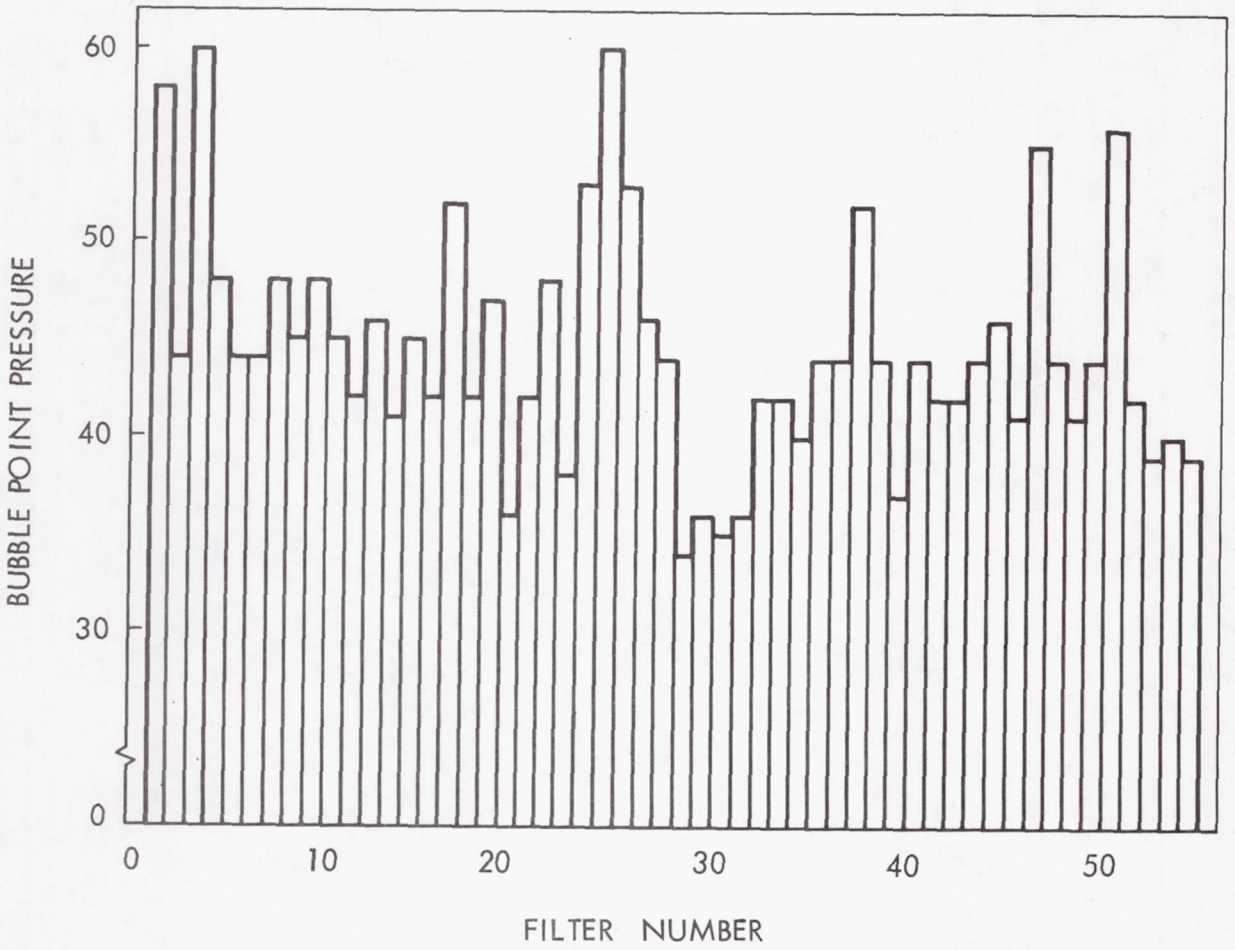

Figure 3-2. The Distribution of the Bubble Point Pressure of the Different Sterilizing Filters Used Through the Test Program

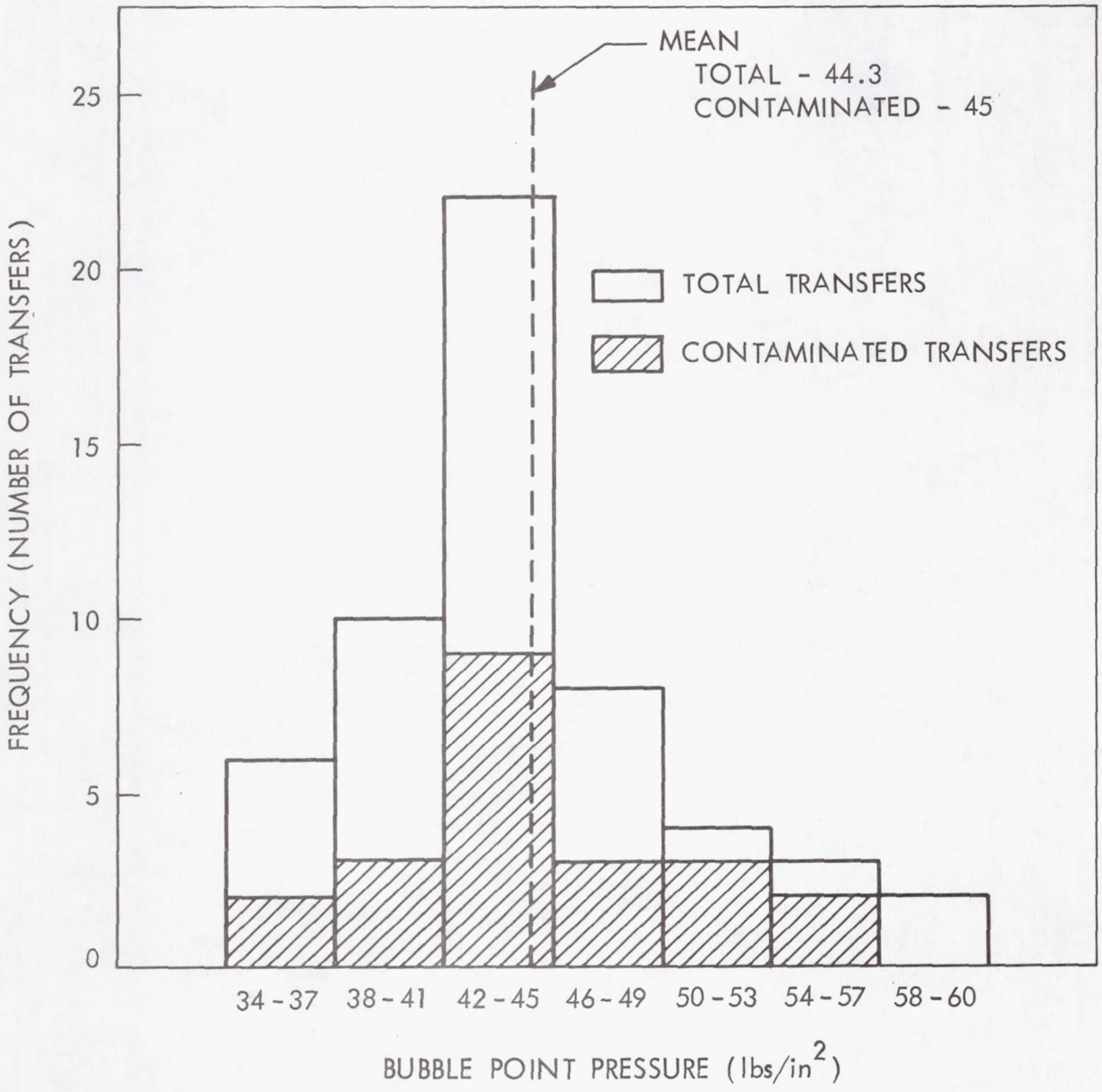

Figure 3-3. Frequency Distribution of Bubble Point Pressures of the Sterilizing Filter System

straight line, indicating that the distributions were normal. After determining that the distributions were normal, a regression-correlation analysis was performed which showed that some correlation existed between bubble point pressure and filtrate sterility, but it was not significant.

These results indicate that under the conditions of this study, there was very little correlation between bubble point pressure and the ability of a membrane filter to sterilize a liquid. However, if the test organism was smaller than the pore size of the sterilizing filter, this would mean that the correlation between bubble point pressure of a filter and the sterility of the filtrate was not tested.

SECTION IV

SUMMARY AND CONCLUSIONS

Efforts to filter biological media, i.e., TSB and peptone water, proved to be unsuccessful on a volume/time basis (see Section III). Referral of the problem to Millipore Corporation corroborated these findings; however, Millipore noted an increase in filtrate volume (up to 1 liter) when a 0.3 μ filter was placed in series before the 0.25 μ filter (personal communication, Dr. Fifield, Millipore Corp.). It may be that media containing protein hydrolysates are unsuitable for sterile filtration. No problem in acquiring a sufficient volume of filtrate in a reasonable time was encountered in the filtration of inoculated water.

Contaminated filtrates were examined in an effort to determine the origin of contamination. Positive identification of the contaminant(s) was not made; however, the observations made indicated that only one type of contaminant was present and that it was identical with the test organism, Pseudomonas aeruginosa. This conclusion was based mainly on morphological considerations and the gram stain.

Tentative identification of the contaminant as being the test organism indicates that the collection portion of the filtration system was sterile at the onset of experimentation and that contamination arose during the actual filtration process. Some possible causes for such an occurrence are the following:

1) A loss of membrane integrity, i.e., rupture or perforation of the membrane.
2) A membrane lacking uniform pore size with some pores large enough to permit passage of organisms.
3) Seepage of liquid around the membrane.
4) An organism smaller than the specified pore size of the filter.

The bubble point test was designed to indicate possible contamination from causes 1, 2, and 3. However, contaminated filtrates were randomly

distributed over a range of bubble points from 34-60 lbs/in^2 while the bubble point for any particular filter remained constant when retested. Therefore, the sensitivity of the bubble point test (in the context of this experiment) as a physical guideline for sterility must be doubted. Loss of membrane integrity may have been a source of contamination if the level of degradation was below the detection sensitivity of the bubble point test. The data indicated that the procedural change of increasing the torque on the filter holder may have reduced contamination. This would indicate the possibility of seepage around the filter, evidence against such a happening being the following:

1) There was no observable leakage from the filter holder.
2) There was no observable loss in pressure when holding at pressure below the bubble point test.

The contaminant was not sized to determine if it was smaller than the specified pore size of the filter, although preliminary experiments pointed in that direction. Since, as noted above, the bubble point for a particular filter was repeatable and the range of bubble points for the different filters was broad, it was concluded that the lack of uniform pore size appeared to be likely candidate for a source of contamination. Additional information to support this conclusion was acquired when three unused 0.25 μ filters (randomly chosen from those on hand) were returned to the manufacturer for quality assurance checks. It was reported that one of the three did not meet the specifications set by the company (personal communication, Millipore Corporation). Due to the scope of this study, a definite identification of the cause(s) of the system failure was not made.

SECTION V

RECOMMENDATIONS

The results and conclusions of this study indicate that further evaluation of filtration as a sterilizing process is required, if this process is to be considered in a liquid sterile insertion system. This evaluation should include defining the physical parameters affecting the reliability of a filter system and developing the criteria for a physical method of verifying the probability of sterility of the filtrate.

The approach requires developing better specifications of filter equipment for acceptance and testing so that the requirements for a reliable filter system can be defined. Further work should include the development of techniques and tests for the determination of: 1) pore size range of the filter membranes, 2) size range of test organisms, 3) sensitivity of physical tests indicating probability of sterility, and 4) verifying that all inserted fluid has passed through the filter medium.

Final efforts should include the development of qualification criteria applicable to all elements of a flight-type sterile insertion system.