Protection Branch Report of Test No. 6-66

A Study of Dry Heat Sterilization of Microorganisms at 105°C

26 January 1966

Prepared by:

Dorothy M. Portner
DOROTHY M. PORTNER
Decontamination Section

Approved by:

Robert K. Hoffman
ROBERT K. HOFFMAN
Chief, Decontamination Section

Herbert M. Decker
HERBERT M. DECKER
Chief, Protection Branch

Charles R. Phillips
CHARLES R. PHILLIPS
Chief, Physical Defense Division

FACILITY FORM 602

N66-17088 (ACCESSION NUMBER) (THRU)

24 (PAGES) / (CODE)

CR 70321 (NASA CR OR TMX OR AD NUMBER) 04 (CATEGORY)

GPO PRICE $ ______

CFSTI PRICE(S) $ ______

Hard copy (HC) 1.00

Microfiche (MF) .50

ff 653 July 65

Physical Defense Division
Fort Detrick, Frederick, Maryland

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At the request of Dr. Carl B. Bruch, NASA Headquarters, the results of a current study on dry heat sterilization at 105° C (221° F) undertaken by this Laboratory, were combined with previously reported data [1/] and are presented here. Extensive dry heat studies by Bruch, et al [2/] and Koesterer [3/] were conducted at temperatures from 80 to 135° C (176-320° F) with most of the effort on temperatures between 115 to 135° C. The time-temperature relationship for dry heat sterilization of space probes has not been firmly established. The low temperature of 105° C may be more advantageous than higher temperatures for sterilization of space probes with their heat sensitive components provided that the time required to attain sterility is practical. Therefore, the study reported here was undertaken to obtain more information on the effectiveness of dry heat at 105° C in sterilizing microorganisms. Thermal death times were determined for (1) natural microbial contamination from aerial fallout or dried bacterial spores on glass surfaces, either embedded or not embedded in plastic; (2) bacterial spores in soil, either embedded or not embedded in plastic; and (3) the natural flora of microorganisms of soil.

MATERIALS AND METHODS

Contamination of Glass

Glass slides (1 x 1½ inches) were contaminated with *Bacillus subtilis* var *niger* spores by placing an aliquot of an aqueous spore suspension upon the surface of each slide and allowing it to dry overnight at ambient relative humidity and temperature. Approximately the same number of slides were inoculated with either a low spore concentration (8.9×10^3 per slide) or a high one (6.4×10^7 per slide).

In addition, glass slides were naturally contaminated with microorganisms by exposure to aerial fallout for eight to ten weeks.

Artificial Contamination of Soil

Two soils, one of a clay consistency and the other a humus garden variety, were used in this study. Both soils were spread in thin layers and sterilized in an autoclave at 120° C and 18 pounds pressure for two three-hour periods. Each soil was divided into two portions and mixed with one of two different aqueous concentrations of Bacillus subtilis var niger spores. A Buchner funnel with a filter paper insert was used to remove the excess liquid from the mud before drying at 50° C overnight. The dry cake was then powdered by grinding with a mortar and pestle. The four prepared samples consisting of two types of soil with two levels of spore contamination were stored in closed containers at room temperature until used.

Plastic

About one-half of the glass slides that were contaminated for this study were embedded in silicone plastic RTV-602* to obtain a covering that would simulate the protection provided microorganisms entrapped in solids. The embedding procedure involved the following steps:

1. The plastic was prepared by mixing 15 drops of catalyst SRC-05 in each 100 grams of RTV-602.

2. Small plates (about 2 inches in diameter and 5/8 inch deep) were filled about one-third full with liquid plastic.

3. After the plastic had set one day at room temperature, the contaminated side of the glass was placed downward on the hardened plastic.

4. Enough liquid plastic was poured over the glass slide to cover it and to fill two-thirds of the plate.

To embed the artificially contaminated soil samples, steps 1 and 2 of the above embedding procedure were followed. After the plastic had set one day at room temperatures, a small amount of plastic was removed from the center to make a depression for about 0.1 gram of soil sample. Liquid plastic was then poured over the soil. All soil and glass samples were placed in the 105° C oven immediately after embedding in the plastic.

Test Procedure

A circulating dry heat oven set at 105° C was used for all samples tested. For exposure to dry heat, three or four small plates, each containing one contaminated slide with or without plastic, were placed in a large covered dish. Small plates containing artificially contaminated soil samples in plastic were also placed in a large covered dish. For testing the clay and garden soils with their natural microbial flora as

* A product of General Electric, Waterford, N.Y.

well as with B. subtilis spores, 0.1 gram samples in glass cups (one cubic centimeter capacity) were placed in a covered dish and exposed to heat. After various exposure periods from 4 to 336 hours, dishes containing the glass or soil samples were removed with sterile tongs from the oven and placed in a plastic chamber. The chamber and its contents (broth blanks, forceps, scalpels, mortars and pestles) had been previously sterilized with ethylene oxide gas. The heated samples were transferred to tryptose broth blanks in this chamber to prevent the possibility of introducing airborne contamination during the transfer procedure. The embedded slides were easily separated from the plastic by use of scalpel and forceps and were placed in the broth without the plastic. The plastic surrounding the embedded soil was cut away and the pile of soil mixed with plastic was placed in a mortar and ground as well as possible with a pestle before transferring to a broth blank.

The samples, following heat treatment, were assayed in two ways. To test for sterility, one set was cultured in broth at 37° C for seven days, after which the broth was examined for microbial growth. The results from this method are reported as the number of contaminated broth samples per total number of samples tested. In addition to the sterility test, at least two samples per exposure period were assayed for viable count in order to determine the level of contamination remaining after exposure to heat for various times. These assays were generally done only for the shorter exposure periods when some contamination would be expected. Control samples, i.e., samples not exposed to heat were also assayed for viable count. All viable count assays were done by placing each sample in a broth blank, shaking vigorously for five minutes, and plating aliquots in tryptose agar for incubation at 37° C for 48 hours before colony counts were made to determine the total number of surviving microorganisms per sample.

RESULTS AND DISCUSSION

The results of the study on dry heat sterilization of microorganisms at 105° C indicate that the time required to achieve sterility varied from 24 to 336 hours. Sterility would be achieved in 24 hours for about 10^6 B. subtilis var niger dried on glass; but when embedded in plastic, the thermal death time increased to 48 hours (Table I). Glass slides contaminated with a high spore concentration required a longer time for sterilization than the slides contaminated with a low spore concentration. However, for the slides embedded in plastic, the level of spore contamination did not appear to have such a marked effect on sterility times. One contaminated slide was still found in the low spore population as well as one in the high after 32 hours (Table I).

For the glass slides contaminated by aerial fallout (not tabulated), the contamination level was only about 40 microorganisms per slide; but even so after a 24 hour heat treatment, contamination was found in one sample out of 14 embedded in plastic. Slides not embedded in plastic were sterilized within 24 hours at 105° C and the embedded slides were sterilized in 48 hours.

Soils artificially contaminated with 10^6 or less B. subtilis var niger spores appeared to be sterile after 48 hours exposure to 105° C (Table II). When the spore level in the soil was increased to 10^7, 96 hours were required to achieve apparent sterility. The clay soil appeared to offer more protection to B. subtilis var niger spores than garden soil. However, the natural flora of garden soil was considerably more difficult to sterilize at 105° C than the clay soil flora (Table III). It required 336 hours to sterilize the garden soil flora in comparison to 120 hours for the clay soil flora.

The data presented show that both plastic and soil offer protection to microorganisms exposed to dry heat. The data in Table IV indicate that the sterility time is not increased when bacterial spores in soil are embedded in plastic. The thermal death time of 48 hours may be a low estimate due to the difficulty in separating the soil from the plastic.

The thermal death times presented in this report substantiate the work of others [2,3] in which they found lower death times for spores dried on carriers than for spores entrapped in solids, and a shorter death time for B. subtilis var niger spores than for some natural soil microorganisms.

It is evident that the T_{90} (or D) values (times to kill 90% of the organisms) for the various systems at 105° C vary considerably depending to the system tested. For example, the T_{90} value for B. subtilis spores on non embedded glass was about 4.5 hours while at the other extreme the T_{90} for natural microbial flora in garden soil was about 56 hours. Because there is such a great variability due to differences in menstruum and organisms one system must be selected for spacecraft sterilization use. Only then can meaningful T_{90} values for specific temperatures be determined which in turn will enable one to estimate the time required to sterilize a spacecraft. The early selection of such a system is also necessary for use in all preliminary procedure evaluations.

It does not seem reasonable however, that the most severe case of sterilizing naturally contaminated soil should be selected for the standard system since piles of soil will not be present on a spacecraft. Furthermore, soils differ too widely in microbial types, numbers, and resistance to be used for a standard reproducible procedure. Because of this fact a controllable artificial contamination is required.

The type of menstruum for holding the organisms should be selected on the basis of universal availability, ease of handling and ease of removing the organisms for assay after treatment. Glass slides appear to be a good candidate surface for this purpose since they can even be finely ground if necessary for assay. It is suggested, however, that the contaminated glass or other surface, be coated with some material such as plastic to make the test more stringent as would be encountered with organisms embedded in solids. The procedure of embedding the glass in plastic described in this study while yielding fairly quantitative results is probably too time consuming and laborious for a standard procedure. For this reason a study has been initiated in this Laboratory to develop a simplified standard procedure.

References

1. Portner, D.M. "Dry Heat Sterilization of Microorganisms at 105° C" Protection Branch Report of Test No. 19-65, June 1965.

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. Microbiology, Vol 4, Washington, D.C. 1963.

3. Koesterer, Martin G. "Thermal Death Studies on Microbial Spores and Some Considerations for the Sterilization of Spacecraft Components" Developments in Ind. Microbiology, Vol. 6, Washington, D.C. 1964.

Table I.

Effectiveness of Dry Heat at 105°C Against Two Concentrations of B. subtilis var niger Spores Embedded and Not Embedded in Plastic

Exposure to 105°C (Hours)	Low Spore Concentration*				High Spore Concentration**			
	Glass Not Embedded		Glass Embedded in RTV-602		Glass Not Embedded		Glass Embedded in RTV-602	
	Sterility Test No.Cont/Total	Avg.Org. Remaining/ Sample	Sterility Test No.Cont/Total	Avg. Org. Remaining/ Sample	Sterility Test No.Cont/Total	Avg.Org. Remaining/ Sample	Sterility Test No.Cont/Total	Avg. Org. Remaining/ Sample
0	-	4,300	-	2,900	-	8,100,000	-	4,800,000
4	15/20	28	21/21	390	6/6	4,100	3/3	14,000,000
8	1/7	-	-	-	8/8	-	-	-
24	0/33	-	5/21	-	1/15	1	10/15	41
32	0/12	-	1/12	-	0/12	-	1/12	-
48	0/8	-	0/13	-	0/15	-	0/15	-

* = Each slide was inoculated with approximately 8,900 B. subtilis var niger spores.

** = Each slide was inoculated with approximately 64,000,000 B. subtilis var niger spores.

- = Not assayed.

Table II.

Effectiveness of Dry Heat at 105°C Against *B. subtilis* var *niger* Spores in Clay and Garden Soils

Exposure to 105°C (Hours)	Clay Soil: Low Spore Concentration, Sterility Test, No.Cont/Total	Clay Soil: Low Spore Concentration, Avg.Org. Remaining/Sample	Clay Soil: High Spore Concentration, Sterility Test, No.Cont/Total	Clay Soil: High Spore Concentration, Avg.Org. Remaining/Sample	Garden Soil: Low Spore Concentration, Sterility Test, No.Cont/Total	Garden Soil: Low Spore Concentration, Avg.Org. Remaining/Sample	Garden Soil: High Spore Concentration, Sterility Test, No.Cont/Total	Garden Soil: High Spore Concentration, Avg.Org. Remaining/Sample
0	-	1,200,000	-	29,000,000	-	43,000	-	1,300,000
24	22/22	193	26/26	34,700	6/27	1	15/23	3
48	0/6	0	6/6	16	0/6	0	0/6	0
72	0/18	0	11/38	1	0/6	0	0/19	0
96	-	-	0/6	0	-	-	-	-
120	-	-	0/4	0	-	-	-	-

- = Not assayed.

Table III.

Effectiveness of Dry Heat at 105°C Against the Microbial Flora of Clay and Garden Soils

Exposure to 105°C (Hours)	Clay Soil		Garden Soil	
	Sterility Test No.Cont/Total	Average Organisms Remaining/Sample	Sterility Test No.Cont/Total	Average Organisms Remaining/Sample
0	-	700,000	-	220,000
24	12/12	610	12/12	510
48	-	-	-	-
72	3/23	1	27/29	22
96	1/14	0	15/15	8
120	0/6	0	8/8	6
144	0/6	0	15/16	6
168	-	-	9/9	3
240	-	-	1/6	0
336	-	-	0/8	0

- = Not assayed.

Table IV.

Effectiveness of Dry Heat at 105°C Against _B_. _subtilis niger_ Spores in Clay Soil Embedded in Plastic

Exposure to 105°C (Hours)	Sterility Test No.Cont./Total	Average Organisms Remaining/Sample
0	-	430,000
24	7/8	5
48	0/8	0
72	0/8	-

- = Not assayed.