

## DEVELOPING A STERILIZATION PROCESS

The objective of a sterilization process is to kill the microorganisms naturally present in the product (product bioburden). The question a D-value study answers is how does the product influence the resistance of suspended organisms during a sterilization cycle? This test is typically performed using calibrated resistant spores. Spores are the most durable of microorganisms and should demonstrate the least impact from the product. SGM Biotech provides this service, however, this information is only one piece of the data necessary to develop a sterilization process. The objective of this document is to provide a general overview on how to correctly use the D-value study data along with Biological Indicators to develop, validate, and monitor a steam sterilization process of a liquid pharmaceutical product.

When validating a cycle for the sterilization of a liquid pharmaceutical product, the following questions need to be answered:

### 1. What is the natural bioburden of the product?

The product may have little or no bioburden depending on the manufacturing conditions, or it may contain high concentrations of multiple organisms. It is very important to learn as much as possible about the bioburden organisms. This information will be critical when determining the parameters of the sterilization cycle. Useful information when characterizing the bioburden includes:

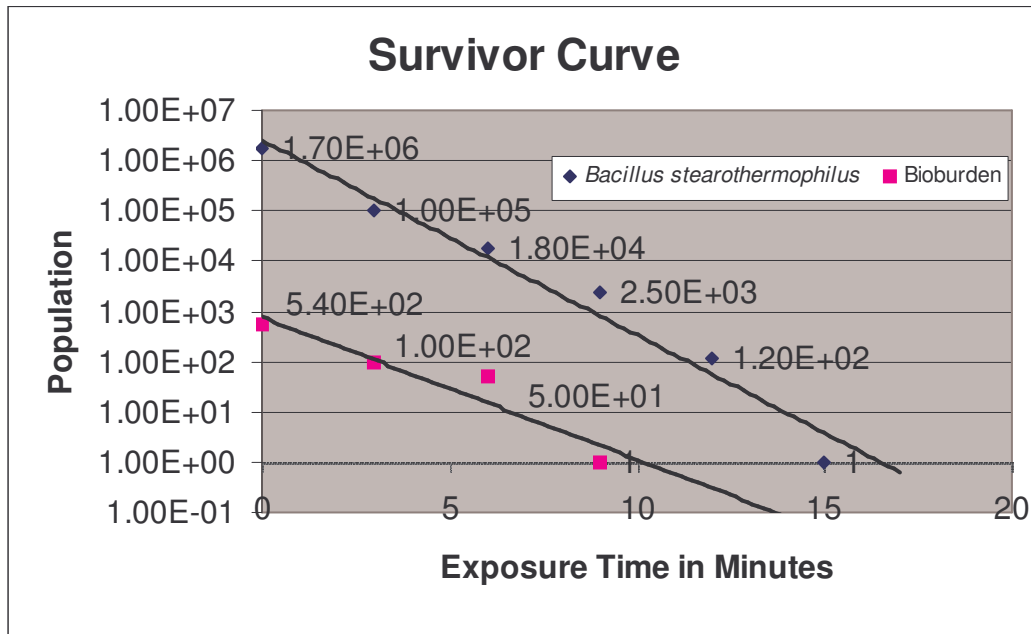
- Total numbers of organisms present just prior to sterilization
- Types of organisms present
- Number of resistant sporeformers present
- Resistance of this bioburden
- Sampling frequency and statistical analysis

### 2. What effect does the product have on the bioburden before sterilization?

This information is useful because it is important to know how the product will influence the behavior of the organisms present. If the product is capable of promoting growth, then over time, relatively few organisms could eventually result in a high number of organisms. This represents a problem if there is a significant time delay between manufacture and sterilization. High numbers of organisms will result in an increase in sterilization time. Alternately the material may be bacteriostatic, bactericidal, sporostatic, or sporicidal.

### 3. How resistant is the bioburden to the sterilization process? (D-value study)

Ideally, once the bioburden organisms have been characterized,  $D_T$ -value studies using these organisms in the product should be performed. Product characteristics such as viscosity, crystal formation, and pH may influence the lethality during of the cycle on the organism. This entire process may not be feasible or possible depending on the circumstances. An alternate method would be to take a conservative approach and perform a D-value study with the product using an organism resistant to the sterilization process. *Bacillus stearothermophilus* is a spore-forming organism widely recognized for monitoring a steam sterilization process, and it may be substituted for the bioburden organism in the D-value study. When this substitution occurs, it is assumed that the *B. stearothermophilus* organisms are more resistant than the bioburden organisms or by design can significantly out number the bioburden organisms. If by chance the bioburden organisms have a higher resistance than *B. stearothermophilus*, then a high number of *B. stearothermophilus* spores should be used. The kill time for the *B. stearothermophilus* would then exceed the kill time for the bioburden organisms. The following chart illustrates this point.



### 4. How does this information help determine the length of a cycle?

The length of the cycle is determined in part by the desired Sterility Assurance Level (SAL), the initial population, and resistance of the organism. For example, if product X contains 100 microorganisms/unit with a  $D_{121}$ -value of 3.0 minutes, then a 24.0-minute exposure would give a SAL of  $10^{-6}$ . The following table illustrates this point.

Exposure Time at 121°C in B.I.E.R. vessel (F <sub>0</sub> )	Number of Surviving Organisms per Unit	Sterility Assurance Level (SAL)
0 minutes	100	Non sterile
3 minutes	10	Non sterile
6 minutes	1	Non sterile
9 minutes	0.1	1 Non sterile in 10
12 minutes	0.01	1 Non sterile in 100
15 minutes	0.001	1 Non sterile in 1000
18 minutes	0.0001	1 Non sterile in 10,000
21 minutes	0.00001	1 Non sterile in 100,000
24 minutes	0.000001	1 Non sterile in 1,000,000

### 5. How does the Biological Indicator fit into this process?

Each lot of biological indicators will vary slightly in its population, resistance, and kill time (exposure time in which zero tested units result in positive). The biological indicators used to monitor a cycle must have a reported kill time equal to or less than the validated cycle time that provides.

### 6. Sterility Assurance Level (SAL)

This value can be calculated based on the Biological Indicator selected. For example, a BI with a 10<sup>6</sup> spore challenge will have to be exposed to a process equal to 12 D-values to yield a SAL of 10<sup>-6</sup>.

This value can be based on calculated resistance of natural bioburden. In this case a BI with a much lower population of spores than the number of bioburden would be selected.

Another approach is to use the bioburden resistance values and to monitor the process with a known Biological Indicator system. This will yield SAL levels that are significantly higher than 10<sup>-6</sup>.

Products that are quite stable to long exposure times at 121° C use the 10<sup>6</sup> BI approach. Products sensitive to heat use the combination bioburden resistance and custom BI challenge to monitor the process.