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# Stability of *Bacillus pumilus* Spore Strips Used for Monitoring Radiation Sterilization

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Dried *Bacillus pumilus* spore strips had a stable D-value of 0.17 to 0.18 Mrad after 1 year at 5 or 25 C, but resistance was lost at 35 C. Refrigeration did not have an adverse effect on resistance to irradiation. The presence of water at the time of initial preparation increased this resistance to 0.23 to 0.25 Mrad. Proper drying is essential to achieve reproducible results in sterility tests using USP-type biological indicators.

The 19th revision of the *United States Pharmacopeia* (5) discusses the use of inoculated carriers (e.g., spore strips) as biological indicators to monitor sterilization and suggests that specific data be gathered on optimal storage conditions and D-values. Accordingly, the endospores of *Bacillus pumilus* (E-601) ATCC no. 27142 were studied with respect to viability and radioresistance.

Spores were prepared by growing *B. pumilus* on the surface of Kolle flasks (Trypticase soy agar [Baltimore Biological Laboratories]) for 10 days at 35 C, after which time spore stains revealed at least 90% sporulation. Growth was scraped off with a glass rod and the cell paste was washed by centrifugation three times with sterile, deionized water. The dense paste was heat shocked for 15 min at 80 C to eliminate vegetative forms, rapidly cooled, and then stored frozen until use. Viable spore counts of frozen suspensions remained unchanged after 4 years at −10 C (cell density approximately $10^{10}$ spores/ml). Carriers were made by diluting the paste in 80% (wt/vol) isopropanol and then inoculating filter strips (40 mm by 8 mm) with 0.1 ml, followed by drying for 5 days at room temperature in a vacuum desiccator over $CaCl_2$. Spore recoveries were determined by triturating macerated strips in a mortar and pestle, followed by dilution and standard plate counts in duplicate (Trypticase soy agar, 35 C, 48 h).

The spore strips were irradiated by a commercial radiosterilization laboratory (Radiation Technology, Inc., Rockaway, N.J.) in a process irradiator that had been programmed for operation in a batch mode using cobalt-60 as the radioisotopic source of gamma irradiation. When in operation, the source plaque, composed of many high-specific-activity cobalt-60 pencils, was remotely raised from its storage pool to the irradiate position within a concrete labyrinth.

The samples to be irradiated were placed at points within the cell, which had been previously dose mapped using both Fricke ferrous sulfate and ceric sulfate dosimeters (3). The dose rate for all irradiations was about 0.45 Mrads per h.

The effects of various storage temperatures on viability and radioresistance are shown in Table 1. The data in Table 1 indicated that filter strips containing dried spores of *B. pumilus* maintained viability for at least 1 year in the refrigerator (5 C) and at room temperature (20 to 25 C). At 35 C, however, viability dropped by 90% after 1 year. Resistance to gamma irradiation remained essentially unchanged for 1 year at 5 C; at 20 to 25 C a similar degree of resistance stability obtained except that a questionable loss was detected after 1 year. These data support the contention that spore strips cannot be indiscriminately stored without regard to time or ambient temperature as is common in the industry. The data, however, do not support the suggestion that refrigeration may cause condensation and conversion to sensitive vegetative forms (2).

The data in Table 2 summarize the fractional spore recoveries obtained by exposure of spore strips to graded sublethal doses of gamma irradiation, using both moist and dry zero-time carriers. Moist carriers were prepared as described above but were not dried over $CaCl_2$ before irradiation.

It is apparent from these findings that the D-value (0.17 to 0.18 Mrad) for dried spores of this strain remained unchanged for at least 1 year at 20 to 25 C. However, improperly dried zero-time spore strips were found to have a D-value of 0.23 to 0.25 Mrad (e.g., failure to

TABLE 1. *Effect of various storage temperatures on viability and radioresistance of B. pumilus E-601 filter paper spore strips*

| Time interval | Viability[a] | | | Resistance (Mrads)[b] | | |
|---|---|---|---|---|---|---|
| | Refrigerated | 20-25 C | 35 C | Refrigerated | 20-25 C | 35 C |
| 0 time | $7 \times 10^5$ | $7 \times 10^5$ | $7 \times 10^5$ | 1.19 | 1.19 | 1.19 |
| 2 months | $9 \times 10^5$ | $5 \times 10^5$ | $6 \times 10^5$ | 1.19 | 1.19 | 1.19 |
| 4 months | $6 \times 10^5$ | $6 \times 10^5$ | $4 \times 10^5$ | 1.19 | 1.19 | 1.19 |
| 6 months | $6 \times 10^5$ | $8 \times 10^5$ | $5 \times 10^5$ | 1.19 | 1.19 | 1.02 |
| 1 year | $5 \times 10^5$ | $4 \times 10^5$ | $4 \times 10^4$ | 1.19 | 1.02 | 0.68[c] |

[a] Average number viable spore per strip (storage in sealed paper envelopes).

[b] Maximum survival dose in megarads as determined by outgrowth of *B. pumilus* in 5 ml of trypticase soy broth incubated at 35 C for 7 days after irradiation in dose increments of 0.17 Mrad (0.51, 0.68, 0.85, 1.02, 1.19, 1.36).

[c] Decrease in resistance to gamma irradiation.

TABLE 2. *Effect of 1 year storage on D-value of B. pumilus spore strips (E-Rad-O-Kit)*

| Dose (Mrad) | Avg spores per strip (freshly prepared) and dried over $CaCl_2$ | $D_{10}$ value[a] | Avg spores per strip (1 yr storage at 20 to 25 C | $D_{10}$ value |
|---|---|---|---|---|
| 0.075 | $1.8 \times 10^5$ | 0.18[b] | $2.2 \times 10^5$ | 0.17 |
| 0.15 | $8.1 \times 10^4$ | | $1.0 \times 10^5$ | |
| 0.30 | $1.3 \times 10^4$ | | $2.1 \times 10^4$ | |
| 0.60 | $1.1 \times 10^2$ | | $1.0 \times 10^2$ | |

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

[b] D-value of 0.23 to 0.25 Mrad for freshly prepared moist spore strips (not dried in vacuum over $CaCl_2$).

desiccate over $CaCl_2$). Such spore strips, however, after 1 year at room temperature had D-values in the 0.17- to 0.18-Mrad range. Data similarly suggesting an apparent radioprotective effect of water have been published by others (1, 4).

The optimum storage conditions for dried *B. pumilus* spore strips were found to be 4 to 10 C for a period up to at least 1 year. Storage at 35 C resulted in losses in both viability and resistance between 6 months and 1 year. *B. pumilus* spore strips can be useful adjuncts in monitoring the efficiency of the radiation sterilization process, as cited in the suggested procedural guidelines of *The United States Pharmacopeia*, vol. XIX (1975). However, the effects of hydration and elevated storage temperature must be considered, lest false positive or false negative results be encountered during sterility tests.

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