Protection Branch Report of Test No. 9-67

Effectiveness of Dry Heat and Ethylene Oxide Gas Upon Spore Contamination Located Between Mated Surfaces and On Exterior Surfaces of Various Materials

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As part of the overall investigation of spacecraft steriliz ation, this comparative study was undertaken to determine whether there is an appreciable difference in the sterilizing effectiveness of dry heat or ethylene oxide gas upon microbial contamination located between mated surfaces in contrast to the contamination located on the external surface of a material. Previous data obtained in this Laboratory [1], and in others [2,3,4] indicated that a significantly longer exposure to dry heat is required to kill microorganisms embedded or entrapped in a material. It is therefore possible that contamination between mated surfaces will also be more difficult to sterilize than the exterior.

Ethylene oxide gas will not penetrate hermetically sealed parts and thus will not sterilize microorganisms entrapped within. However, this gas is an excellent sterilant for external surfaces. If it becomes necessary to replace or repair a part after an interplanetary space vehicle is heat sterilized, ethylene oxide gas might be used to assure sterilization in the event an undetected break in integrity occurred during the sterile insertion process. If it can be shown that ethylene oxide penetrates and sterilizes the interfaces of mated surfaces the reliability of this process will be greatly increased.

This paper presents results obtained for both dry heat and ethylene oxide gas upon contamination located both between mated surfaces and on external surfaces of various materials.

MATERIALS AND METHODS

The study was divided into four parts, three of which concerned sterilization with dry heat and the fourth with ethylene oxide gas.

Test Organism

An aqueous spore suspension of *Bacillus subtilis* var *niger* was used to contaminate each sample tested in this study.

Dry Heat Sterilization

All samples used in the three series of tests described below were placed in large covered dishes and transferred to a convection dry heat oven at 125C or 105C. The exposure period was recorded as starting when the oven temperature was again at 125C or 105C, a lag time of about 15 and 60 minutes respectively. Considerably more material was placed in the 105C oven thus requiring a longer lag time.

The First Dry Heat Tests

These tests were conducted at 125C on the following materials: round-head steel machine screws (1/4-20 and 1 inch long) with appropriate nuts, stainless steel strips (1 x 2 x 4/64 inches) and glass slides (1 x 2 x 3/64 inches). The threads of the screws and the center area of the steel and glass surfaces were contaminated with the spore suspension and allowed to dry at 1% relative humidity overnight. Before heat exposure the surfaces were mated. Three nuts were placed on each screw to cover the contaminated threads. Each contaminated steel strip or glass slide covered with a sterile piece of appropriate material was clamped together with binder clips. To test the effectiveness of dry heat upon contamination located on the exterior surface of a material, nuts were placed near but not on the contaminated screws. A sterile stainless steel strip or glass slide was placed under the appropriate contaminated material and then clamped together with binder clips. Thus, in both instances, the same quantity of material was exposed to dry heat for one hour. After the heat treatment, the nuts were removed from the screws and the binder clips from the steel and glass pieces. Each sample, consisting of a screw and three nuts, or two steel strips, or two glass slides, was aseptically transferred to 0.05% Tween 20 blanks. The samples were then shaken and assayed for microorganisms by the pour plate method using trypticase soy agar as culture medium. Colony counts were made after the plates had incubated at 32C for 72 hours. The controls, corresponding samples not exposed to heat, were also assayed in the manner described above.

The Second Dry Heat Tests

These tests were conducted at 105C for various exposure periods (4, 24 and 48 hours) so that any possible difference between death rates for interior and exterior contaminated surfaces would be magnified. The only material used was glass (1 x 1 x 3/64 inch squares). Like the first dry heat tests, the center area of the glass surfaces were contaminated with the spore suspension and allowed to dry at 1% relative humidity overnight. Before heat exposure, the glass squares were arranged to form samples in two different thickness, with contamination located either on the interior or exterior surface. For the interior, the contaminated surface was placed in the center of two or

six pieces of glass and held firmly together with binder clips. For the exterior, the contaminated surface was on top of a stack of two or six pieces of glass and also held together with binder clips. For the bacteriological determination, the binder clips were removed from the samples and two pieces of glass, the one with the contaminated surface and the piece nearest to it, were assayed for microorganisms in the manner described above.

The Third Dry Heat Tests

These tests were conducted at 125C on the materials that were used for the tests with ethylene oxide gas. Stainless steel squares ($1\frac{1}{2} \times 1\frac{1}{2} \times 5/64$ inches) were designed with a hole at the center-edge of each side so that pairs could be bolted together. The glass squares used here were the same as those described in the second dry heat tests. The center area of the steel and glass surfaces were contaminated with a spore suspension and allowed to dry at 33% relative humidity overnight so that the spores would be equilibrated at a relative humidity optimum for ethylene oxide sterilization. For the mated surfaces, each contaminated steel square was covered with another steel square and bolted together, and each contaminated glass square was covered with another glass square and clamped together with binder clips. A contaminated steel or glass square was placed upon a corresponding sterile square with the contamination located on the exterior of the respective material. The bacteriological assay for these tests was conducted in the manner described above.

Ethylene Oxide Sterilization

Stainless steel and glass squares were prepared in the same manner as those described for the third dry heat tests. These samples, along with the materials to process them (taped-sealed Tween 20 blanks, forceps and a socket wrench) were placed in an airtight plastic chamber [5] fitted with glove ports and rubber gloves, a screw type door, and small inlet tubes. A gaseous ethylene oxide-freon mixture was admitted into the closed chamber. After a four-hour exposure to ethylene oxide (approximately 337 mg/liter) at ambient temperature, the chamber was then flushed with filtered sterile air for 16 hours to remove all ethylene oxide gas. With the aid of tools, the mated surfaces were separated. Each sample consisting of two pieces of steel or glass was placed in a Tween 20 blank and then removed from the chamber to perform the bacteriological assay by the same procedure as described for the dry heat tests.

RESULTS AND DISCUSSION

The results of this study are summarized in Tables I and II. In all three series of tests, dry heat appeared as effective against spore contamination located between mated surfaces as against spore contamination located on the exterior surface of the various materials tested (Table I). Ethylene oxide gas was also equally effective against spore contamination located between mated surfaces or on the exterior surface of steel or glass (Table II). For both dry heat and ethylene oxide tests, exposure periods were selected that would reflect a decrease in population but not sterility so that numerical comparisons could be made. There appears to be no evidence that extended exposure period will be necessary to sterilize mated surfaces. However, if the surfaces are hermetically sealed, sterilization can not be attained with ethylene oxide gas and longer exposure periods with dry heat are needed to kill microorganisms entrapped in solids [1,2,3,4].

References

1. Protection Branch Report of Test No. 19-65: "Dry Heat Sterilization of Microorganisms at 105° C", dated 7 June 1965. Ft. Detrick, Md.

2. Bruch, C.W., M.G. Koesterer, and M.K. Bruch. "Dry Heat Sterilization: Its Development and Application to Components of Exobiological Space Probes". Developments in Ind. Microb., Vol. 4, 1963.

3. Koesterer, M.G. "Thermal Death Studies on Microbial Spores and Some Considerations for the Sterilization of Spacecraft Components". Developments in Ind. Microbiol., Vol. 6. 1964.

4. Fourth Quarterly Report of Progress on Research Project R-36-015-001 "Ecology and Thermal Inactivation of Microbes In and On Interplanetary Space Vehicle Components". April 1966. RATSEC, Cincinnati, Ohio.

5. Protection Branch Report of Test No. 7-60: "A Technique for the Investigation of Bacterial Contamination Inside Electronic Components", dated 11 March 1960. Ft. Detrick, Md.

Table I. The Effectiveness of Dry Heat Upon B. subtilis var niger Spore Contamination Located On and Between Surfaces of Various Materials

Dry Heat Tests	Temp.	Material and Quantity	Exposure To Heat (Hrs)	Number Spores/Sample Location of Contamination On Surface	Between Surfaces*
1st	125C	1 screw and 3 nuts	0	519,000	519,000
			1	194	204
		2 stainless steel strips	0	704,000	704,000
			1	209	349
		2 glass slides	0	826,000	826,000
			1	206	189
2nd	105C	2 glass squares	0	887,000	887,000
			4	50,100	67,300
			24	1	7
			48	0	0
		6 glass squares	0	887,000	887,000
			4	59,400	79,000
			24	2	1
			48	0	0
3rd	125C	2 stainless steel squares	0	460,000	460,000
			1	< 1	6
		2 glass squares	0	870,000	870,000
			1	27	25

Note: For the 1st tests, each entry is an average of 4 samples.
For the 2nd tests, each entry is an average of 16 samples.
For the 3rd tests, each entry is an average of 12 samples.

* All flat surfaces were held together with binder clips except the stainless steel squares which were bolted on all four sides.

Table II. The Effectiveness of Ethylene Oxide Gas* Upon B. subtilis var niger Spore Contamination Located On and Between Stainless Steel or Glass Surfaces

Material	Exposure To ETO (Hrs)	Number Spores/Surface Location of Contamination On Surface	Between Surface
Stainless Steel	0	331,000	331,000
	4	5	44
Glass	0	859,000	859,000
	4	4	5

Note: Each entry is an average of 12 samples.

* 337 mg/liter of air.