Protection Branch Report of Test No. 13-67

Quantitative Spore Recoveries from Diatomaceous Earth Pellets Used as Protective Material in Dry Heat Sterilization Studies

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Early in the investigation of problems involved in spacecraft sterilization this Laboratory showed that some electronic components are internally contaminated with microorganisms [1]. Since such components will be used in the construction of an interplanetary spacecraft they must be capable of withstanding some sterilization procedure. Of major concern however, is assessing such components for contamination or sterility. At this time it is technically impossible to completely pulverize or dissolve a component and liberate in a viable state all entrapped living microorganisms.

It has been conclusively demonstrated by Bruch, Koesterer and Bruch [2] that microorganisms contained in a protective medium, such as soil, dental inlay material, etc., are more resistant to heat inactivation than are organisms unprotected. For this reason it can be correctly assumed that microorganisms entrapped in an electronic component would be more difficult to kill. In an effort to obtain death rates and D values for entrapped microorganisms subjected to dry heat, attempts have been made to simulate contaminated electronic components by incorporating bacterial spores in Eccofoam plastic, dental inlay material, lucite, silicone potting compounds and the like. Unfortunately lengthy and complex procedures are required to free microorganisms from such solids for assay, and invariably a considerable loss of organisms is incurred for one reason or other.

It is recognized that no material will be truly representative of all or perhaps even many electronic components. Thus it seems desirable to use a material that will be easy to handle and give quantitative and reproducible results. Diatomaceous earth appears to be such a material from the data given in this paper comparing the bacterial recoveries obtained from diatomaceous earth pellets and from glass surfaces after exposure to dry heat at 105 or 125 C for various periods.

MATERIALS AND METHODS

Diatomaceous Earth Pellets

About 100 ml of Bacillus subtilis var niger spore suspension (about 5×10^6 spores/ml) were mixed with 16 grams of diatomaceous earth. To obtain homogeneity, the mixture was prepared by mixing increments of 10-12 ml of spore suspension and 1-2 grams of diatomaceous earth. The thick moist mixture was stored in a covered jar at 5 C until ready to use. On storage, the mixture may become too dry to use; but by merely adding water, it can be made pliant again without loss in spore concentration.

A 2-ml syringe, modified by cutting the tip off flush to the bottom of the barrel, was used to make the pellets. The thick moist diatomaceous earth-spore mixture was packed into the syringe with a spatula; immediately, it was extruded in $\frac{1}{2}$ cc portions onto a glass plate, severing the portions with a scapel. The pellets were then placed in an oven at 75 C for $2\frac{1}{2}$ hours to harden and dry.

Test Procedure

Pieces of glass ($\frac{1}{2}$ square inch area) were contaminated with a B. subtilis var niger spore suspension and placed along with the moist diatomaceous earth pellets in a 75 C oven for $2\frac{1}{2}$ hours. The dry pellets and contaminated glass were exposed to dry heat at 105 or 125 C for various periods. After the heat treatment, each sample to be assayed for viable count was placed in a jar containing 25 ml of 0.05% Tween 20 solution and glass beads. Each sample was then shaken mechanically for 10 minutes and then assayed by the pour plate method using trypticase soy agar as culture medium. Plates were incubated at 32 C for 72 hours before colony counts were made. Samples not subjected to the heat treatment were also assayed in the same manner.

To verify sterility, some samples that were subjected to dry heat for prolonged periods, were placed in jars containing 25 ml of trypticase soy broth and glass beads. These broth samples were also shaken mechanically for 10 minutes so that the diatomaceous earth pellets would disintegrate before they were incubated at 32 C for seven days and then checked for bacterial growth.

RESULTS AND DISCUSSION

The results of this brief study indicate that the death rate for B. subtilis var niger spores exposed to dry heat at 105 C or 125 C is considerably slower when the spores are in diatomaceous earth than when they are on the exterior glass surface (Table I). Each figure represents the average of 3-9 determinations. D values calculated from these results were 240 and 400 minutes for spores on glass and diatomaceous earth respectively at 105 C, and, 17 and 28 minutes for spores on glass and diatomaceous earth respectively at 125 C.

Table II shows that comparable recoveries can be obtained with diatomaceous earth pellets. The ease of preparation and the reproducibility of the samples are factors that suggest that diatomaceous earth pellets would be a good solid to use to obtain quantitative measurements on the efficiency of dry heat sterilization.

References

1. Phillips, C.R. and Hoffman, R.K. 1960. "Sterilization of Interplanetary Vehicles" Science 132, 991-995.

2. Bruch, C.W., Koesterer, M.G., and Bruch, M.K. 1963. "Dry Heat Sterilization: Its Development and Application to Components of Exobiological Space Probes". Dev. Ind. Microbiol. 4, 334-342.

Table I.

Effect of Dry Heat at 105 C and 125 C Upon Spores in Diatomaceous Earth and On Glass

Exposure to Heat (Hours)	105 C		125 C	
	Diatomaceous Earth	Glass	Diatomaceous Earth	Glass
0	1,260,000	2,880,000	1,260,000	330,000
1			11,300	108
$2\frac{1}{2}$			5	0
5			< 1	0
22			0	0
24	1,680	1		
68	0	0		

Note: (1) B. subtilis var niger spore preparations used in tests at 105 C were different than those used in tests at 125 C.

(2) Each entry is an average of 3-9 determinations.

Table II.

Comparison of the Reproducibility of Samples of Diatomaceous Earth Mixed with Spores

Samples	Wet Preparation X 10^6	Dry Preparation* Initial Count** X 10^6	Dry Preparation* Final Count*** X 10^6	Dry Preparation* Exposed to 105 C for 24 hours Count X 10^3
1	4.24	4.79	3.49	4.87
2	4.35	3.64	3.69	1.43
3	5.15	5.16	3.92	3.08
4	4.39	4.58	3.05	2.56
5	4.42	3.49	3.48	2.42
6	4.55	5.52	3.90	2.73
7	4.21	3.88	4.28	2.68
8	3.65	3.72	3.89	3.15
Average	4.37	4.35	3.71	2.87

* All samples were dried at 75 C for $2\frac{1}{2}$ hours.

** Assayed immediately after drying.

*** Assayed 24 hours after drying.