Bioburden

Bioburden is a measure of microorganism recovery in a unit of product or substance (raw material, solution, surface, etc.). It is the initial population of microorganisms prior to being subjected to a sterilization procedure; thus, bioburden is the Y-intercept of the microbial death kinetic plot. Bioburden determination in a laboratory for *D* value determination is relatively reproducible compared with bioburden determination in a compounded product solution prior to filtration. In the manufacturing environment, most microorganisms are difficult to recover (grow). Bioburden recovery on surfaces using swabs or Rodac plates is usually very poor; on the order of 30% to 40% of what might actually present.

In manufacturing environments, the expected maximum level of microorganisms prior to filtration is not more than 10 CFUs/100 mL solution. United States Pharmacopeia (USP) General Chapter <1111> provides guidelines for raw materials, excipients, and bulk drug substances with a proposed bioburden limit of not more than 1000 CFU/g or mL.

D Value

The *D* value (decimal point reduction value) is the time or dose required for a one log reduction in the microbial population under specific conditions. An example of a plot of microbial population versus time or dose is shown in Figure 17-2 where it is demonstrated how the *D* value is obtained. In reality, a linear regression line is calculated, as microbial population versus time data is rarely linear. The *D* value is calculated from this best fit line from the microbial reduction data over time or dose. Several complicated mathematical approaches are available for accurately calculating the *D* value (1). If the *D* value is one minute, this means that it required one minute at a given temperature for the microbial population to be reduced by one log unit. The *D* value is dependent on many factors including type of microorganism, temperature or dose, and the medium/substance containing the microorganism. *D* values for a variety of microorganisms at 121°C are given in Table 17-2. Note the differences in the time required to reduce different species at the same temperature. Table 17-2 also shows the effect of type of sterilization method on the *D* value of the same microorganism (*B. subtilis var. niger*) showing the effectiveness of different treatments and different temperatures on microbial level reduction.

D values for some biological indicator organisms used currently are shown in Table 17-1. Over the years these values have increased indicating the natural tendency of microbial life to develop resistance to methods used to destroy them.

Z Value

The *Z* value is the number of degrees or dosage units required for a one log reduction in the *D* value. The *Z* value measures resistance of the microorganism to the sterilization source. Figure 17-3 shows a logarithmic plot of *D* value for a particular microorganism versus temperature for a heat sterilization process. The steeper the slope the more resistant is the indicator organism. The conventional *Z* value used for steam sterilization is 10°C (2).

Table 17-2A *D* Values for Different Bacterial Spores by Steam Sterilization

Spore	***D* value range (minutes at 121°C)**
Bacillus stearothermophilus	1.5–3.0
Bacillus subtilis ATC 5230	0.3–0.7
Bacillus coagulans	0.4–0.8
Clostridium sporogenes	0.4–0.8

Original source unknown. Information obtained from Kenneth E. Avis course notes, University of Tennessee, 1980. *D* Values may no longer be accurate, but purpose of the table is to point out relative differences among bacterial spores to steam sterilization.