

Microbiological Evaluation of the Mobile Biological Isolator System

Daniel M. Taylor
Frank Morelli
William Neiderheiser
Wallace M. Tratz

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Childrens Hospital of Los Angeles (CHLA) was responsible for patients, sterile isolation rooms, and for sampling the patients' microflora. Mrs. R. Moldan, Supervisor for the isolation rooms, provided the technical support for the Childrens Hospital.

The actual laboratory work, except where otherwise specified, was conducted by Frank Morelli (Bioscience Lab Manager), William Neiderheiser, and Wallace M. Tratz of the Bionetics Corporation under JPL Contract No. 954429.

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ABSTRACT

Evaluations on critical components of the Mobile Biological Isolation System were performed. HEPA filter efficiency and suit integrity were found to withstand repeated ethylene oxide (ETO) sterilizations. The minimum ETO sterilization time required to inactivate all contaminant organisms was established at four hours. Two days of aerating at 120°F was found to dissipate all harmful ETO residuals from the suit. Donning and doffing procedures were clarified and written specifically for isolation rooms.

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INTRODUCTION

The Mobile Biological Isolation System was originally designed for a child with severe combined immune deficiency (SCID). Children born with SCID only rarely survive to the age of one because of their impaired ability to resist infection. David, a child with SCID, has now survived to the age of eight in a sterile isolation room supplied with laminar filtered air.

Studies with leukemia patients confined to sterile isolation rooms, in conjunction with antibiotics, antiseptics, and other microbial reducing agents and techniques, have shown the value of this sterile environment for the control of infections. Normally patients undergoing chemotherapy lose resistance to infection in a manner similar to SCID's. However, significant research and therapy is currently being conducted combining chemotherapy with isolation room technology, since this has been found to reduce infection rates.

One shortcoming of this treatment, however, is that it provides no protection for patients when moved outside of the laminar airflow room. In the case of pediatric patients such removal for medical reasons (i.e., X-rays) is often necessary. In addition, it was considered of psychological benefit for the patients to be able to leave the clean room enclosure for play with other children and brief excursions. The Mobile Biological Isolation System, by providing sterile filtered air and acting as a biological barrier against contamination, would enable patients to engage in activities outside of the laminar flow environment with a minimal risk of environmentally induced infection. However, to ensure that this system with all its auxiliary components and back-up subsystems would provide a safe environment, a variety of tests were performed.

OBJECTIVE

The objective of this effort was to evaluate the integrity of the Mobile Biological Isolation System to ensure its proper functioning as a biological barrier against contamination. This effort involved a number of tasks:

TASK I: Evaluation of HEPA filters efficiency after repeated ETO sterilization.

TASK II: Evaluation of suit integrity after repeated ETO sterilization.

TASK III: Determination of the minimum time required to sterilize the suit.

TASK IV: Determination of the minimum aeration time necessary to dissipate harmful ETO residuals.

TASK V: Recommended protocol for the determination of patient microflora.

TASK VI: Donning and doffing procedures.

ABSTRACT OF EACH TASK

TASK I: EVALUATION OF HEPA FILTER EFFICIENCY AFTER REPEATED ETO STERILIZATION

The major component of the MBIS system is the HEPA filter utilized to remove all viable organisms from contact with susceptible patients. Thus, the efficiency and reliability of these filters is of paramount importance.

The objective of this task was to determine if repeated ethylene oxide (ETO) sterilization induces filter degradation thereby decreasing filter effectiveness. Twenty-five ETO sterilization cycles were scheduled, each followed by a Freon TF aerosol test, since the DOP method might possibly contaminate the filters. After 18 ETO and Freon tests, no marked degradation or loss of filter efficiency was noted. The additional seven tests were scheduled for completion in conjunction with actual excursions of patients at Childrens Hospital.

TASK II: EVALUATION OF SUIT INTEGRITY AFTER REPEATED ETO STERILIZATION

The objective of this task was to determine if repeated ETO sterilization (up to 120°F) would produce leaks in the suit or otherwise degrade the suit material, since the suit has numerous seams, some sewn and some glued, representing potential weak points. To test for this possibility leak tests were performed according to JSC specifications. The snoop liquid (bubble) method was found to be more practical than the halogen detection method. After each ETO sterilization the snoop test showed no major leaks or suit degradation. Several very minor leaks observed have been confirmed by JPL to be inherent to the suit material and of no immediate concern. In any case, all future excursions should be preceded by a leak test.

TASK III: DETERMINATION OF THE MINIMUM TIME REQUIRED TO STERILIZE THE SUIT

Prior to each excursion the suit must be ETO sterilized to guarantee a safe environment for each patient. The objective of this task was to determine the minimum sterilization time, with assurance of sterility, necessary to kill all viable organisms within the suit. This was accomplished by using Bacillus subtilis var. niger spores as the indicator organism. Spore concentrations of 10^2 , 10^4 , 10^6 and 10^8 were placed within the suit in critical areas (boot, glove, dome, groin and chest) and sterilized with ethylene oxide (ETO) for various periods of time. Initial results showed that three hours of sterilization would inactivate all spores. However, in order to include a margin of safety, an additional hour was added, establishing four hours for the sterilization time. Five consecutive ETO runs consistently gave 100 percent kill, confirming this standard.

TASK IV: DETERMINATION OF MINIMUM AERATION TIME NECESSARY TO DISSIPATE HARMFUL ETO RESIDUALS

Because ethylene oxide may be absorbed into the suit material and is toxic, accurate determination of its concentration after sterilization was necessary. In addition, ETO reacts with chloride ions and water to form several potentially harmful residuals, ethylene chlorohydrin (ETCH) and ethylene glycol (ETG). Federal standards have set the maximum permissible level for skin contact at 250 ppm for ETO, 250 ppm for ETCH and 1,000 ppm for ETG. However, good policy would entail patient contact to be well below these limits accomplished by aerating the suit at 120°F forced air flow for given periods of time.

The objective of this task was to determine the minimum aeration time necessary to dissipate ETO and its residuals to well below harmful concentrations. Concentrations were determined with gas chromatography according to the method of Spitz and Weinberger after 0, 1, 2, 3 and 4 days of aeration.

Immediately after sterilization, the ETO concentration was above the allowable limits generating some concern, particularly for the glove, dome and seam. Concentrations of ETCH and ETG were below the limits but present. Two days of aeration reduced the ETO concentrations to zero and for ETCH and ETG to essentially trace quantities. Consequently two days of aeration was established as the minimum aeration time for patient use.

TASK V: RECOMMENDED PROTOCOL FOR THE DETERMINATION OF PATIENT MICROFLORA

The objective of this task was to support Childrens Hospital of Los Angeles (CHLA) in a joint effort to ensure system reliability of MBIS. On August 7, 1978 the suit was delivered to CHLA. The JPL protocol for sampling skin microflora was submitted to the Bacteriological Department. The plan consists of pre- and post-bacteria sampling of both the patient and the suit.

TASK VI: DONNING AND DOFFING PROCEDURES

At this point in time four subjects have been processed. Donning and doffing procedures have been clarified and rewritten to fit the particular needs of CHLA isolation rooms. An excursion outside the isolation room to the ground level lasted approximately one hour. Although the subject was comfortable and the excursion considered successful, the subject did not wish to continue. Excursions outside the hospital have not been completed. However, arrangements for such excursions will be made.

TASK I
EVALUATION OF HEPA FILTER EFFICIENCY AND STABILITY
AFTER REPEATED ETO STERILIZATION

INTRODUCTION

A major component of the MBIS system is the High Efficiency Particulate Air (HEPA) filters utilized to remove all viable organisms from contact with susceptible patients. HEPA filters are rated as 99.97 percent efficient in removing 0.3 micron particles or larger which provides an effective barrier to microbial contamination of filtered air. Since the MBIS system was designed to permit maximum mobility while maintaining a sterile environment, the air quality, efficiency, and durability of these filters is of paramount importance to the system and patient.

One potential problem, however, is that the HEPA filters require ETO sterilization at 120°F for up to four hours prior to each patient excursion. Considering the numerous excursions and, hence, sterilizations necessary and possible resulting decomposition of the filter media and adhesives binding the media to the filter case, the probability of filter degradation and corresponding loss of efficiency as a consequence has not been adequately determined.

OBJECTIVE

The objective of this task was to determine if repeated ethylene oxide (ETO) sterilization would induce filter degradation thereby decreasing filter efficiency and reliability.

MATERIALS

Ethylene oxide gas sterilizer
DOP (dioctylphthalate)
Freon-12
Sinclair-Phoenix JM 2000
Royco 225 particle counter

Laminar flow bench
Squirrel cage blower
Iso-kinetic probe
Smoke generator
Filter holder (plenum)

METHODS

Although a variety of methods were available for evaluating filter efficiency, most of which involve challenging filters with chemicals, particulates, or various microbes, several popular chemical techniques were considered; DOP and Freon.

The DOP method, developed by the Army Chemical Corps, has gained wide acceptance and is today extensively employed by filter manufacturers, the aerospace industry, the Atomic Energy Commission, and many other users of HEPA clean systems. This method utilized a vaporized organic liquid referred to as DOP (dioctylphthalate), which is a plasticizer used by the chemical industry to impart flexibility to various products. For some applications the DOP method conveys significant advantages but it does bleed through the HEPA filters as well as contributing organic material to an environment specifically requiring low organic contamination.

For environments requiring low organic levels, such as with the MBIS system, an alternative leak test method was available; the Freon-12¹ method developed by A. R. Walters of the Northrop Corporation and J. C. Hurgeton of Bio-Netics Corporation (Reference 1). This method challenges filters with Freon-12 and the filtered air is monitored with a Royco 225 counter. It is fast, inexpensive, and reported nearly as accurate as DOP. Also, no potential harmful residuals remain to contaminate the filtered air, as Freon readily evaporates and has no apparent effect on the filter media. For these reasons Freon-12 was the method of choice.

PROCEDURE

A series of Freon leak tests on a single HEPA filter following each ETO sterilization was the test plan; specifically, eighteen consecutive tests were

¹Trademark of E.I. duPont de Nemours and Co., Inc.

performed. However, prior to the initiation of this schedule, a comparison test of DOP and Freon methods were evaluated.

The set-up for comparison involved the following. A HEPA filter was positioned in a filter holder attached to a squirrel cage blower, smoke generator, and DOP source, in that order. A Sinclair-Phoenix JM 2000 particle counter was calibrated according to manufacturer specifications. DOP was volatilized in the smoke generator and forced through the filter with positive pressure by the blower. In a similar manner Freon-12 was introduced to the filter. Both chemicals were monitored by hand with an iso-kinetic probe attached to the Sinclair-Phoenix. First, the central portion of the filter was scanned, then the sides adhering the filter media to the filter case were monitored and the results recorded.

The results (Figures 1 and 2) showed a close comparison between the two methods, although DOP was slightly more sensitive, and indicated that DOP and Freon can be interchanged according to preference. Moreover both methods demonstrated a high degree of filtering efficiency for the filter tested.

For the eighteen consecutive Freon determinations, a separate apparatus (Figure 3) was employed. All Freon challenges were performed in a laminar flow bench. Freon-12, under pressure, was forced into the blower which further volatilized the Freon. The blower also forced the Freon through the filter with positive pressure.

In this testing mode, a single HEPA filter was ETO autoclaved at 120°F for four hours and immediately tested for efficiency with the above methodology. This procedure was repeated for eighteen consecutive runs.

RESULTS

The results of the eighteen Freon tests (Table 1) showed all readings were well below the Federal Standard of 0.01%. Furthermore, there appeared to be an increase in filter efficiency during the testing program. This is a known phenomenon of HEPA filters; they tend to increase in filter efficiency due to particulate matter blocking the larger pores of the filter media, thereby allowing progressively smaller pores for filtration.

CONCLUSION

After eighteen repeated ethylene oxide sterilizations at 120°F, Freon tests on the filtered air indicated no marked degradation or loss of filter efficiency, but in fact, showed a slight improvement.

REFERENCES

1. Walters, A. R. and J. C. Hurgeton, HEPA Filter Leak Testing Using Freon TF Aerosol, prepared by Northrop Corporation, Electronics Division, Precision Products Department, Norwood, Massachusetts, and by Bio-Netics Corporation, Langley, Virginia.

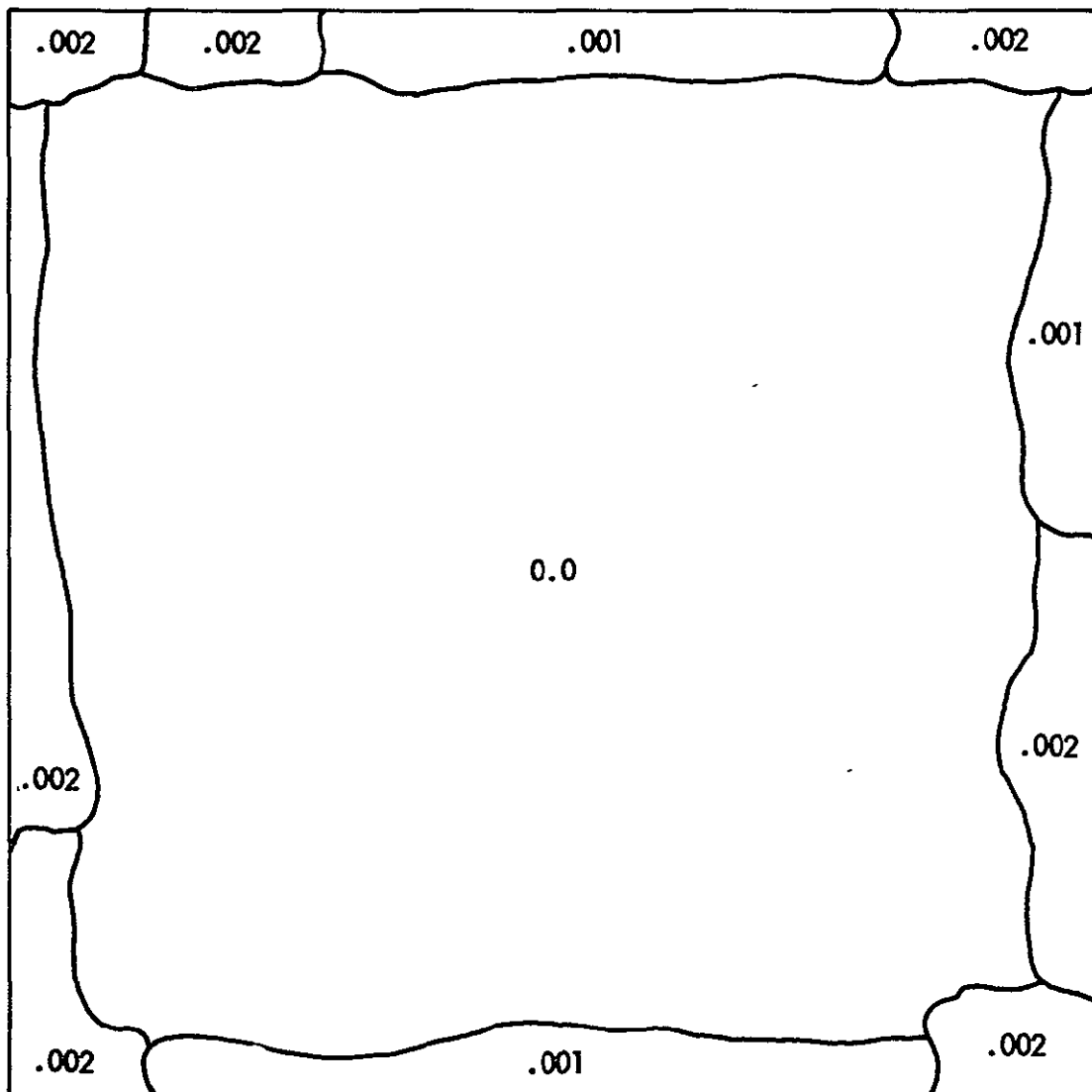


FIGURE 1. DDP Leak Test on HEPA Filter showing percent of upstream concentration through filter. Tolerance limit is 0.01 percent.

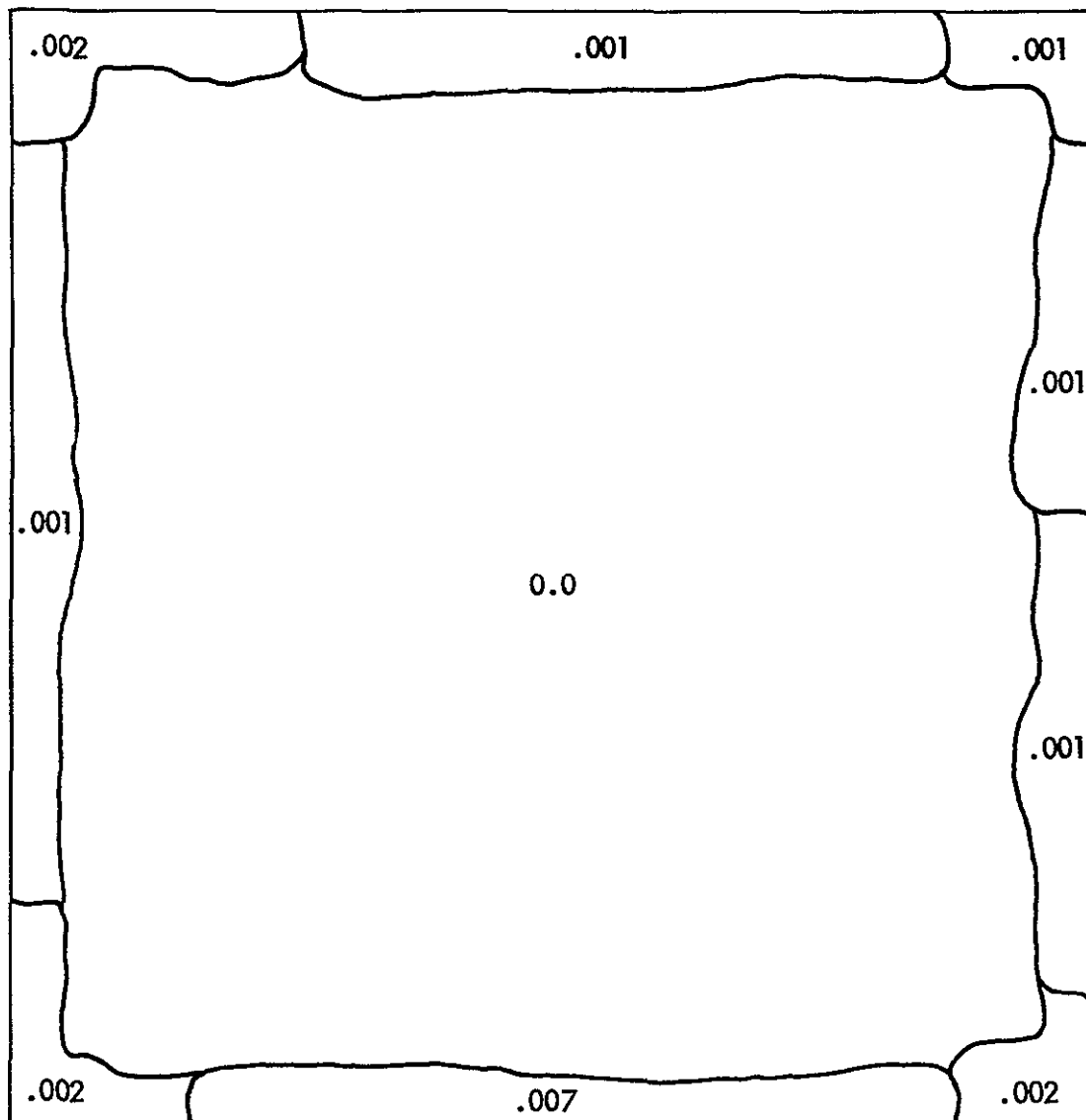


Figure 2. Freon-12 Leak Test on HEPA Filter showing percent of upstream concentration through filter. Tolerance limit is 0.01 percent.

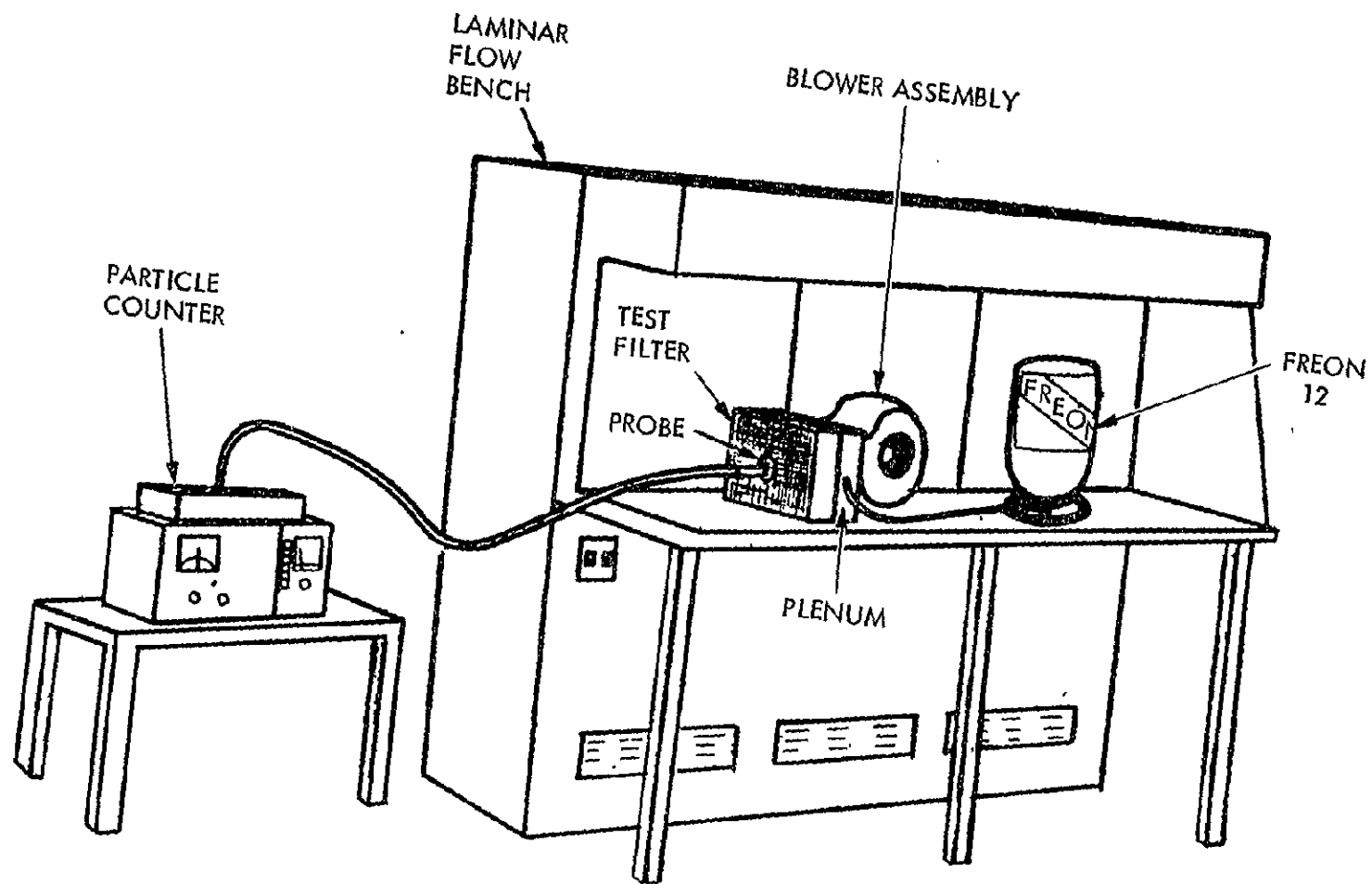


FIGURE 3. Freon Test Set-Up in Laminar Flow Bench

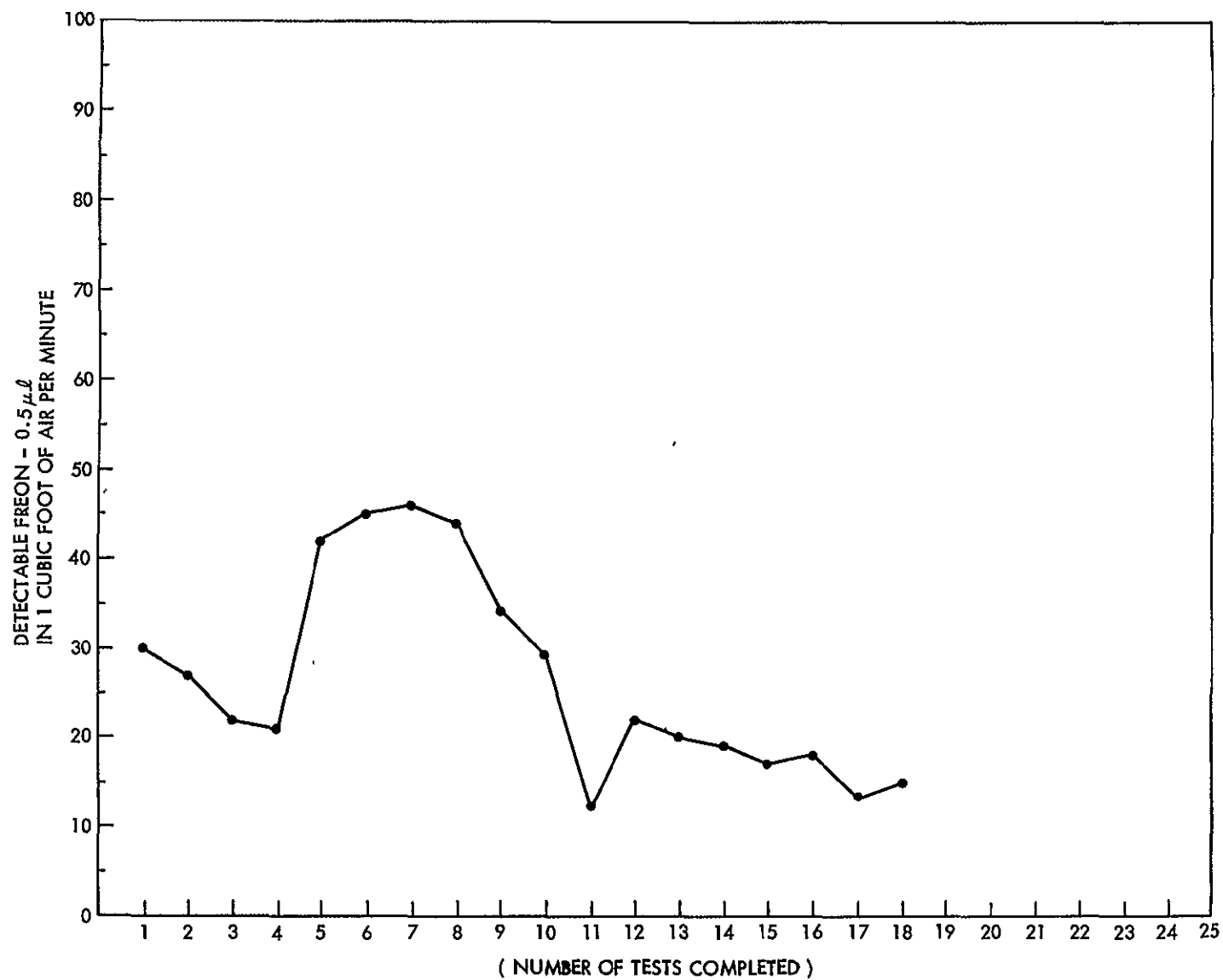


TABLE 1. Results of eighteen consecutive Freon tests, each after a ETO Sterilization.

TASK II
EVALUATION OF SUIT INTEGRITY AFTER REPEATED
ETO STERILIZATION AT 120°F

INTRODUCTION

The Mobile Biological Isolation System was designed to withstand temperatures as high as 130°F, but higher temperatures or prolonged exposures could possibly damage the suit. The upper limit of temperature resistance of the MBIS suit is primarily determined by the Neoprene adhesives (Reference 1) used in the construction of the dome, boots, gloves and seams. Since those high temperatures are known to degrade adhesive bondings, this may reduce the performance of the suit to act as a biological barrier by causing leaks.

However, high temperatures could not be avoided because (ETO) sterilization uses moderate heat over several hours, which could conceivably cause degradation of the suit. To introduce a margin of safety against possible temperature variations of the autoclave, the sterilizing temperature was purposely reduced from 130 to 120°F. Moreover, the effects of repeated sterilizations even at this lower temperature have not been adequately determined and remain for analysis.

OBJECTIVE

The objective of this task was to determine if repeated ETO sterilization at 120°F degrades the adhesives binding the suit or suit materials resulting in major or minor leakage.

MATERIALS

6-year-old suit	Halogen leak detector (GE Type H-10)
9-year-old suit	Liquid snoop
Blower (squirrel cage)	Duct tape
Tygon tubing	Freon-12

METHOD

The fundamental methodology for assaying possible suit degradation was the performance of leak tests on the suit after each sterilization cycle. Two

methods were employed for this purpose. The first method utilized Freon-12 and a Halogen Leak Detector. The second method employed a liquid leak detector (snoop) which bubbles when in contact with a leak. Both methods were used after each of 5 sterilization cycles on both the 6-year-old and 9-year-old suits.

To conduct these tests the exhaust ports on the two MBIS suits were sealed with duct tape. The squirrel cage blower was connected to the inlet hose of the suit dome and turned on, producing a differential pressure in the suit of 0.15 psi. The closed ports were then tested with snoop liquid leak detector to reveal any possible leaks around the duct tape. No leaks were observed.

In this testing mode (Figure 4), Freon-12 was introduced into the system by way of the blower and hose assembly. The outer surface of the suit was then scanned with a halogen leak detector to determine the presence or absence of Freon-12 and all leaks were plotted per attached drawing.

In a similar manner the snoop leak detector was used to locate bypasses of the suit, except Freon-12 was not used. The snoop liquid was brushed over the entire surface of the suit and all bubbles indicating leaks were recorded per attached drawing.

RESULTS

Of the two methods used, the snoop leak detector proved to be the most rapid and accurate. The Freon method utilizing a Halogen Leak Detector worked well but was more difficult to differentiate gases other than Freon-12 interfering with the instrument.

The tests on the 6-year-old suit (Figures 5 and 6) indicated only one very slightly detectable leak on the right wrist (Figure 5). This leak was observed in the first test and did not increase due to repeated ETO sterilizations. All other areas of the 6-year-old suit were, by both methods used, completely devoid of leaks during the entire testing series.

The 9-year-old suit (Figures 7 and 8) similarly revealed a minor leak in the chest area (Figure 7), which also did not increase in severity with repeated sterilizations. Even after 5 ETO sterilization cycles no other leaks were observed.

CONCLUSION

The two minor leaks observed did not increase or further degrade due to sterilization at 120°F. Furthermore, JSC has confirmed their presence and also confirmed that they are of no immediate concern but inherent properties of the suit material and construction.

More importantly, after repeated sterilization no additional leaks were produced at these operating temperatures.

These tests demonstrate the ability of the MBIS suit to withstand ETO sterilization temperatures of 120°F and not degrade. Further, even very slight leaks present no problem to the suit functioning as a biological barrier, as microbes cannot enter the sterile environment of the suit due to positive pressure from the suit toward the exterior.

Consequently it was concluded that repeated ETO sterilization at 120°F does not detrimentally affect or degrade the integrity of the MBIS suit. However, as a safety precaution, a leak test should be performed after each succeeding sterilization and excursion.

REFERENCES

1. Mobile Biological Isolation System Final Test Report for Engineering Evaluation and Acceptance for Operational Use. Prepared by Fred R. Spross. Report No. DE-TA-007. National Aeronautics and Space Administration, Lyndon B. Johnson Space Center, Houston, Texas. October 20, 1976.

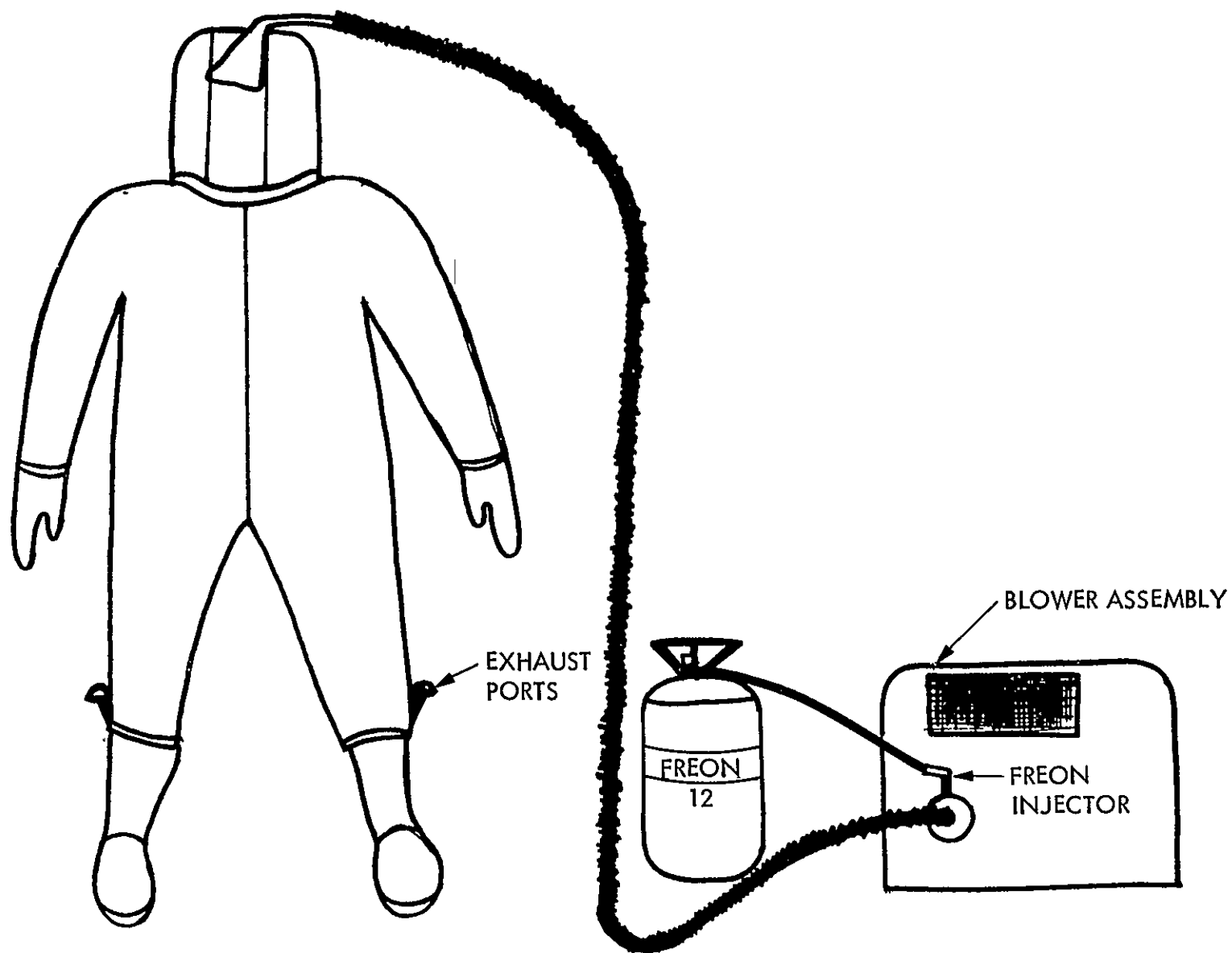


FIGURE 4. Freon Leak Test Set-Up.

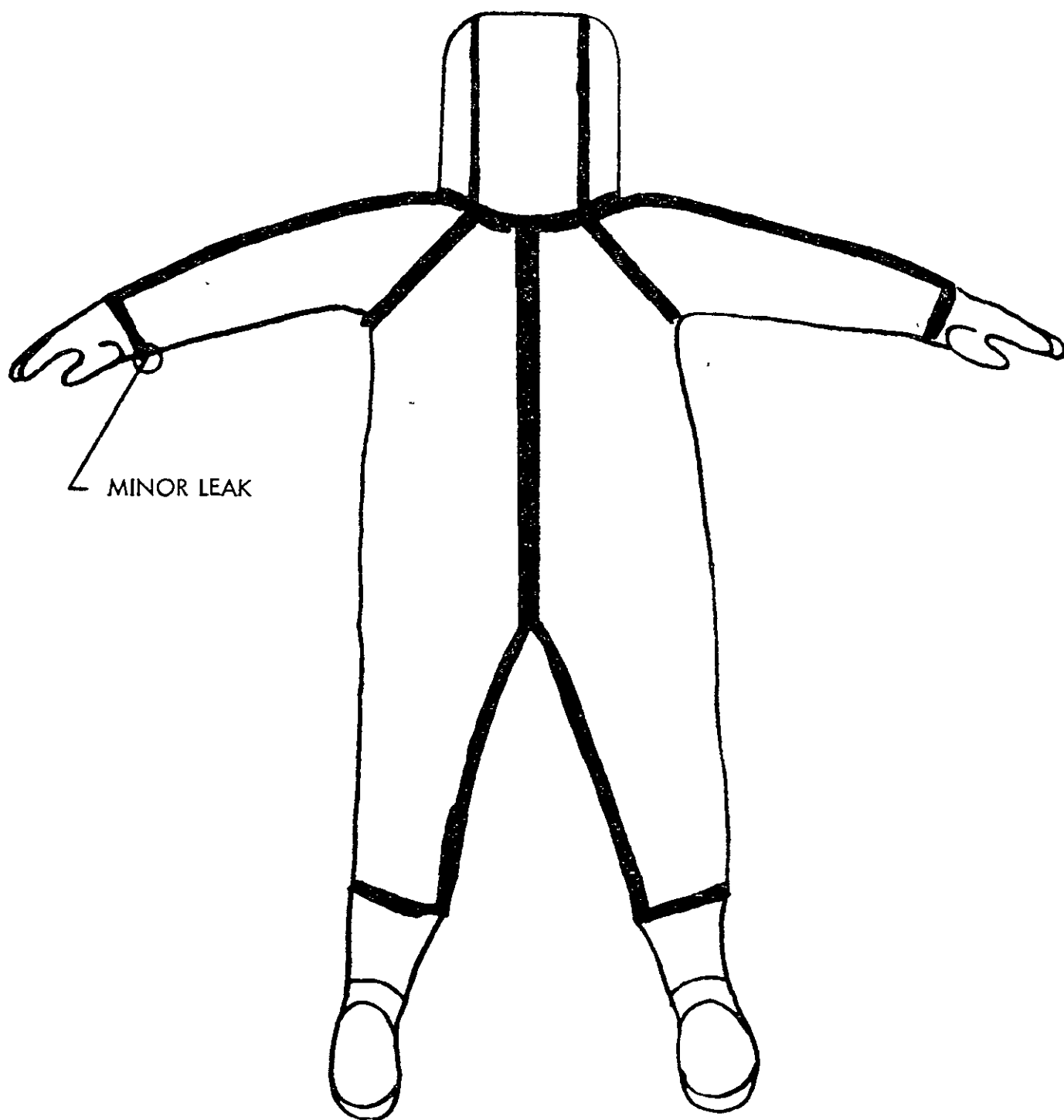


FIGURE 5. Front of 6-year-old suit showing a minor leak on right wrist.

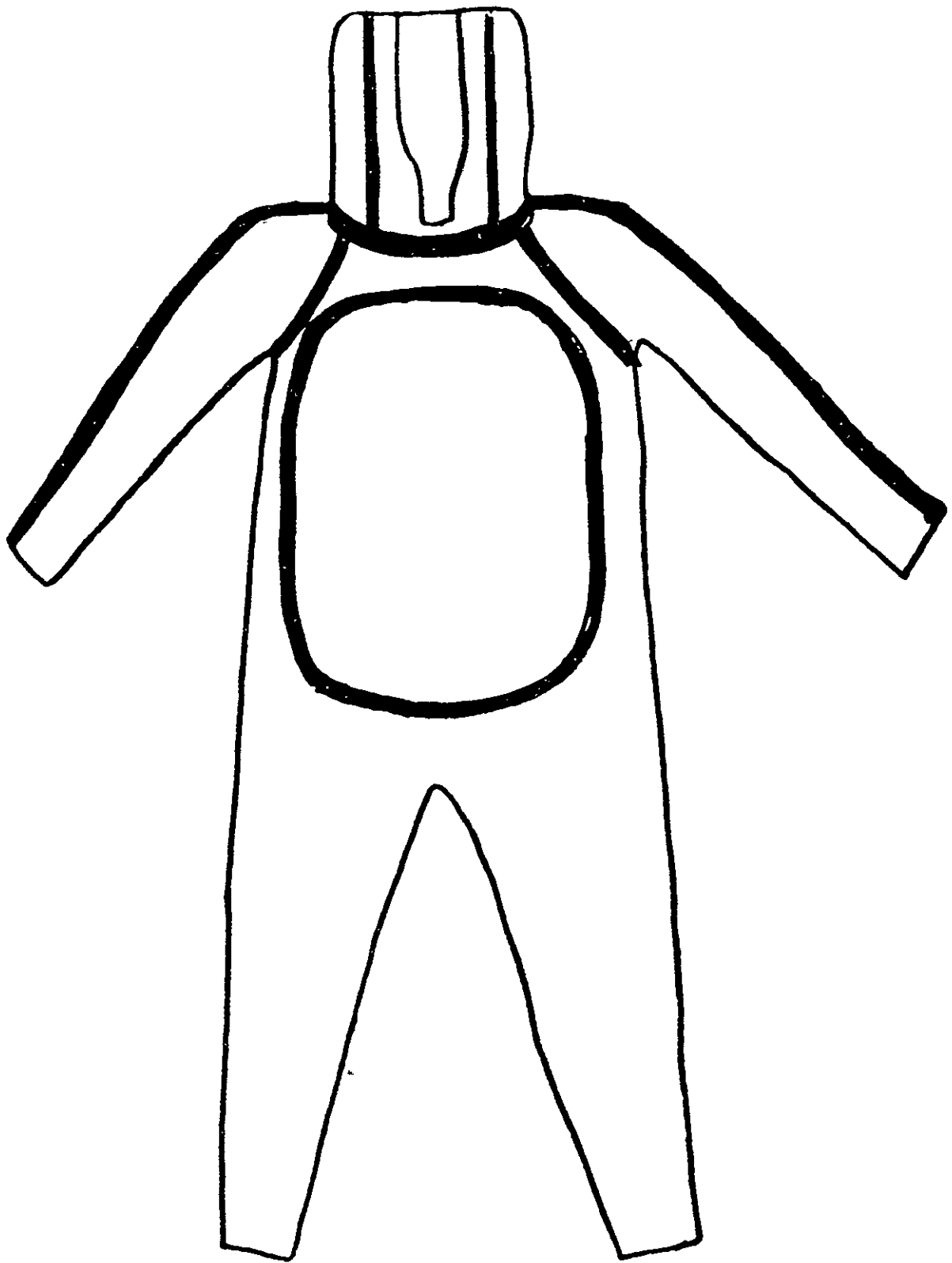


FIGURE 6. Back of 6-year-old suit showing no leaks.

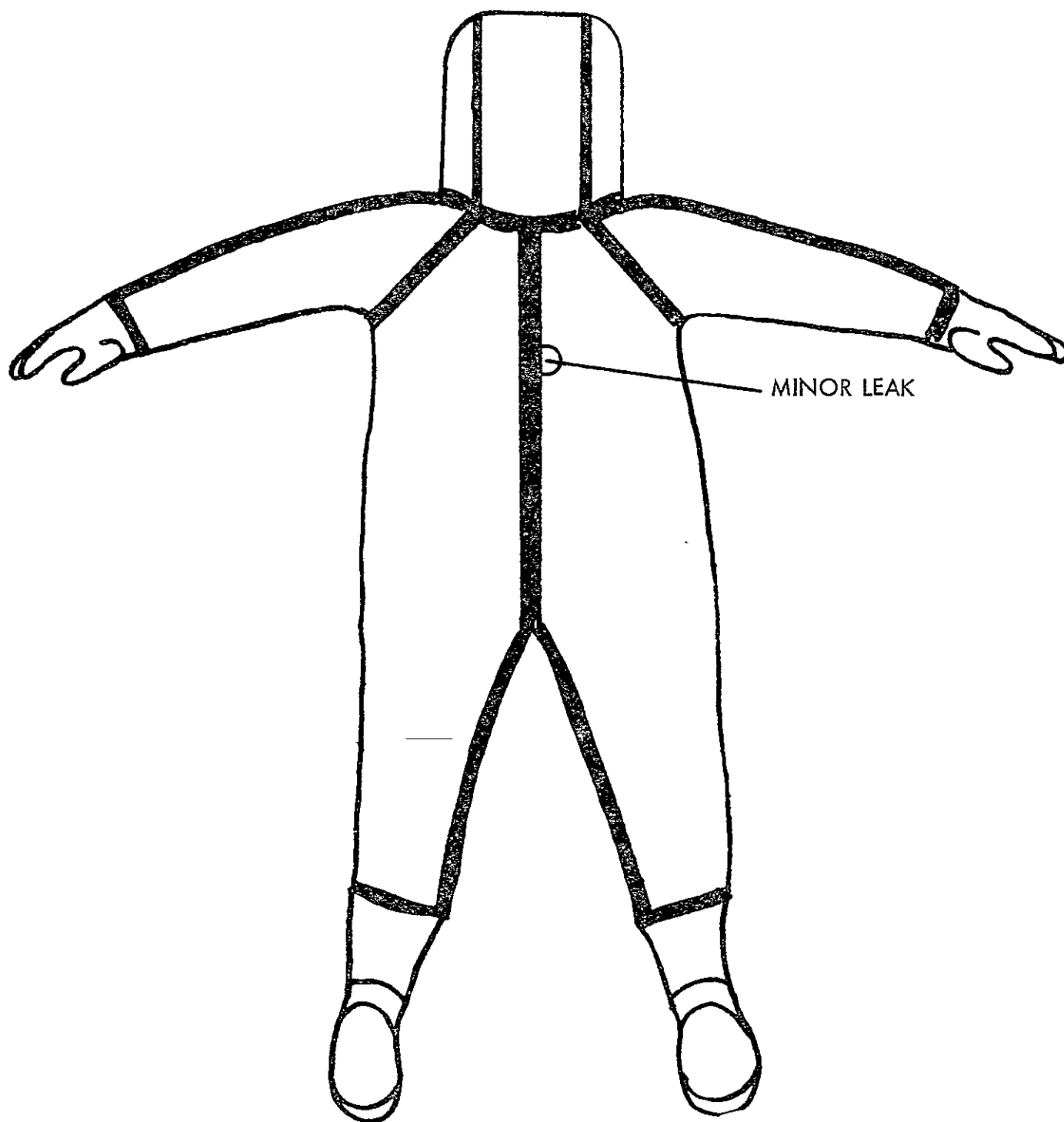


FIGURE 7. Front of 9-year-old suit showing a minor leak in chest area.

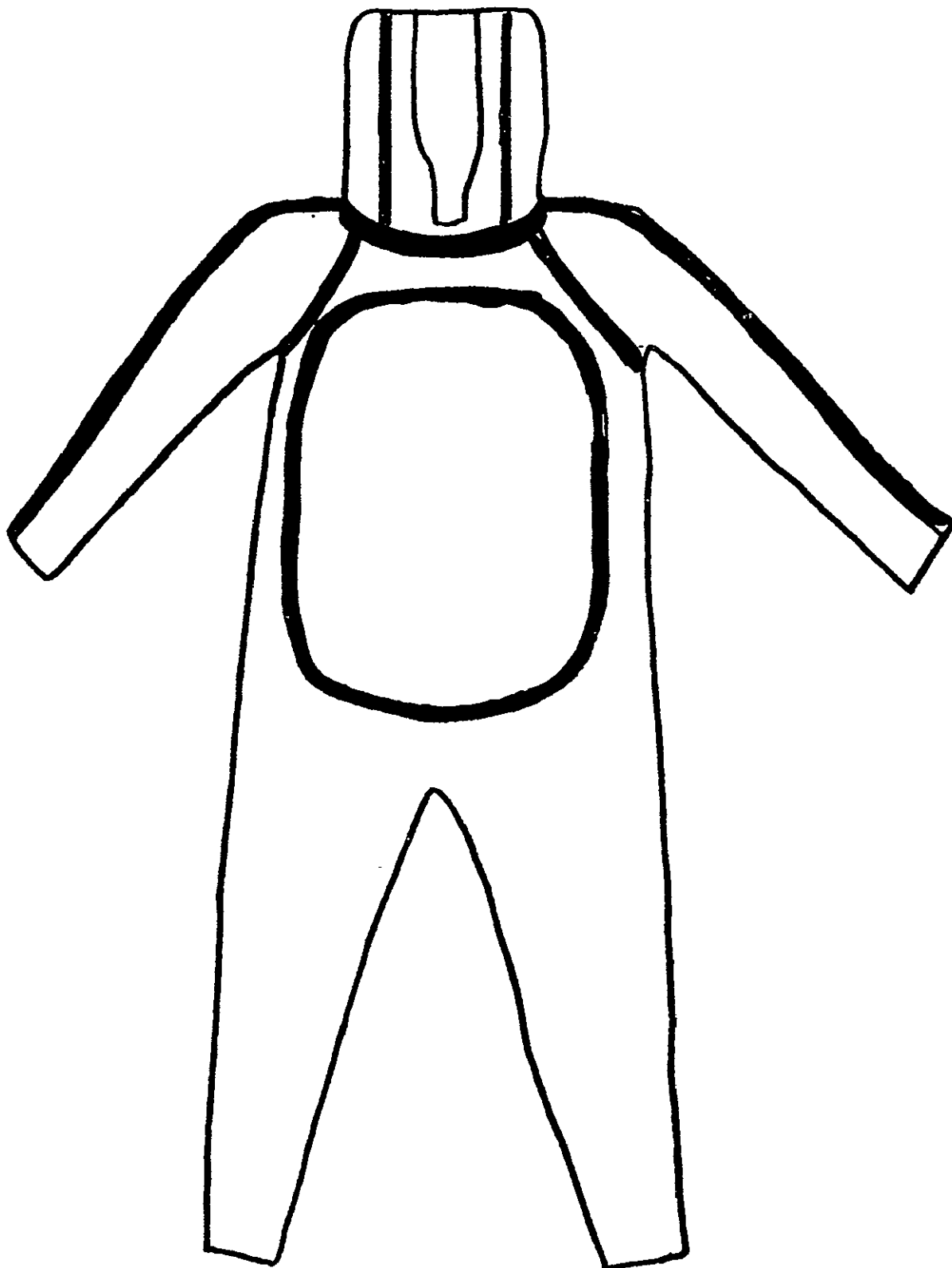


FIGURE 8. Back of 9-year-old suit showing no leaks.

TASK III
DETERMINATION OF THE MINIMUM
TIME REQUIRED FOR
ETO STERILIZATION OF THE SUIT

INTRODUCTION

Because of patient sensitivity to microbes it is absolutely essential that the suit be sterilized to insure that all viable organisms are effectively inactivated. The importance of complete sterilization prior to patient use cannot be over emphasized.

However, heat sterilization was not possible with the Mobile Biological Isolation Suit because of plastic degradation at high temperatures. Consequently, the most feasible method of sterilization at lower temperatures was the utilization of ethylene oxide (ETO) gas, which has been shown to be lethal even to the most resistant of spores, and yet not destructive to many heat sensitive materials.

Significantly there are two important limitations with ETO sterilization. Firstly, even ETO sterilization requires a moderate temperature, usually at 130°F, and this temperature, although not harmful to plastics, may possibly be detrimental to adhesives used in construction of the suit. This may be particularly significant if the suit is submitted to numerous ETO cycles. To compensate for this factor, the ETO operational temperatures were lowered from 130°F to 120°F, which, however, will increase sterilization time.

Secondly, ETO sterilization is a function of contact time. With heat sterilization, contact time is not as important due to the fact that all elements within the autoclave quickly equilibrate to the high operating temperature. With gas sterilization, however, contact time is dependent upon diffusion of ETO to all crevices and pockets of the material to be sterilized.

This is precisely the problem of this task: how long should the contact or sterilization time be for complete sterilization of the suit, considering that when the suit is folded and wrapped in surgical cloth gas, diffusion into all areas will be greatly reduced?

OBJECTIVE

The objective of this task is to determine the optimum ETO sterilization time required to guarantee complete inactivation of all organisms in all positions of the MBIS suit.

MATERIALS

Ethylene Oxide Autoclave

B.S.N. spore strips (10^2 , 10^4 , 10^5 , 10^6 and 10^8)

Trypticase Soy Broth Tubes (10ml)

Incubator (37°C)

Surgical Muslin Cloth

METHODS

To test for complete ETO inactivation a rather resistant and hardy spore, Bacillus subtilis var. niger (B.S.N.), was selected as the test organism. The spores were obtained as prepared strips with the following concentrations; 10^2 , 10^4 , 10^6 and 10^8 . These viable B.S.N. spore strips were placed in critical areas of the suit (Figure 9): the dome, boot, glove, chest and groin. Additional B.S.N. spore strips (10^5) were placed in the filter hose and filter. Corresponding spore strips not exposed to ETO were used as controls.

The suit was then folded, double wrapped in surgical muslin cloth, and placed in ethylene oxide (ETO) autoclave for various periods of time: 2, 3, and 4 hours. All ETO temperature and pressure data were monitored on charts to assure that the temperature never exceeded 120°F and stored for future reference (see Figures 10 through 16).

After sterilization the spore strips were aseptically removed from the various suit positions, placed in sterile trypticase soy broth and incubated

at 37°C. Incubation lasted from 3 to 7 days before the results were scored.

Growth, denoted by cloudy broth, was indicative by insufficient exposure and sterilization of ETO gas, and was hence, scored positive (see Table 2). No growth, denoted by clear broth, indicated sufficient ETO exposure and complete inactivation of all spores and was thus scored negative.

RESULTS

At no time did temperatures exceed 120°F (Figures 10 through 16).

A two-hour ETO cycle (Table 2) showed incomplete inactivation of the spores, particularly for the 10^6 and 10^8 spore strips within the boot and glove areas. Apparently these appendages are the most critical areas of the suit for gas sterilization. ETO may penetrate into these areas more slowly than 2 hours and also the thicker rubber of the boots and gloves may absorb ETO, thereby decreasing the ETO concentration and its bactericidal effectiveness. The minimum sterilization time to kill all viable B.S.N. spores was three hours (Table 2).

Although a three-hour cycle appeared sufficient to inactivate all B.S.N. spores, at least in the single test, an additional hour would ensure complete sterilization and provide a margin of safety. Consequently five consecutive four-hour tests were performed, resulting in 100 percent mortality for all B.S.N. spores in all suit positions (Table 2).

CONCLUSION

These tests confirm that a four-hour ETO sterilization time is sufficient to guarantee complete inactivation of all organisms when tested with B.S.N. spores.

POSITION	CONCENTRATION OF SPORES	2 hr. run	3 hr. run	1 st 4 hr. run	2 nd 4 hr. run	3 rd 4 hr. run	4 th 4 hr. run	5 th 4 hr. run
Dome	10^2	-	-	-	-	-	-	-
	10^4	-	-	-	-	-	-	-
	10^6	-	-	-	-	-	-	-
	10^8	+	-	-	-	-	-	-
Boot	10^2	-	-	-	-	-	-	-
	10^4	-	-	-	-	-	-	-
	10^6	+	-	-	-	-	-	-
	10^8	+	-	-	-	-	-	-
Glove	10^2	-	-	-	-	-	-	-
	10^4	-	-	-	-	-	-	-
	10^6	+	-	-	-	-	-	-
	10^8	+	-	-	-	-	-	-
Chest	10^2	-	-	-	-	-	-	-
	10^4	-	-	-	-	-	-	-
	10^6	-	-	-	-	-	-	-
	10^8	-	-	-	-	-	-	-
Groin	10^2	-	-	-	-	-	-	-
	10^4	-	-	-	-	-	-	-
	10^6	-	-	-	-	-	-	-
	10^8	-	-	-	-	-	-	-
SUIT	10^2	+	+	+	+	+	+	+
CONTROL	10^4	+	+	+	+	+	+	+
	10^6	+	+	+	+	+	+	+
	10^8	+	+	+	+	+	+	+
	10^5	+	+	+	+	+	+	+
Hose	10^5	-	-	-	-	-	-	-
	10^5	-	-	-	-	-	-	-
HOSE CONTROL	10^5	+	+	+	+	+	+	+
Filter	10^5	-	-	-	-	-	-	-
	10^5	-	-	-	-	-	-	-
FILTER CONTROL	10^5	+	+	+	-	-	-	+
PERCENT KILL		40%	100%	100%	100%	100%	100%	100%

(-) negative means no growth.

(+) positive means growth confirmed.

TABLE 2. Results of ETO sterilization of suit with B.S.N. spores as the indicator organisms.

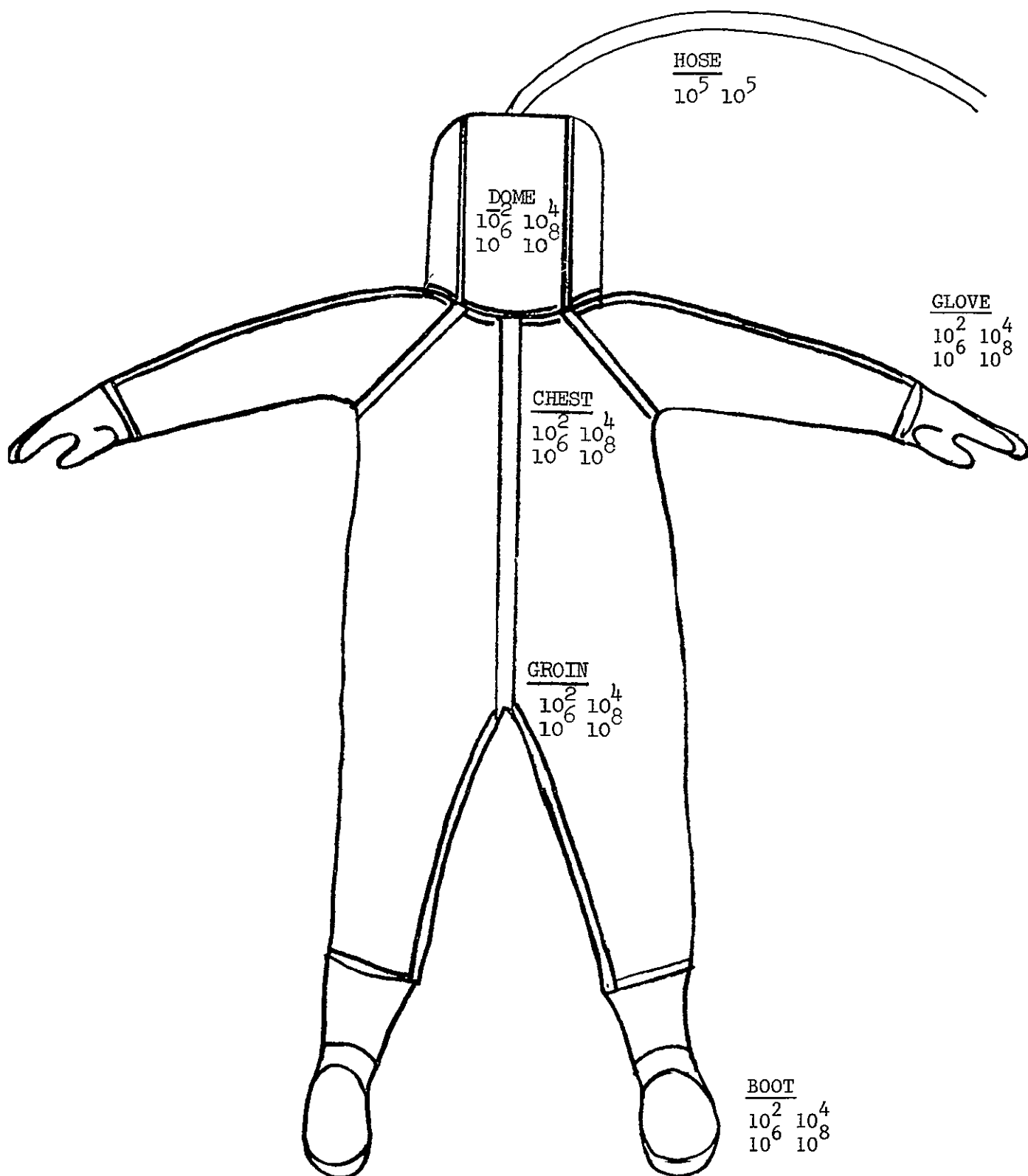


FIGURE 9. Position of B.S.N. Spores

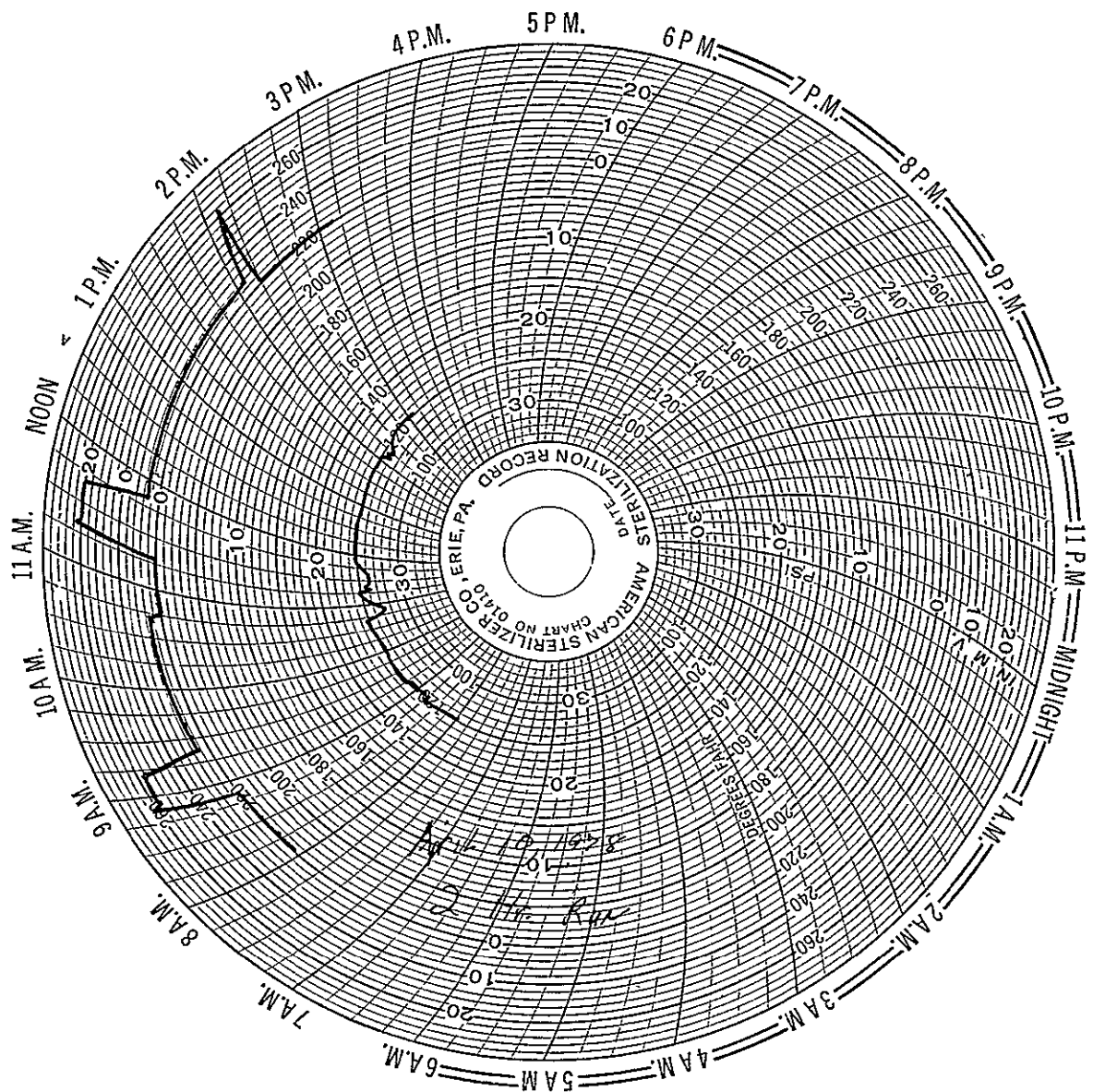


FIGURE 10. Two-hour ETO sterilization chart showing temperature less than 120°F.

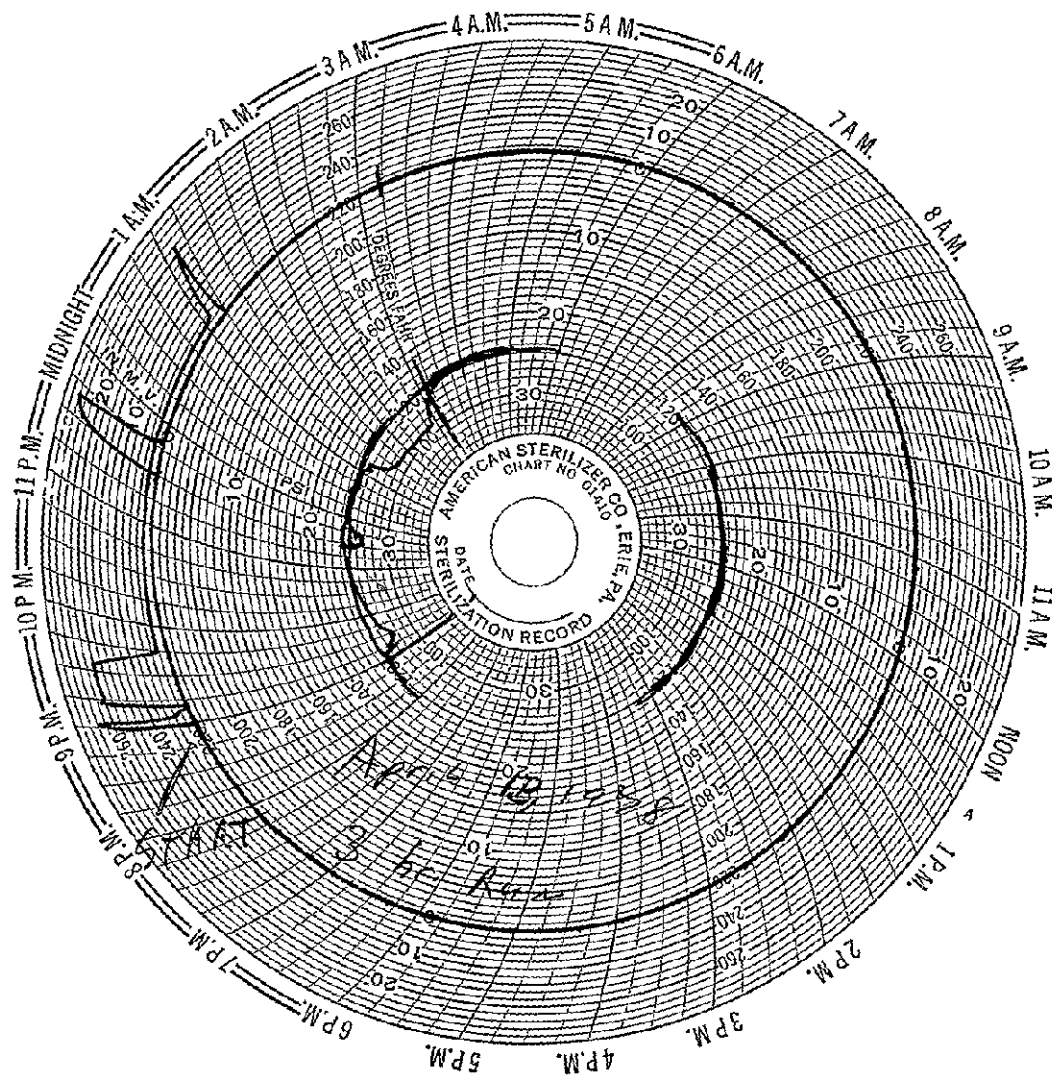


FIGURE 11. Three-hour ETO sterilization chart showing temperature less than 120°F.

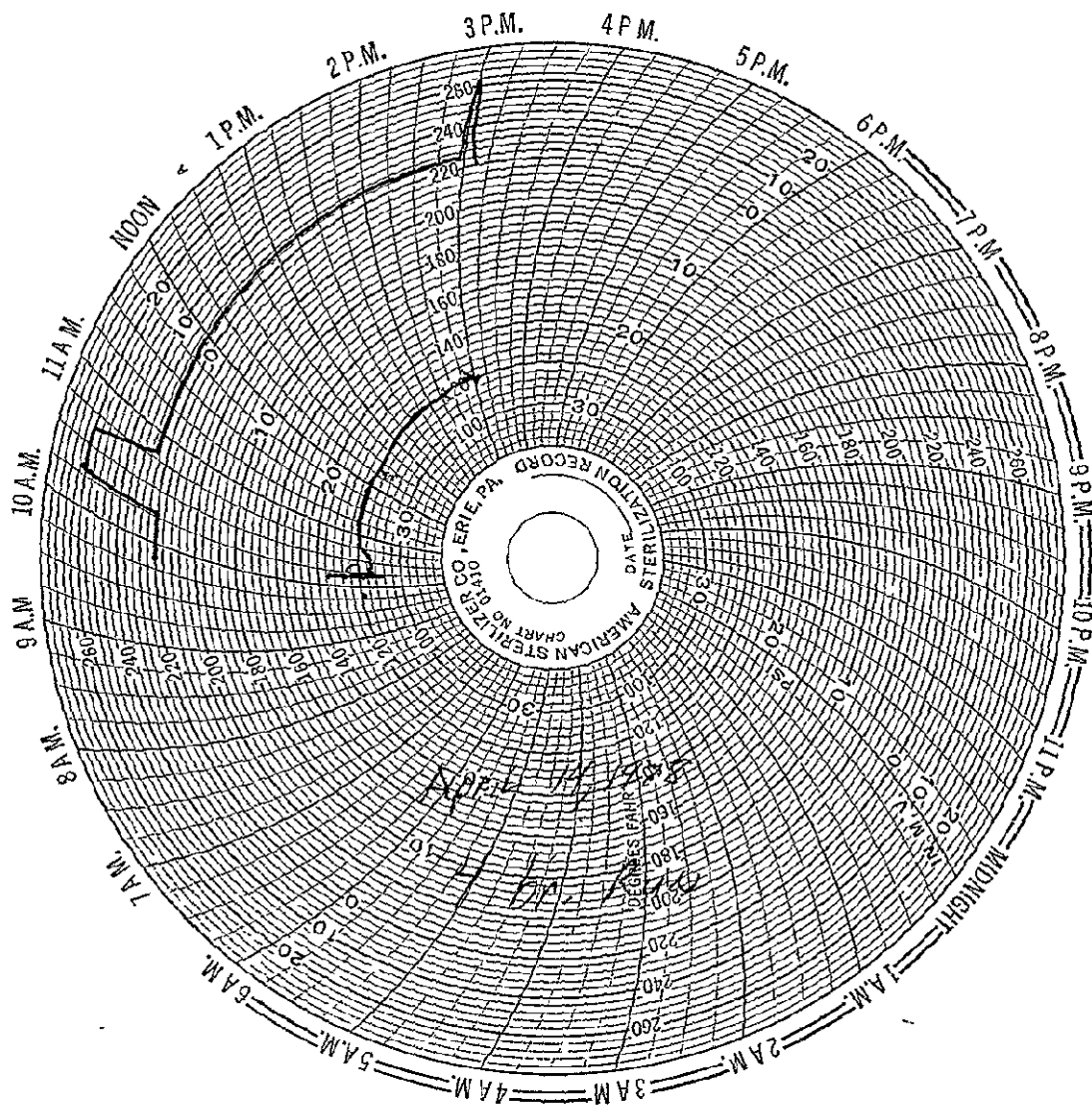


FIGURE 12. First four-hour ETO sterilization chart showing temperature less than 120°F.

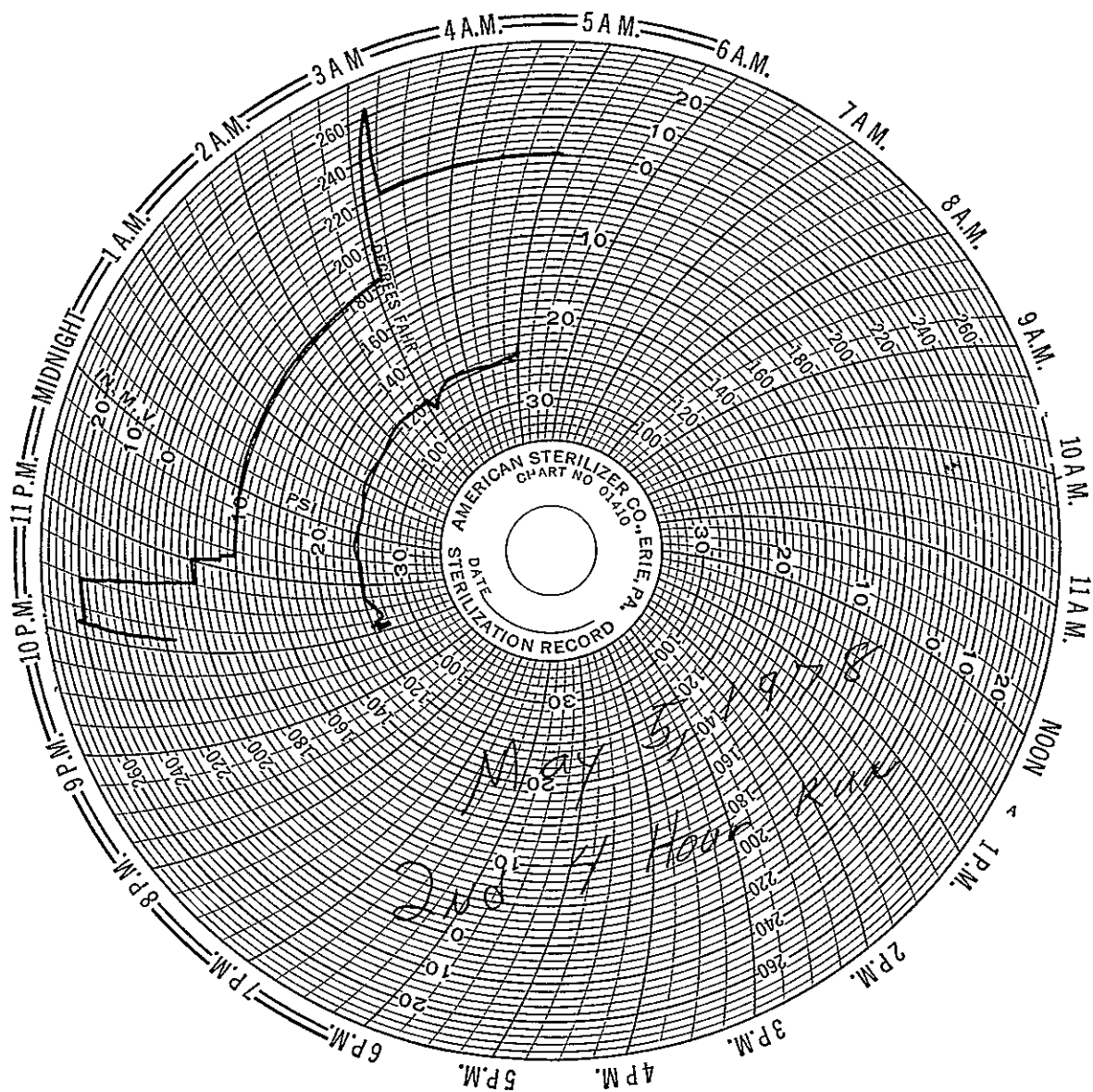


FIGURE 13. Second four-hour ETO sterilization chart showing temperature less than 120°F.

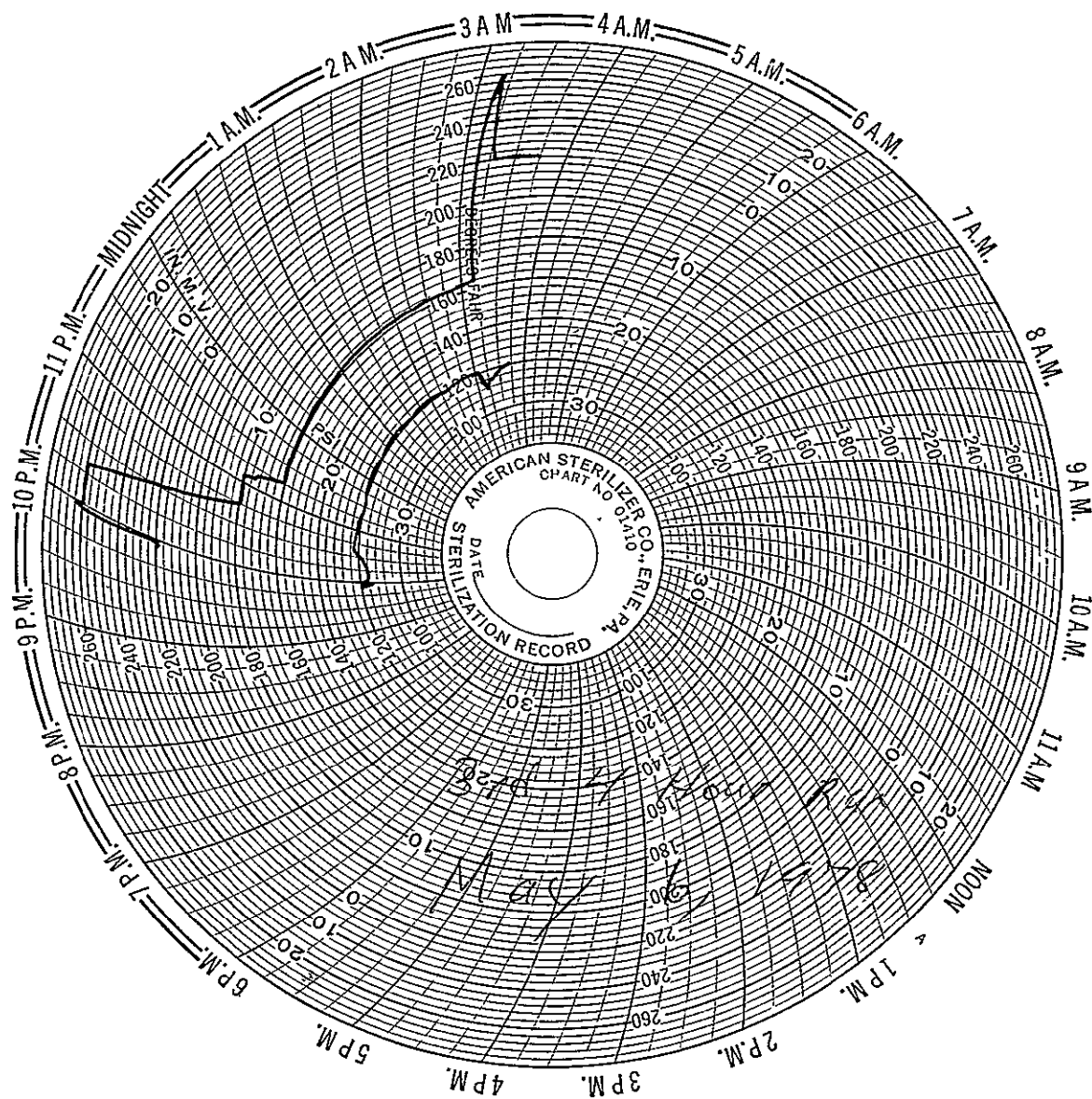


FIGURE 14. Third four-hour ETO sterilization chart showing temperature less than 120°F.

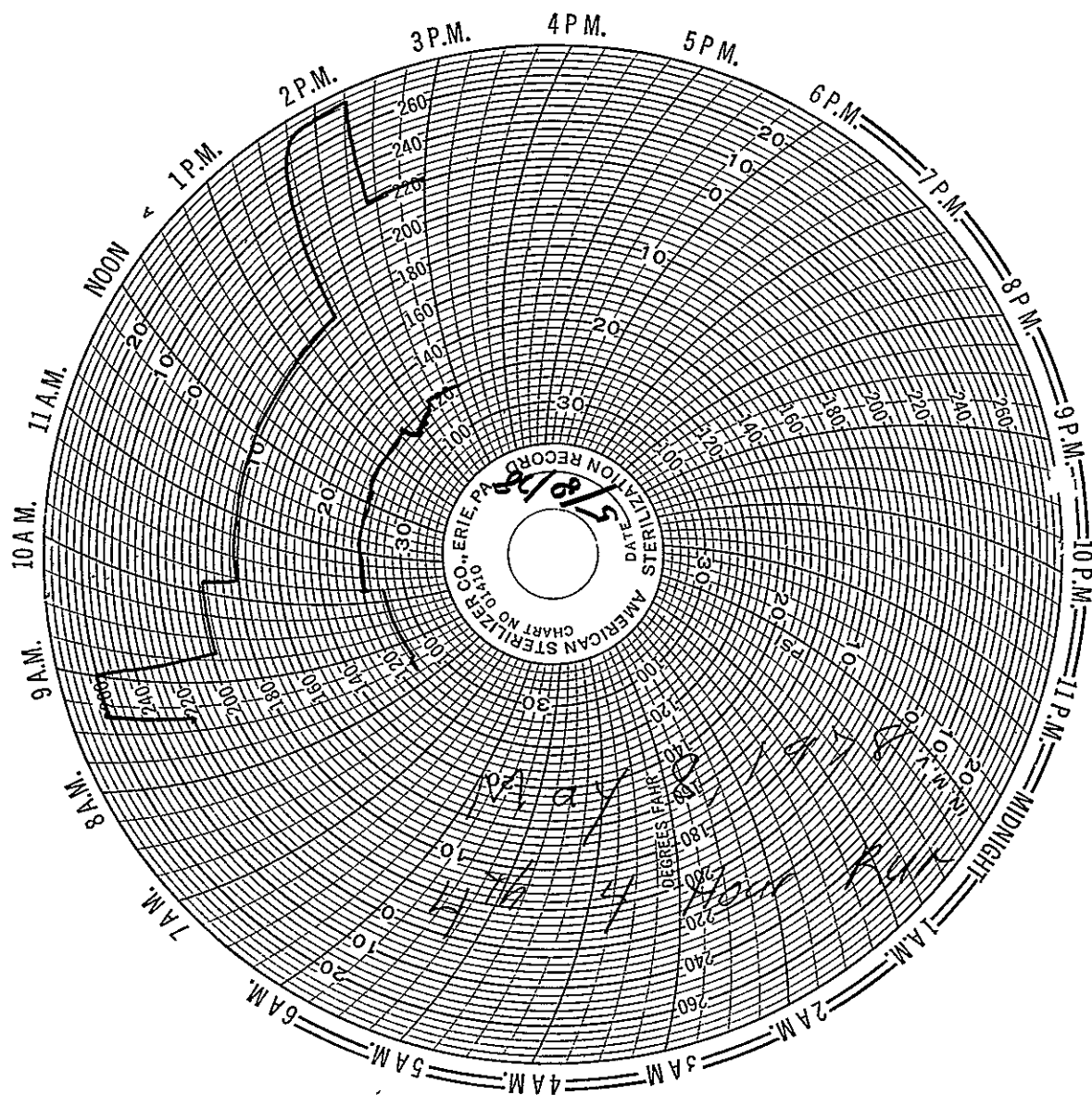


FIGURE 15. Fourth four-hour ETO sterilization chart showing temperature less than 120°F.

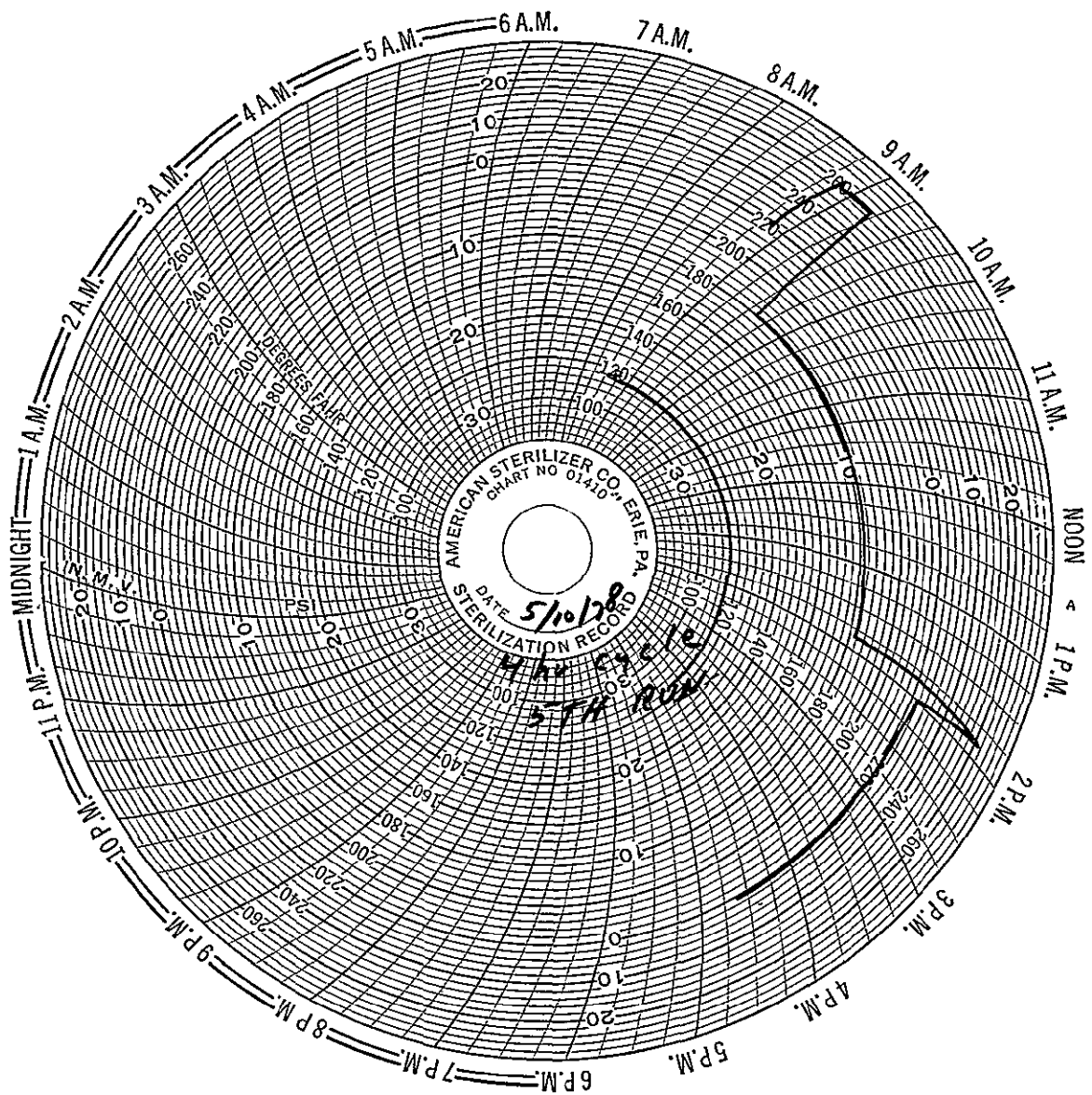


FIGURE 16. Fifth four-hour ETO sterilization chart showing temperature less than 120°F.

TASK IV
DETERMINATION OF MINIMUM AERATION TIME NECESSARY
TO DISSIPATE HARMFUL ETO RESIDUALS

INTRODUCTION

Ethylene oxide (ETO) sterilization of the MBIS suit produces harmful residuals differentially absorbed in the various suit components. Because ETO is toxic, even at low concentrations, accurate determinations of the residual suit concentrations were absolutely mandatory before patient use. In addition ethylene oxide can react with chloride ions present in the suit to form ethylene chlorohydrine (ETCH) and with water to form ethylene glycol (ETG), both of which are also toxic.

Since this possible toxicity to patients coming in contact with a sterilized suit depends on the concentration levels, gas chromatography (Reference 2) was used for the low-level residual determinations. Permissible safe levels established by the FDA (Reference 1) for devices contacting skin are:

Ethylene Oxide	250 ppm
Ethylene Chlorohydrin	250 ppm
Ethylene Glycol	1,000 ppm

In anticipation that these toxic residuals induced by ETO sterilization may exceed maximum exposure limits, aerating the suit for various periods of time was expected to dissipate these residuals from the suit. Further, dissipation could be accelerated by a forced warm air flow, shortening the minimum aeration time.

OBJECTIVE

The objective of this task was two-fold:

1. To determine the initial concentrations of ETO, ETCH and ETG in the MBIS suit immediately after sterilization.
2. To determine the minimum aeration time required to dissipate these residuals from the suit to well below FDA safety limits.

EQUIPMENT AND MATERIALS

The following equipment and chemicals were used for the gas chromatographic determination of ethylene oxide, ethylene chlorohydrin, and ethylene glycol levels:

Varian Gas Chromatography #1440

Chromosorb 101 column for Ethylene Chlorohydrin and Glycol

Chromosorb 102 column for Ethylene Oxide

Autolab Minigrator (Spectra-Physics)

Hewlett-Packard Chart Recorder 7127A

Bubble Meter

Ethylene Oxide

Ethylene Chlorethanol (Eastman Kodak 131-250)

Ethylene Glycol (Eastman Kodak 133)

MBIS suit samples

Hamilton 1 μ l syringes #7001

Silicone Septa W-9 (Applied Science #15432)

30 ml hypo-vials (Pierce Chemical #12944)

Silicone Rubber Septa (Pierce Chemical #13237)

Aluminum caps and cap crincher

METHODS

Since the measurement of the minute concentrations of ET0, ETCH and ETG absorbed in the suit material required data in the nanogram range, gas chromatography techniques were utilized as the most appropriate and accurate.

The three gases used for chromatogram determinations were regulated with the aid of a bubble meter. Helium was the carrier gas with a flow rate of 30 ml/min., while air was set at 300 ml/min. and hydrogen at 30 ml/min.

Temperatures were also important, as slight variations produced larger differences in the measured results. The detector temperature for all residuals was adjusted to 200°C. For the analysis of ethylene oxide, the oven temperature was 100°C and the injector was 120°C. For ethylene chlorohydrin and ethylene glycol determinations, the oven was 160°C and the injector was 195°C.

The chromatography columns were conditioned prior to use. Chromosorb 102, used for the detection of ethylene oxide, was conditioned for 1 day in a 200°C oven with a continuous helium flow of 30 ml/min. After 24 hours 1 µl of acetone was injected into the column every 15 minutes for approximately 3 hours. Chromosorb 101, used for ethylene chlorohydrin and ethylene glycol, was conditioned as above except that the 1 µl injections consisted of distilled water.

For instrument calibration, standard solutions of the three residuals in the nanogram range were prepared. Several drops of ethylene oxide and ethylene chlorohydrin were separately weighed in clean beakers and the volumes were adjusted to 1 liter. Serial dilutions of these stock solutions were then made from 120 ng/µl down to 12 ng/µl ethylene oxide and from 102 ng/µl to 5.25 ng/µl ethylene chlorohydrin (see Table 3).

Because ethylene glycol is an alcohol not blending itself to serial dilutions, a different method was used for the ETG standards. Six concentrations were individually weighed using a lamda micropipette. Each succeeding concentration was approximately doubled, giving ethylene glycol standards

from 156 to 30 ng/ μ l (see Table 3).

One μ l of these standards was injected into the appropriate column and the corresponding data was later used to construct a calibration curve, relating gas chromatography readings with the concentration of residuals in nanograms.

Two instruments were used to monitor gas chromatography outputs: (1) a chart recorder, and (2) a computing integrator (Spectra-Physics Minigrator). A comparison of these two methods revealed the most accurate and also the potential variations inherent within both systems.

A series of 1 μ l injections with a standard 50 ng/ μ l solution of ethylene chlorohydrin was used. Both the chart recorder and the minigrator were simultaneously monitored. The peak heights of the chart recorder were later measured in centimeters. The area under the curve was automatically calculated by the minigrator which had the following programmed parameters; PW at 15, SS at 20, TP at 20 and T_1 at 100.

Test results verified narrow confidence intervals for both methods. The peak heights of the chart recorder were within ± 6 percent while the minigrator units were within ± 3 percent, which are the expected variations for future chromatography analyses. In addition these statistics include variations of the injector syringe, variation of the various temperatures and gas flow parameters, and human variations.

Because the minigrator is more accurate, easier to use, and quicker, it was the method of choice for all sample analyses.

PROCEDURE

The determination of residuals within the suit after sterilization required: autoclaving suit samples, suspending the samples in solution, injecting 1 μ l of solution into the appropriate column, recording the results in nanograms, and calculating residual concentrations in parts per million (ppm).

Uniform samples of the five suit materials, boot, glove, dome, suit and seam were cut with a cork borer. Twenty representatives of each material were weighed giving the following data:

Boot	0.0791	$\pm$ 0.016	grams
Glove	0.0651	$\pm$ 0.0025	grams
Dome	0.0938	$\pm$ 0.0009	grams
Seam	0.2439	$\pm$ 0.0046	grams
Suit	0.0798	$\pm$ 0.0026	grams

Statistical analysis showed consistent weights with narrow confidence intervals having a maximum variation of ± 3.8 percent. This uniformity justified allowing the above averages to be used in later tests for calculating ppm without reweighing each sample individually.

All suit materials were placed in their corresponding suit locations, for example, the dome sample was positioned in the dome, etc., wrapped in surgical cloth, and ETO autoclaved for 4 hours. One sample of each material was removed and immediately analyzed for ETO, ETCI and ETG concentrations. The remaining samples were left in the suit which was placed in a 120°F forced air aerator.

Each day thereafter for 4 days, samples were removed from the suit and analyzed. One series of samples was placed in 5 ml of acetone and analyzed

for ethylene oxide. A second series was placed in 5 ml of distilled water and allowed to soak for 24 hours before ethylene chlorohydrin and ethylene glycol determinations were made.

Analysis consisted of injecting 1 μ l of sample solution into the gas chromatography unit. The resulting minigrator readings were recorded and later converted into ng/ μ l using the prepared calibration curves. Parts per million (ppm) were calculated from the following formula:

$$\text{ppm} = \frac{0.5 \times \text{ng}/\mu\text{l}}{\text{average weight of sample (g)}}$$

RESULTS

The initial ethylene oxide concentrations without any aeration were higher than the maximum FDA limits of 250 ppm (Table 4), particularly for the glove, dome and seam. This justified aeration to dissipate this toxic gas. After aerating for 1 day with a forced air flow, the ethylene oxide levels were reduced to minor concentrations. After 2 days all suit samples were zero (Table 4).

With no aeration, ethylene chlorohydrin was well below the limit of 250 ppm, the highest concentration was 84 ppm in the seam. Aerating reduced these low concentrations. However, all ethylene chlorohydrin was not dissipated until after 4 days (Table 4).

Ethylene glycol was well below the maximum concentrations of 1,000 ppm in all suit materials (Table 4). Most readings were below the lowest detectable levels, between less than 45 to less than 169 ppm.

Although non-aerated ethylene oxide concentrations were higher than ethylene chlorohydrin and ethylene glycol, it is dissipated more quickly from the suit materials probably because it is a gas, whereas ethylene

chlorohydrin and ethylene glycol are liquids at room temperature and slower to dissipate.

CONCLUSION

The objective of this task was to determine the minimum aeration time necessary to dissipate ethylene oxide and its residuals to well below harmful concentrations. Concentrations were determined by gas chromatography after 0, 1, 2, 3 and 4 days of aeration. Immediately after sterilization, ethylene oxide concentrations were above the allowable FDA limits, generating some concern, particularly for the glove, dome and seams. Concentrations of ethylene chlorohydrin and ethylene glycol were below the FDA limits but present. Two days of aeration reduced the ethylene oxide concentrations to zero and for ethylene chlorohydrin and ethylene glycol to essentially trace quantities. Consequently, two days of aeration was established as the minimum aeration time for patient use.

REFERENCES

1. Ethylene oxide, ethylene chlorohydrin and ethylene glycol: Proposed maximum residue limits and maximum level of exposure, Federal Register. Department of Health, Education and Welfare, Food and Drug Administration Friday, June 23, 1978, Part V.
2. Spitz, H. D. and J. Weinberger, Determination of Ethylene Oxide, Ethylene Chlorohydrin and Ethylene Glycol by Gas Chromatography, Journal of Pharmaceutical Sciences, 60 (2): 271-274, 1971.

ETHYLENE OXIDE STANDARDS

<u>ng/μl</u>	<u>Minigrator Readings</u>
120	10,481
60	5,146
36	3,083
24	1,991
12	1,059

ETHYLENE CHLOROHYDRIN STANDARDS

<u>ng/μl</u>	<u>Minigrator Readings</u>			
	<u>#1</u>	<u>#2</u>	<u>#3</u>	<u>Average</u>
105.	3,354	3,597	3,857	3,603
79	2,881	2,628	2,775	2,761
52.5	1,850	2,641	1,945	1,812
26	906	853	885	881
15.75	721	581	660	654
10.5	305	268	277	283
5.25	91	58	88	79

ETHYLENE GLYCOL STANDARDS

<u>ng/μl</u>	<u>Minigrator Readings</u>				
	<u>#1</u>	<u>#2</u>	<u>#3</u>	<u>#4</u>	<u>Average</u>
156	2,715	2,349	2,340	3,004	2,602
90	1,081	2,163	1,170	1,283	1,175
63	853	762	800	654	763
55	603	587	612	541	587
48	438	256	549	421	416
30	179	190	200	222	198

TABLE 3. CALIBRATION CURVE RELATING MINIGRATOR UNITS TO ng/ μ l

ETHYLENE OXIDE IN PPM (Limit is 250 ppm)

	<u>Aeration time in days</u>				
	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
BOOT	167	13	0	0	0
GLOVE	438	0	0	0	0
DOME	560	16	0	0	0
SEAM	271	0	0	0	0
SUIT	119	0	0	0	0

ETHYLENE CHLOROHYDRIN IN PPM (Limit is 250 ppm)

	<u>Aeration time in days</u>				
	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
BOOT	<19	0	0	0	0
GLOVE	62	38	35	<23	0
DOME	0	0	0	0	0
SEAM	84	27	18	15	0
SUIT	38	<19	<19	<19	0

ETHYLENE GLYCOL IN PPM (Limit is 5,000 ppm)

	<u>Aeration time in days</u>				
	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
BOOT	<139	<139	152	170	0
GLOVE	<169	0	<169	0	23
DOME	<117	<117	0	0	0
SEAM	< 45	0	< 45	0	<45
SUIT	<138	<138	<138	0	0

TABLE 4. RESIDUAL CONCENTRATIONS OF BOOT, GLOVE, DOME, SUIT and SEAM

TASK V

RECOMMENDED PROTOCOL FOR THE SAMPLING OF SKIN MICROFLORA

The following protocol is recommended for analysis of microflora before and after each patient excursion. A total of nine locations should be sampled.

1. Neck - Microflora samples from the back of the neck should be taken with sterile cotton swabs moistened in trypticase soy broth (TSB). Washing should be made of a 4 x 5 cm area by rubbing thoroughly. After sampling, the swab should be inserted into a test tube containing 5 ml of sterile TSB and sonicated for 5 minutes. Subsequently the solution should be diluted to 10^0 , 10^1 , 10^2 , 10^3 and 10^4 with TSB and plated on nutrient agar. After three days of incubation at 37°C, plate counts should be made and converted to organisms per cm^2 .
2. Hair - Microflora samples from hair in the region behind the head should be taken with sterile cotton swabs moistened in trypticase soy broth (TSB). Washing should be made of approximately a 4 x 5 cm area by rubbing portions of hair. After sampling, the swab should be inserted into a test tube containing 5 ml of sterile TSB and sonicated for 5 minutes. Subsequently the solution should be diluted to 10^0 , 10^1 , 10^2 , 10^3 and 10^4 with TSB and plated on nutrient agar. After three days of incubation at 37°C, plate counts should be made and converted to organisms per cm^2 .
3. Axilla - Microflora samples from the armpits (axilla) should be taken with sterile cotton swabs moistened in TSB by rubbing both armpits thoroughly. After sampling, the swabs should be inserted into a test tube containing 5 ml of sterile TSB and sonicated for 5 minutes. Subsequently the solutions should be diluted to 10^0 , 10^1 , 10^2 , 10^3 and 10^4 with TSB and plated on nutrient agar. After three days of incubation at 37°C, plate counts should be made and converted to organisms per armpit.

4. Groin - Microflora samples from the region of the groin should be taken with sterile cotton swabs moistened in trypticase soy broth (TSB). Washing should be conducted by thoroughly rubbing the indicated area of skin. After sampling, the swab should be inserted into a test tube containing 5 ml of sterile TSB and sonicated for 5 minutes. Subsequently the solution should be diluted to 10^0 , 10^1 , 10^2 , 10^3 and 10^4 with TSB and plated on nutrient agar. After three days of incubation at 37°C, plate counts should be made.
5. Throat Swab - Microflora samples from the tonsil area of the throat should be taken with a dry, sterile cotton swab. Sample collection is accomplished by rubbing the swab on the surface of the tonsils. After sampling, the swab is inserted into a sterile test tube containing 5 ml of sterile TSB and sonicated for 5 minutes. This solution should subsequently be diluted to 10^0 , 10^1 , 10^2 , 10^3 , 10^4 , 10^5 and 10^6 in TSB and 4 ml plated on the surface of nutrient media. Following this, one ml of the remaining sample is placed into 5 ml of nutrient broth and incubated at 37°C for 6 hours. After incubation, the sample is streaked onto blood agar plate to isolate Beta hemolytic streptococci.
6. Gargle Samples - Microflora samples should be taken from the mouth and oral cavity by washing once with 10 ml of PBS solution. Washing is conducted by gargling in the recesses of the mouth and the region of the pharynx for 30 seconds. After this the liquid is collected and divided equally. One part will subsequently be diluted to 10^0 , 10^1 , 10^2 , 10^3 , 10^4 and 10^5 and plated on the surface of nutrient media. The remaining portion of the original sample should be centrifuged at 3,000 rpm for 15 minutes. Withdraw the supernatant and plate approximately 1.5 ml of the residual on nutrient media.

7. Auditory Canals - Microflora are sampled from both ear channels by rubbing with a sterile cotton swab moistened in PBS. After sampling, the swabs should be inserted into a test tube containing 5 ml of sterile TSB and sonicated for 5 minutes. Subsequently the solutions should be diluted to 10^0 , 10^1 , 10^2 , 10^3 and 10^4 with PBS and plated on nutrient agar. After three days of incubation at 37°C, plate counts should be made.
8. Nostrils - Microflora from the recess of the nose should be taken with the aid of a dry sterile cotton swab. During this procedure, the mucous should be taken from the forward and middle part of both nasal channels. After sampling, the swab should be inserted into a test tube containing 5 ml of sterile TSB and sonicated for 5 minutes. Subsequently the solution should be diluted to 10^0 , 10^1 , 10^2 , 10^3 and 10^4 with TSB and plated on nutrient agar.
9. Toe Webs - Microbial samples from between the toes of both feet should be collected by rubbing between the toes with a sterile cotton swab moistened in TSB. After sampling, the swab should be inserted into a test tube containing 5 ml of sterile TSB and sonicated for 5 minutes. Subsequently, the solution should be diluted to 10^0 , 10^1 , 10^2 , 10^3 and 10^4 with TSB and plated on nutrient agar.

TASK VI
DONNING AND DOFFING PROCEDURES

The following donning and doffing procedures were developed at Childrens Hospital at Los Angeles by Mrs. R. Moldan of CHLA, Bill Carmine of JSC and by Bionetics personnel. Children of similar age and heights as chemotherapy patients were used in the development of these procedures.

SUIT DORNING

Three attendants should directly assist in both donning and doffing. One nurse should "suit up" and enter the PT room. The other two attendants should assist the patient outside the red lines near the door. Care and caution should be maintained at all times to minimize any contamination.

- a. Verify that subject is prepared and positioned to don suit.
- b. Unzip protective flap.
- c. Unsnap the 3 retention snaps and secure the sleeves to prevent rolling.
- d. Switch BLSS SI from norm to emer.
- e. Unroll sleeves and release velcro fasteners.
- f. Place boots on the floor or low bench and hold top of suit up from entry sleeve at shoulders.
- g. Subject should place feet in legs of suit and stand up into boots.
- h. Have subject bend forward at the waist and enter the upper torso with the head and shoulders.
- i. Have subject stand and pull suit onto the body.
- j. Have subject don one arm at a time.
- k. Fit the subjects fingers into the gloves.
- l. Fasten velcro fasteners on entry sleeve.
- m. Switch BLSS SI to norm.
- n. Fold edges of entry sleeve in and secure velcro.
- o. Begin rolling entry sleeve as tightly as possible slowly toward

- the subjects torso while suit air is expelled from the leg vents.
- p. Secure the 3 restraint straps.
 - q. Close zipper on protective flap.
 - r. Assist subject in exiting the room. Assist subject into cover garment - legs first, then arms.
 - s. Fasten short air hose tether straps to shoulders of suit under cover garment.
 - t. Fasten cover garment straps across torso back - fasten velcro at back of neck and ankles.
 - u. Run long air hose tether straps over shoulders, under arms and fasten.
 - v. Verify differential pressure gauge is indicating between 0.15 and 0.30 psi.

SUIT DOFFING

- a. A low bench should be positioned inside of PT room near the doorway and a sterile blanket placed on top. The bench should be extended slightly beyond the doorway but not beyond the outside red lines.
- b. First attendant sets up sterile basin with Biomet bactericide on a sterile field just outside the room.
- c. Have the subject stand with back to doorway of room, just outside the red line.
- d. Second attendant dons sterile gloves, and using sterile washcloth, wipes the entire protective flap and zipper area with Biomet.
- e. Second attendant unzips suit back and unsnaps retention straps. Unroll the sleeve and hold top and bottom of sleeve taut.
- f. First attendant dons sterile gloves and wipes outside of entire sleeve with Biomet.

- g. Nurse inside room assists subject through open sleeve and attendants outside assist by holding suit while subject is doffing.
- h. Have subject remove arms from suit, one at a time.
- i. have subject bend at waist, remove head from helmet, and sit back on end of bench, leaning back to remove upper torso from suit.
- j. Attendant will pull suit and blanket covering bench from doorway area.

