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# BIOBURDEN DYNAMICS: THE VIABILITY OF MICROORGANISMS ON DEVICES BEFORE AND AFTER STERILIZATION

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The term *bioburden* is used to define the number of microorganisms on a device or drug prior to sterilization. Since desiccated organisms do not survive indefinitely on an inert or hostile substance, it was the purpose of this study to examine the viability of a diverse bioburden spectrum, including organisms not normally assayed in sterility testing. The rates of death at room temperature were determined for a number of microorganisms inoculated onto the surface of a high-density polyethylene (HDPE) hip cup. Additional tests were performed with filter paper strips impregnated with *Bacillus* endospores and incubated at various temperatures. The effects of product substrate on irradiation sterilization and the effects of bioburden location on EtO sterilization were also studied.

## Inoculation Methods

**Bacteria and Fungi.** Broth cultures of bacteria (Table I) and the yeast *Candida albicans* were incubated at 35°C for 24 to 48 hours and then diluted 1:100 in sterile saline and sterile distilled water. The purpose of dilution was to reduce the organic substrate of the broth, thereby countering any protection it might provide the organisms. Then 0.1 ml of each of the diluted cultures was inoculated onto the surface of high-density polyethylene hip cups.

*Aspergillus niger* spores were harvested with a sterile saline and Triton X-100 solution from a 10-day-old culture grown on low-pH, high-carbohydrate agar. This spore suspension was diluted 1:10 in saline, and 0.1 ml of the suspension was then inoculated onto each of the hip cups.

The same method used to harvest *A. niger* spores was also used to prepare endospores of *Bacillus pumilus* and *Bacillus subtilis* subsp. *niger*.[1,2] The endospores were then suspended in 80% isopropanol and inoculated onto various devices and filter paper strips. The $D_{10}$ values were determined after processing the endospores with either gamma irradiation or EtO.

**Viruses.** A frozen pool of viruses maintained at −70°C was thawed, and 0.1 ml was inoculated onto each of the hip cups. The following methods of propagation were employed. Herpes simplex virus type I (HSV I) was propagated in H.Ep.2 cells fed with Earle's Minimum Essential Medium (EMEM) supplemented with 2% calf serum, as well as penicillin and streptomycin to a final concentration of 100 units/ml and 100 μg/ml, respectively. Cell sheets were incubated stationary at 35°C for up to 10 days, and the virus was

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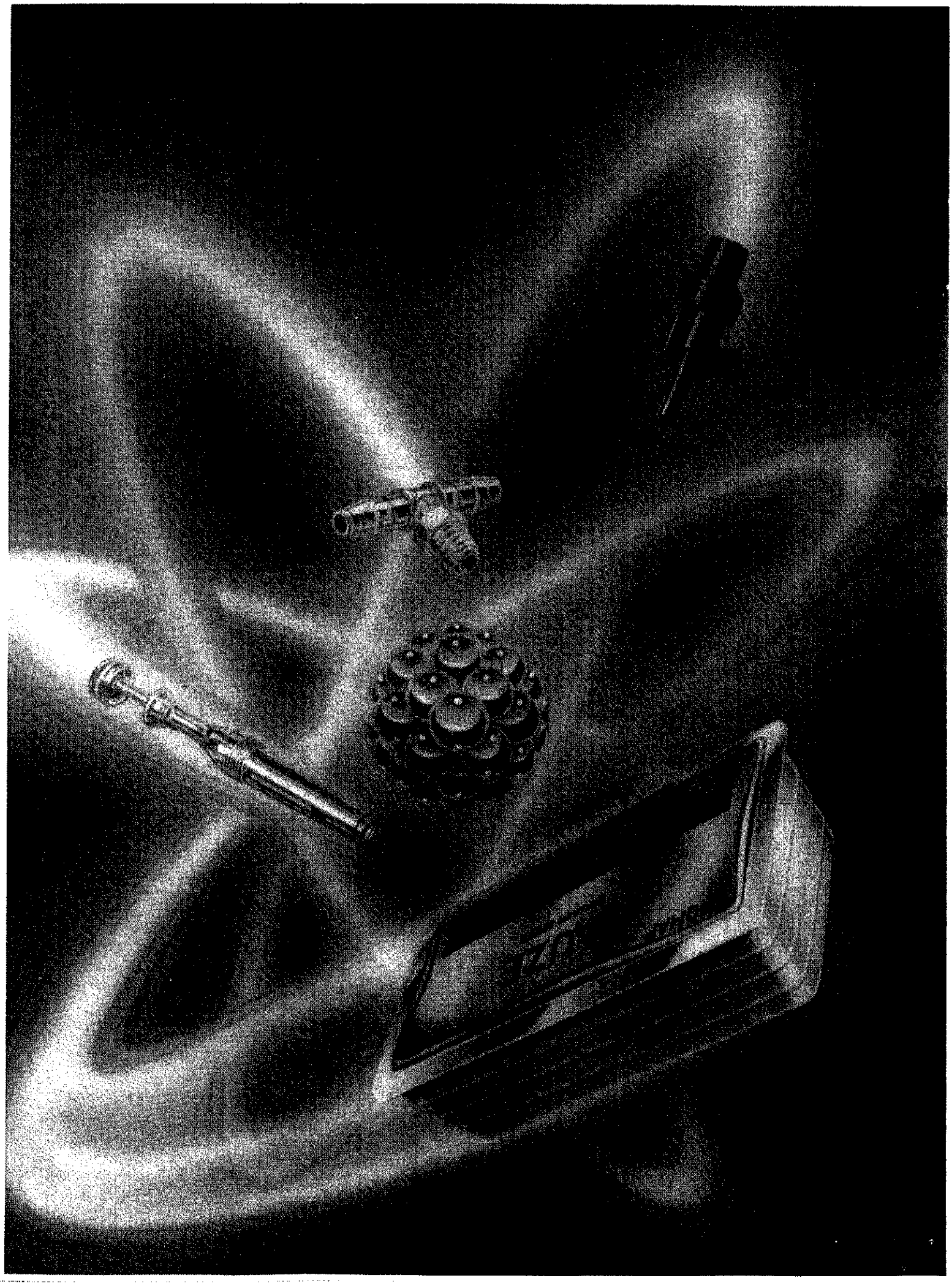

detected microscopically by cytopathic effect (CPE).

Vaccinia virus and poliovirus type I (Mahoney) were both inoculated into the human diploid fibroblast cell line MRC-5 and fed with EMEM supplemented as before. Again, cell sheets were incubated at 35°C for up to 10 days and then observed for CPE.

Influenza $A_2$ virus (Japan 305/57) was propagated in the chorioallantoic cavity of 12-day-old chick embryos. The inoculated eggs were incubated stationary for 48 hours at 35°C and then refrigerated overnight. The chorioallantoic fluid (CAF) was harvested from the air sac locus. The presence of influenza virus in the CAF was determined by mixing 0.5 ml of CAF with 0.5 ml of a 0.5% saline suspension of guinea pig red blood cells and observing for gross signs of hemagglutination (the Salk reaction).

All the inoculated products were air-dried and held at room temperature until bioburden recovery. Further details on virus propagation and maintenance can be found in Grunberg and Prince.[3]

### Bioburden Recovery

A modification of the NASA/FDA aerobic protocol was employed.[4] The devices were placed into shaking vessels containing an elution fluid (EF) of 0.85% saline supplemented with 1% Tween 20. The samples were agitated for 30 minutes on a reciprocal shaker at 280 strokes per minute (in some cases a stomacher or vortex was used), and then 5-ml aliquots were plated to soybean casein digest agar pour plates. The total volume plated was equal to at least 25% of the volume of EF, so

| Organism | | CFU After Number of Hours at 21°C, RH 40–50% | | | $D_{10}$ Value[c] | Saline Lag Time (h)[d] |
|---|---|---|---|---|---|---|
| | | $N_0$ (0-Time) | $N_1$ (6 h) | $N_1$ (24 h) | | |
| *Staphylococcus aureus* (ATCC 6538) | Saline[a] | $1.9 \times 10^5$ | $1.6 \times 10^5$ | **$1.0 \times 10^4$** | 19 h | 6 |
| | Water[b] | $1.6 \times 10^5$ | | **$<1.0 \times 10^1$** | 4.5 h | |
| *Micrococcus luteus* (GBL 27) | Saline[a] | $1.2 \times 10^4$ | $1.8 \times 10^4$ | **$3.9 \times 10^3$** | 48 h | 6 |
| | Water[b] | $1.4 \times 10^4$ | $1.3 \times 10^3$ | **$1.2 \times 10^3$** | 22 h | |
| *Escherichia coli* (ATCC 8739) | Saline[a] | $1.2 \times 10^6$ | **$3.0 \times 10^1$** | $<1.0 \times 10^1$ | 78 min | <6 |
| | Water[b] | $1.1 \times 10^6$ | **$<1.0 \times 10^1$** | $<1.0 \times 10^1$ | 59 min | |
| *Salmonella pullorum* (ATCC 10398) | Saline[a] | $1.0 \times 10^6$ | $1.5 \times 10^4$ | **$4.0 \times 10^1$** | 5.5 h | ~6 |
| | Water[b] | $4.1 \times 10^5$ | **$9.0 \times 10^1$** | $1.0 \times 10^1$ | 100 min | |
| *Candida albicans* (ATCC 10231) | Saline[a] | $1.8 \times 10^5$ | $1.3 \times 10^5$ | **$1.2 \times 10^4$** | 20 h | 6 |
| | Water[b] | $3.2 \times 10^4$ | **$<1.0 \times 10^1$** | $<1.0 \times 10^1$ | 80 min | |
| *Propionibacterium acnes* (ATCC 6919) | Saline[a] | $1.6 \times 10^5$ | $1.6 \times 10^4$ | **$<1.0 \times 10^1$** | 4.5 h | ~6 |
| *Aspergillus niger* (ATCC 16404) | Saline[a] | $4.0 \times 10^5$ | $9.4 \times 10^4$ | **$8.4 \times 10^2$** | 9 h | ~6 |
| *Bacillus pumilus* (spores) (ATCC 27142) | | $1.0 \times 10^6$ | $1.1 \times 10^6$ | $1.0 \times 10^6$ | ~3 yr (see Table II) | ≥1 yr at RT |
| *Bacillus subtilis* subsp. *niger* (spores) (ATCC 6633) | | $1.1 \times 10^6$ | $1.0 \times 10^6$ | $1.0 \times 10^6$ | ~3 yr (see Table II) | no data |

[a]Organism inoculated in 0.85% NaCl suspension.
[b]Organism inoculated as suspension in distilled water.
[c]Calculated by

$$D_{10} = \frac{\text{time}}{\log_{10} N_0 - \log_{10} N_1}$$

The $N_1$ values used for these calculations are shown in boldface.
[d]Arbitrarily taken to be the time during which the count remained the same or did not decline appreciably in relation to the major die-off; note the protective effect of saline.

*Table I: Desiccation $D_{10}$ values for bacteria, yeast, and endospores on high-density polyethylene hip cups.*

| Time Interval | Viability[a] | | | $Log_{10}$ Survivors[c] |
|---|---|---|---|---|
| | 4°–8°C | 20°–25°C | 35°C | |
| 0 | $7 \times 10^5$ | $7 \times 10^5$ | $7 \times 10^5$ | 5.85 |
| 2 mo | $9 \times 10^5$ | $5 \times 10^5$ | $6 \times 10^5$ | nd[b] |
| 4 mo | $6 \times 10^5$ | $6 \times 10^5$ | $4 \times 10^5$ | nd |
| 6 mo | $6 \times 10^5$ | $8 \times 10^5$ | $5 \times 10^5$ | nd |
| 1 yr | $5 \times 10^5$ | $4 \times 10^5$ | $4 \times 10^4$ | nd |
| 3 yr | nd[b] | $6.9 \times 10^4$ | nd[b] | 4.85 |
| 6 yr | nd | $2.6 \times 10^3$ | nd | 3.41 |
| 8 yr | nd | $2.5 \times 10^2$ | nd | 2.39 |
| 13 yr | nd | $<1.0 \times 10^1$ | nd | nd |

[a]Average number of viable spores per strip (stored in sealed paper envelopes).

[b]No data.

[c]Average $D_{10}$ value for 3, 6, and 8 yr is 2.6 yr.

*Table II: Effect of storage temperatures on viability of spores of* B. pumilus *on E-601 filter paper strips.*

that a 100-ml elution was dispensed as five 5-ml pour plates. The cultures were incubated at 35°C for 48 hours or at room temperature for 5 days, after which time the number of colonies were enumerated and then multiplied by the plating factor to give the total number of colony-forming units (CFU) per device. In cases of mixed, unknown bioburden, the cultures were incubated for 48 hours at 35°C followed by 3 additional days at room temperature (20°–25°C). For the virus, 10-fold serial dilutions of EF were inoculated into either cell cultures or embryonated eggs, which were then incubated at 35°C for the appropriate length of time.

$D_{10}$ values were determined by the log-intercept method on least-square fits of dose-survivor curves, as well as by the Stumbo equation,

$$D_{10} = \frac{\text{t or Mrad}}{\log_{10} N_0 - \log_{10} N_1}$$

## Results and Discussion

**Bacteria and Fungi.** Table I presents the "die-off" of the various organisms at 6 and 24 hours after inoculation. In most cases, a 1- or 2-log decline was seen within the first 6 hours. Note that saline provided greater viability to gram-negative and gram-positive bacteria than did distilled water. One might therefore expect that bioburden organisms vectored to a substrate from nasal shedding, saliva, or perspiration (with the accompanying concentration of electrolytes) might be more resistant to die-off than organisms vectored from air, water, or other salt-free media.

As shown in Table I, *Staphylococcus aureus* and *Micrococcus luteus* were the vegetative bacteria that were most resistant to drying, with *C. albicans* also quite resistant. As expected, the two species of endospores retained full viability. Moreover, the gram-negative rods and *Propionibacterium acnes* were moderately sensitive to desiccation, with *A. niger* spores even more resistant than the gram-negative rods.

These organisms can be ranked according to decreasing resistance to desiccation. The endospores were the most resistant (approximately 3 years), followed by *M. luteus* (48 hours), *C. albicans* (20 hours), *S. aureus* (19 hours), *A. niger* spores (9 hours), *Salmonella pullorum* (5.5 hours), *P. acnes* (4.5 hours), and *Escherichia coli* (a little over 1 hour). These findings agree with additional laboratory results on the ambient bioburden of a variety of devices, including high-density polyethylene hip cups, needles, syringes, catheters, gauze, muslin, sutures, and disposable thermometers. These analyses had generally yielded gram-positive bacilli and cocci, particularly *Bacillus* and *Micrococcus;* enteric and nonfermenting bacilli and anaerobes were found infrequently. Thus, the resistance data summarized in Table I are sufficiently predictive of various biotypes during a routine bioburden survey.

If one assumed that a device was harboring $10^2$ of every organism named in Table I, then applying the saline $D_{10}$ values for a 5-log loss would result in the following times for the device to clear itself of each organism, assuming that $10^{-3}$ is the clearing point: *E. coli*, 6 hours; *S. pullorum*, 16 hours; *P. acnes*, 30 hours; *A. niger*, 48 hours; *S. aureus*, 4 days; *C. albicans*, 4 days; *M. luteus*, 10 days. It should be pointed out that these clearance times assume a linear death rate of microorganisms on hostile devices. If exponential death does not occur in all cases, vegetative forms may still be isolated after 10 days.

These values indicate that the resistant micrococcal bioburden is significantly reduced after approximately 10 days quarantine, leaving essentially an endospore population. One might assume, therefore, at least a 10-day clearance point for vegetative bacteria. The presterilization clearing of vegetative bioburden is well known; the data presented here are simply an attempt to define the *rate* at which such viability loss occurs. The endospore loss for inoculation of *B. pumilus* spores onto filter paper strips is shown in Table II. These data illustrate the stability of a pure spore population, the viability of endospores (already seen in Table I), and the extension of this viability to at least 1 year. The $D_{10}$ value for *B. pumilus* spores was approximately 3 years.

Thus, the common vegetative bioburden most resistant to desiccation consists of *Micrococcus* (death measured in hours) and *Bacillus* spores (death measured in years). If one assumes the die-off rates and bioburden dynamics postulated here, a product harboring $10^1$ spores should have a sterility assurance level (SAL) of $10^{-3}$ after 12 years.

**Viruses.** The data given in Table III are from a 48-hour die-off experiment with various lipophilic and hydrophilic viruses. The results were not entirely expected. Since virions need a living host, it was always assumed that they would not survive for more than a few hours on inanimate surfaces. However, the data clearly demonstrate short-term resistance to desiccation with three of the four agents surviving for 24 hours. Influenza, a lipophilic myxovirus, was the most sensitive to desiccation. Herpesvirus, another lipophilic agent, showed an unexpected degree of resistance with a $D_{10}$ value of 7 hours. The high $D_{10}$ value for poliovirus (13.5 hours) was not unexpected. *Vaccinia* showed an intermediate level of resistance.

The absence of virus testing in sterility assays is justified by the present data. However, if one wanted to validate that a device substrate was hostile to viruses, the biological indicator of choice would be poliovirus. If one assumes a poliovirus bioburden of $10^2$ and a $D_{10}$ value of 14 hours, then it would take 70 hours to achieve a virus-free assurance level of $10^{-3}$. We may

| Virus | Host | $ID_{50}$'s per Device[a] | | | $D_{10}$ Max[b] |
|---|---|---|---|---|---|
| | | $N_0$ (0-time) | $N_1$ (24 h) | $N_1$ (48 h) | |
| Influenza $A_2$ J-305/57 (Asian) GBL 505 | Chick embryo | $1.0 \times 10^7$ | $<1.0 \times 10^1$ | — | 4 h |
| HSV-I (Sabin) GBL 504 | H.Ep.2 | $5.0 \times 10^7$ | $5.0 \times 10^5$ | $<1.0 \times 10^1$ | 7 h |
| Vaccinia (Wyeth) GBL 506 | MRC-5 | $5.0 \times 10^6$ | $1.0 \times 10^5$ | $<1.0 \times 10^1$ | 8.5 h |
| Polio I (Mahoney) GBL 507 | MRC-5 | $1.0 \times 10^6$ | $8.0 \times 10^4$ | $5.0 \times 10^1$ | 13.5 h |

[a] $ID_{50}$ = infectious dose 50% per device as calculated by the method of Reed and Muench from the data obtained in cell cultures or in chick embryos.
[b] $D_{10}$ estimated from

$$D_{10} = \frac{\text{time}}{\log N_0 - \log N_1}$$

$N_1$ values taken from 48-h recovery data, except for influenza virus, which was taken from 24-h recovery data.

*Table III: $D_{10}$ values for viruses on high-density polyethylene hip cups after 24 and 48 hours at room temperature.*

| Organism | ~$D_{10}$ Value | Susceptibility Group[a] |
|---|---|---|
| *E. coli* | 1 h | A |
| *P. acnes*<br>Influenza A virus<br>*S. pullorum* | 4–5 h | B |
| *A. niger*<br>Herpes simplex I<br>Vaccinia virus | 7–9 h | C |
| Poliovirus | 13 h | D |
| *S. aureus*<br>*C. albicans* | 20 h | E |
| *M. luteus* | 48 h | F |
| *B. pumilus* spores | ~3 yr | G |

[a] Increasing resistance to ambient desiccation.

*Table IV: Microbial grouping of desiccation viability.*

| Inoculated Product | $D_{10}$ Value (Mrads, duplicate determinations) | | |
|---|---|---|---|
| Devices | | | |
| 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 | $\bar{X}_{25} = 0.18$[c] | |
| 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[a] | 0.16, 0.17, 0.18 | | $\bar{X}_2 = 0.21$ |
| Drugs and Natural Substances | | | |
| Gum powder | 0.15 | | |
| Tuberculin (Tine test) | 0.17 | | |
| Potatoes | 0.17 | | |
| Ophthalmic liquid (water in oil emulsion) | 0.20 | $\bar{X}_7 = 0.24$ | |
| L-lysine powder | 0.35 | | |
| Ig G powder | 0.4 | | |
| Aloe concentrate (ambient flora)[b] | 0.26 | | |

[a]E-Rad-O-Kit® spore strips. Values were the same regardless of position in the tote box during continuous irradiation.
[b]Based on natural bioburden.
[c]These average values for gamma irradiation constitute the value known as $D_{gamma}$ in the article. Positional effects were not noted; values were the same at various inoculation sites (e.g., for 1.0-ml syringe: inside barrel at annular ring, 0.17 Mrad; outside surface, 0.17 Mrad).

*Table V: $D_{10}$ values of dried spores of* B. pumilus *(ATCC 27142) on various medical devices.*

assume, therefore, that 3 days is the eradication or clearing point for viruses.

**Spore Resistance to Radiation and EtO.** In Table IV, microorganisms are grouped according to susceptibility to desiccation. It is clear from the data presented in Tables I through IV that if one waits 3 days for viral clearing and 10 days for vegetative bacterial clearing, then the device to be sterilized will harbor essentially an endospore population. Since the endospore is thus the real target of sterilization by EtO and ionizing irradiation, it is important that spore resistance to these procedures be discussed.

Table V summarizes the effects of product substrate on spore resistance to ionizing irradiation. The data show relatively uniform $D_{10}$ values for the *B. pumilus* spores (ATCC 27142). Note, however, the slowly increasing values for $D_{10}$ from top to bottom, suggesting that a substrate or a radioprotective phenomenon is influencing spore resistance. For example, gauze is easier to sterilize than catgut suture. And the results with aloe plant extract demonstrate the radioprotective capacity of natural substances—also evidenced when spores of *B. pumilus* are incorporated into gamma globulin or lysine. Moreover, the chemical nature of the irradiated substrate is more important in determining resistance to radiosterilization than is the exposure position of the bioburden. For example, it was no more difficult to kill spores on the inside of a plastic syringe at the annular ring between the plunger and the barrel than on the outside surface. This was not found to be the case, however, with sterilization by EtO.

Table VI shows the effects of bioburden location when EtO is the sterilizing agent. $D_{10}$ values obtained for the spores of *B. subtilis* subsp. *niger* (ATCC 9372) are meaningless without reference to their location in or on the product. In addition, inoculated product is sometimes more resistant to sterilization by EtO than is a spore strip or inoculated carrier. The similarity in re-

sistance between inoculated carriers and inoculated products must not be assumed, but must be determined on a case-by-case basis.

### Immediate and Retained Bioburdens

There are two kinds of bioburdens encountered prior to sterilization: the *immediate* bioburden and the *retained* bioburden. The immediate bioburden (the bioburden that exists immediately after product manufacturing) contains not only endospores and desiccation-resistant cocci, but also gram-negative organisms, fungi, and viruses. The retained bioburden (the bioburden that is retained after the product has incubated for several days or weeks) consists, for the most part, of *Bacillus* spores and a few gram-positive cocci.

The product containing the retained bioburden is the least challenging to a sterilization process. Therefore, the immediate bioburden, the worst-case situation, should be tested when bioburden information is needed to validate a sterilization process, as in the AAMI B-1 method[5] or dose-survivor method for radiation sterilization.[2] The bioburden should be determined within 24 hours and certainly less than 10 days after manufacture of the product. However, if retained rather than immediate bioburden is desired as the basis for dose setting, then experiments of the $10^{-2}$ ($D^*$) audit type should be run on a product whose lag time from manufacture to sterilization is similar to the lag time from manufacture to analysis of bioburden.[5]

### Steps for Contamination Control

It can generally be assumed that viruses will clear from a nonpermissive device in 3 days and that the majority of vegetative bacteria will clear to a $10^{-3}$ SAL by 10 days. Keeping the endospore population to a minimum and preventing the vectoring of electrolyte-protected bacteria are important aspects of contamination control. If one wants to achieve an EtO cycle or a

| Device | Position of Spore Strip or Inoculated Spores | $D_{10}$ Value (min)[a] | |
|---|---|---|---|
| Free spore strip (100% EtO) | — | 2–3 | |
| Gauze pad (100% EtO) | On pad | 2–3 | |
| Dialysis bag (100% EtO) | Inside bag | 3.5 | |
| Suture (12/88) | Inside blister pack | | |
| Wet with preservative | | 3–11 | |
| Wet without preservative | | 22 | |
| Dry | | 30 | |
| Cardiac pacing wire (100%) | On surface of wire | 8 | |
| Pipette, 1-ml glass (12/88) | Inside plugged pipette | | |
| Spore strips | | 4 | |
| Inoculated product | | 10 | $\bar{X} = 17$[b] |
| Collagen pad (100%) | On pad (encapsulated) | 21 | |
| Aspirating tube (12/88) | | | |
| Spore strips | In strip pack or inside tube | 11 | |
| Inoculated product | Spores inside tube | 16 | |
| Lap sponge (100%) | — | 27 | |
| Polyethylene disposable nurser bottle (12/88) | Inside plastic bottle of a compressed roll | 35 | |
| Syringe (100%) | At annular ring on depressed plunger | 40 | |
| Stopcock assembly (100%) | Inside stopcock | 42 | |

[a]Calculated from either $D_{10} = \dfrac{\text{time}}{\log N_0 - \log N_1}$ or $D_{10} = \dfrac{\text{time}}{\log_{10} N_0 - \log_{10} \ln \left[\dfrac{\text{no. samples}}{\text{no. negative}}\right]}$

when quantitative plate count analyses were obtained from spore strips and when fractional sterility tests were performed on spore strips, respectively.

[b]This average value for EtO treatment is referred to as $D_{EtO}$ in the article.

*Table VI: $D_{10}$ values of spores of* B. subtilis *subsp.* niger *(ATCC 9372) after EtO treatment (RH = 50%; temperature = 105°–115°F; mg/L EtO = 225–600).*

| Bioburden ($10^x$) | Log (x + 6) | Time for SAL $10^{-6}$ (min)[a] |
|---|---|---|
| $10^0$ | 6 | 102 |
| $10^1$ | 7 | 119 |
| $10^2$ | 8 | 136 |
| $10^3$ | 9 | 153 |

[a]Calculated by log (x + 6) × $D_{EtO}$ (17 min).

*Table VII: Steps for contamination control, EtO.*

| Bioburden ($10^x$) | Log (x + 6) | Mrads for SAL $10^{-6}$[a] |
|---|---|---|
| $10^0$ | 6 | 1.26 |
| $10^1$ | 7 | 1.41 |
| $10^2$ | 8 | 1.68 |
| $10^3$ | 9 | 1.89 |

[a]Calculated by log (x + 6) × $D_{gamma}$ (0.21 Mrad).

*Table VIII: Steps for contamination control, gamma radiation.*

gamma irradiation process with an SAL of $10^{-6}$, the following steps (demonstrated in Table VII and Table VIII) may be useful:

1. Wait 10–20 days after manufacture; then perform aerobic bioburden on 30 units of the product and calculate the average count.
2. For radiation sterilization, if the average aerobic count is $10^x$, multiply the average $D_{10}$ value ($D_{gamma}$) for *B. pumilus* as given in Table V by x + 6 and consider this the dose in megarads for a $10^{-6}$ SAL. Multiply $D_{gamma}$ by x + 2 for the $10^{-2}$ audit dose and perform 100 sterility tests. If less than 2 out of 100 tests are positive, consider the $10^{-6}$ dose to be correct. The value of $D_{gamma}$ is taken from Table V as 0.21 Mrad.
3. For EtO sterilization, multiply the average $D_{10}$ value ($D_{EtO}$) for *B. subtilis* as given in Table VI by x + 6 and consider this the sterilization time for a $10^{-6}$ sterility assurance level. Multiply $D_{EtO}$ by x + 2 for the $10^{-2}$ audit time and perform 100 sterility tests. If less than 2 out of 100 tests are positive, consider the $10^{-6}$ dose to be correct. The value of $D_{EtO}$ is taken from Table VI as 17 minutes.
4. For additional safety, add the value of one $D_{gamma}$ or one $D_{EtO}$ in the irradiation or EtO process shown in step 2 or step 3.

Thus, there is little reason to believe that devices manufactured under GMP will not achieve an SAL of $10^{-6}$ after approximately 2.5 hours of exposure to EtO under typical chamber conditions or after exposure to approximately 2.0 Mrad.

## Conclusion

Microorganisms can be grouped according to their desiccation resistance, with *E. coli, Salmonella, Propionibacterium,* and Influenza A virus among the most sensitive to drying, and *S. aureus, C. albicans, M. luteus,* and poliovirus among the most resistant. Moreover, organisms dried onto a device from saline show increased viability compared with water controls.

After 10 days on a device, a bioburden will consist essentially of endospores and gram-positive cocci such as *Micrococcus.* In fact, *Bacillus* endospores are indifferent to desiccation at room temperature for at least 1 year. Thus, the sterilization process is really aimed at eradicating the endospores. It has been shown that the chemical nature of the device or diagnostic substrate is an important determinant in radiation sterilization; and for EtO sterilization, the position of the bioburden in or on the device is an equally important determinant.


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