Army Bio Labs

Protection Branch Report of Test No. 19-65

Dry Heat Sterilization of Microorganisms at 105° C

7 June 1965

Prepared by:

Dorothy M. Portner

DOROTHY M. PORTNER
Decontamination Section
Physical Defense Division

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

Physical Defense Division
Fort Detrick, Frederick, Maryland

Protection Branch Report of Test No. 19-65

CHC

Dry Heat Sterilization of Microorganisms at 105° C

27517

The effectiveness of dry heat at 105° C (221° F) against different levels of microbial contamination on glass surfaces, either embedded or not embedded in plastic, was investigated. ~~Previous dry heat studies by Bruch, et al [1] and Koesterer [2] were conducted at temperatures from 80° to 160° C (176° — 320° F) with emphasis on temperatures between 115° to 135° C.~~ Limited tests were run at 105° C and those which were performed were done with spores on exposed surfaces or organisms in soil. No death rates were determined on organisms embedded in plastic. The reason for conducting further studies at this low temperature was that Dr. Imshenetsky indicated ~~(personal communication to Laurence B. Hall at COSPAR meeting)~~ the Russian spacecraft would be sterilized by exposure to 105° C for a few days. This temperature is considerably lower than any temperature NASA has considered for dry heat sterilization of space probes. Therefore, the investigation reported here was undertaken to obtain more information on the effectiveness of dry heat sterilization at this lower temperature.

Author

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.

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

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

solids. The embedding procedure involved the following stages:

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.

Test Procedure

Three to four small plates, each containing one contaminated glass slide with or without the embedding plastic, were placed in a large covered dish and transferred to the 105° C circulation dry heat oven. Approximately two hours lapsed before the oven temperature rose to 105° C again; at which time, the exposure period was recorded as starting. After various exposure periods from 4 to 48 hours, the large covered dishes containing the samples were removed, with sterile tongs, from the oven and placed in a plastic chamber. The chamber and its contents (broth blanks, scapels, and forceps) had been previously sterilized with ethylene oxide gas. The glass slides 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 scapel and forceps and were placed in the broth without the plastic. In addition to culturing samples in broth to determine sterility, other samples exposed to heat were assayed for viable count. Control samples, i.e., contaminated glass slides, embedded and not embedded in plastic for one or two days at room temperature, were also assayed for viable count. All assays were done by the pour plate method and cultured in tryptose agar at 37° C for 48 hours before colony counts were made. Broth samples were incubated at 37° C for seven days before checking for bacterial growth.

RESULTS AND DISCUSSION

The results obtained for dry B. subtilis var niger spores subjected to dry heat at 105° C are summarized in Table I. Apparent sterility was achieved for all samples after 48 hours exposure to 105° C. This sterility time is comparable to the time estimated from Koesterer's thermal

death rate curve for B. subtilis var niger [2]. It is not surprising that a considerable longer time was required to kill spores embedded in plastic than those exposed on surfaces since others [1,2] have also observed slower death rates for spores entrapped in solids in tests conducted at higher temperatures.

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 as marked an effect on the sterility time. 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 that had been 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.

The data from this study, while not extensive in number, do indicate that the resistant B. subtilis var niger spores even if embedded in plastic can be killed when subjected to dry heat at 105° C for 48 hours. Thus space probes could probably be sterilized with dry heat at a temperature as low as 105° C in a few days as the Russians have indicated. In the light of these results, the NASA time-temperature requirements for heat sterilization might be reconsidered. The important factor in heat sterilization of a spacecraft is not the external temperature but the temperature at the site of the microorganisms within the vehicle. When a spacecraft is placed in an oven there may be a considerable time lapse before the most inaccessible and best insulated areas reach the desired temperature. With an extended exposure time for the heat cycle one would have more confidence that the entire spacecraft reached the desired temperature and would indeed be sterilized.

References

1. Bruch, Carl 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.

2. 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 [a]				High Spore Concentration [b]			
	Glass Not Embedded		Glass Embedded in RTV-602		Glass Not Embedded		Glass Embedded in RTV-602	
	No. Cont/Total	Org/Sample	No. Cont/Total	Org/Sample	No. Cont/Total	Org/Sample	No. Cont/Total	Org/Sample
0	c	4,300	c	2,900	c	8,100,000	c	4,800,000
4	15/20	28	21/21	390	6/6	4,100	3/3	14,000,000
8	1/7	c	c	c	8/8	c	c	c
24	0/33	c	5/21	c	1/15	1	10/15	41
32	0/12	c	1/12	c	0/12	c	1/12	c
48	0/8	c	0/13	c	0/15	c	0/15	c

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

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

c = Not assayed.