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D-Values of *Bacillus pumilus* Spores on Irradiated Devices (Inoculated Product)

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The D-values of *Bacillus pumilus* spores on various devices ranged from 0.14 to 0.23 Mrads. The majority of devices displayed D-values equal to or less than the value obtained on filter paper. Increased resistivity was also encountered.

The D-value of *Bacillus pumilus* spores was reported to be 0.17 to 0.18 Mrads when tested on dry filter paper strips (5). This value was stable for at least 1 year. Spore strips have gained wide acceptance as biological indicators in testing the adequacy of ^{60}C and other sterilization processes (3, 4). Another type of biological indicator, the inoculated product, has also become widely used in the sterility testing of drugs and devices, and its superiority to the inoculated carrier has been suggested (1, 2, 4, 8). Little information is available, however, on the D-values of the spores of *B. pumilus* on inoculated products. Bruch has stressed the importance of this determination for drugs and devices undergoing sterilization (1, 2). He has pointed out that our ability to predict the probability of sterility at any one dose is related to our knowledge of the background count ("bioburden") and the D-value of either a standard organism or the ambient microbial population. I have determined the D-values of the spores of *B. pumilus* on a variety of device substrates and have compared these values wtih spore strips and water controls.

Spores of *B. pumilus* E-601 (ATCC 27142) were prepared and stored as suspensions in 80% (vol/vol) isopropanol as previously described (5). All devices were inoculated with approximately 10^6 spores per g or per unit. Inoculated substrates were air-dried at 20 to 25°C for 1 week. Syringes were inoculated into the lumen of the barrel nearest the depressed annular ring. Absorbent substrates were inoculated uniformly with 0.1 to 0.5 ml of spore suspension to yield 10^6 spores per g. Sutures were inoculated by slowly pipetting spores along the coiled surface. Plastic blood bags were inoculated on the interior surface, and methylmethacrylate polymer was triturated with the spore suspension by using a mortar and pestle to yield 10^6 spores per g. Verification of the number of spores inoculated and retained was determined 1 month later on at least 10 replicates of each inoculated product. Theoretical and found recovery values were found to be close (± 0.33 log).

Surviving spores were determined by maceration of the irradiated substrate in a Waring blender (3 min) containing 50 to 100 ml of sterile saline or Trypticase soy broth. The number of viable spores in the elution fluid was determined by the membrane filtration technique (0.45 μM) or by plating a series of 5-ml portions with Trypticase soy agar (35°C, 48 h). The volume of fluid filtered or the number of 5-ml portions plated was a function of the number of survivors estimated for a particular radiation dose.

The devices were irradiated by a commercial radiosterilization laboratory (Radiation Technology, Inc., Rockaway, N.J.) in a batch mode, using ^{60}Co as the radioisotopic source of gamma irradiation (5, 6). All dose titrations were performed in duplicate at 0.05, 0.1, 0.2, 0.4, 0.6, and 1.0 Mrads. Five determinations were made at each point, and the average number of survivors was plotted as $\log_{10}$ against dose.

Table 1 summarizes the D-values on the various substrates in ascending order of relative resistance. It can be seen that the values obtained on all devices were close to the control filter paper values. A possible radioprotective effect was seen for the aqueous suspension and catgut sutures. It was apparent that dry inoculated carriers (spore strips) and dry inoculated products displayed similar but not identical patterns of resistivity. Chemical nature and moisture content must be considered before one can assume that the values reported here apply for all inoculated products, since it is known that physicochemical properties of the substrate affect resistivity (5, 7).

Similar studies with drugs, other devices, diagnostics, and semisolid dosage forms are in progress. Dose-survivor curves must be plotted for a wide spectrum of drugs and devices before a table of standard values can be used as a guide to assess a priori the relative radioresistance of an article to be sterilized. It should be pointed

TABLE 1. *D_{10}-values of dried spores of B. pumilus ATCC 27142 on various medical devices*

Device (inoculated product)	D_{10}-value (Mrads, duplicate determinations)[a]
Bone cement (methylmethacrylate polymer)	0.14, 0.16
Plastic blood bag (low-density polyethylene)	0.14, 0.15
Gauze	0.14, 0.16
1.0-ml syringe (polypropylene)	0.15, 0.16
Muslin	0.15, 0.17
Surgical sponge (polyurethane)	0.15, 0.18
Acetabular prosthesis (high-density polyethylene hip cup)	0.17, 0.18
Suture, silk	0.17, 0.18
Pyrex tube, silicone-coated	0.18, 0.19
Suture, catgut (beef intestinal collagen)	0.22, 0.23
Water, distilled	0.25, 0.26
Filter paper control[b]	0.16, 0.17, 0.18

[a] Megarads required to reduce the initial population 1 $\log_{10}$ or 90% as determined by method of least squares after plotting $\log_{10}$ against dose.

[b] E-Rad-O-Kit spore strips (Gibraltar Biological Laboratories).

out that D-values for ambient or reference organisms are useful predictors of sterilization but that routine monitoring of bioburden is essential in relating these values to a dose commensurate with low probability of contamination.

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