STERILITY ASSURANCE-GATHERING MICROBIOLOGICAL DATA

BIOMERIEUX CASE STUDY

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PIONEERING DIAGNOSTICS

BIOMÉRIEUX

- **What does Sterility mean?**
- **What is Sterility Assurance? How is it calculated?**
- **Understand how we collect data to support Sterility Assurance**
- **Review some specific data collected on some of bioMerieux's products**
- **Discuss why bioMerieux's products present a unique challenge in collecting useful microbiological data**
- **Understand how SAL is affected by the manufacturing process**

What is Sterility?

- **Free of viable microorganisms**

- **Sterilization Methods:**
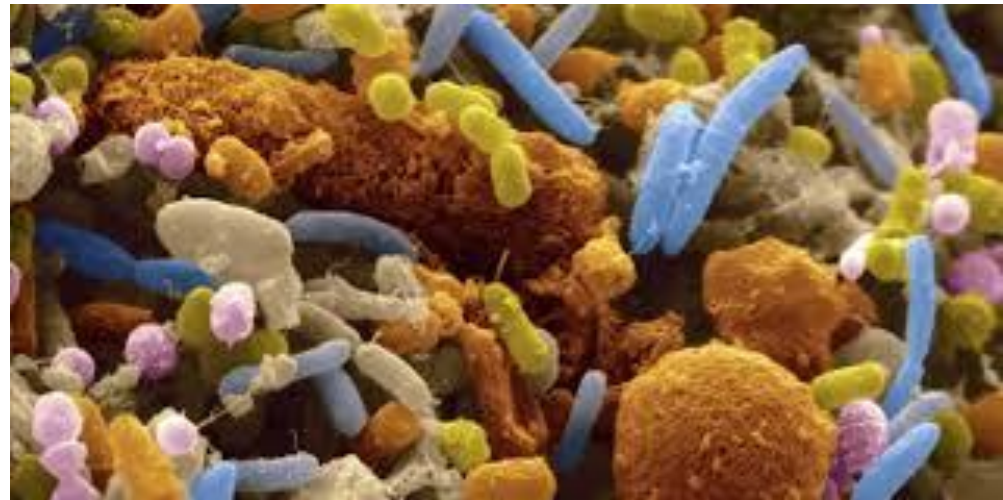 - Heat
    - Steam Sterilization, Dry Heat Sterilization
  - Chemical
    - Ozone, Ethylene Oxide
  - Radiation
    - Gamma, UV

- **Sterilization is not an absolute**

Sterility Assurance is a probability, not an absolute

- We are not able to examine every single device produced for sterility
- Sterilizing agents (heat, radiation, etc) will produce predictable reduction of microorganisms of known resistance and population
- The degree of sterility can be calculated and is often expressed as the probability of a non-sterile unit (PNSU) or **Sterility Assurance Level (SAL)**

3 pieces of information to obtain an SAL

- Bioburden of your product
- Resistance to the sterilizing agent of that bioburden
- The amount of sterilizing agent applied

- **Bioburden: the number of organisms present on a device. Often, only the number of sporulating organisms is counted as these are the organisms that may present resistance the sterilizing agent**

- **D value: The D-value of an organism is essentially its resistance to the sterilizing agent. The D-value is the amount of sterilizing agent necessary to reduce the population by 90%.**

- **Fo: a measure of thermal lethality. In simplest terms, it is the *equivalent* exposure time at 121.1 C, calculated using the *actual* exposure time at variable temperature.**

$$\text{Log SAL} = (-F / D) + \text{Log N}$$

A quick example

- A medical device contains 10^3 CFU of Bacillus *subtilis*.
- The D_{121}-value of *B. subtilis* was found to be 1.0.
- The device is autoclaved for 12 minutes at 121.1C. The resulting Fo was found to be 10.0.

What is the SAL?

Log SAL = (-F / D) + Log N

Log SAL = (-10.0/1.0) + Log (10^3)

Log SAL = -10.0 + 3

Log SAL = -7.0

SAL = 10^{-7}

The probability of a non-sterile unit is 1 in 10,000,000.

A quick example

How does the SAL change as the bioburden or D-values change?

- The company begins a construction project which opens the facility to some outside bioburden. The bioburden of the product increases to 10^4 CFU per device of *B. subtilis*.

What is the SAL?

Log SAL = (-F / D) + Log N

Log SAL = (-10.0/1.0) + Log (10^4)

Log SAL = -10.0 + 4

Log SAL = -6.0

SAL = 10^{-6}

The probability of a non-sterile unit is 1 in 1,000,000.

How do we collect data?

- **Collecting microbiological data for bioburden and D-value analysis should be carefully considered and can vary greatly depending on the manufacturing process and type of device or pharmaceutical.**

- **Points to consider:**
  - Regular production run sampling or "worst-case"?
    - May depend on if you are validating a sterilization process or if you are demonstrating process control

  - What can contribute to bioburden changes in your process?
    - Seasonality
    - operator involvement
    - hold times
    - cleaning processes
    - incoming materials
    - Supplier changes
    - Utilities

Microbiological Media produced at bioMerieux, Lombard
- Plated growth media
- Bottles and tubes of growth media, dilution buffers, sterile water
- Some selective media

Most products are terminally sterilized
- Steam, Irradiation

Products are heat sensitive

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■ **In comparison to other medical devices or pharmaceuticals, media presents a unique challenge in determining SAL**

■ **Because media is intended to grow microorganisms, capturing accurate bioburden or spore counts is difficult**

■ Careful control must be applied to the manufacturing process to control the bioburden prior to sterilization

■ Media is often used as the "worst-case" for qualifying filling lines for pharmaceutical products

■ Greater control must be placed on hold times

bioMerieux – Case Study

Two studies were carried out to provide the site with more accurate bioburden and microbial resistance data to support current steam sterilization Fo values and SAL

Bioburden Study

- Bioburden was sampled immediately prior to sterilization for a range of products. Sporulating organisms were quantified and identified

Hold Time Studies

- Hold times have a direct impact on the bioburden of the product before sterilization. The effects of hold times on bioburden, product appearance, pH were assessed

bioMerieux – Case Study

Bioburden

- **Samples were taken over a 1 year period from products immediately before sterilization.**
  - Approximately 80% of samples taken did not contain organisms above the detection limit
    - Indicates that prior to sterilization the bioburden is generally very low
  - Of the 20% of samples that yielded measurable bioburden, 50% contained sporulating organisms.
    - If this seems high, it is likely because the product is exposed to a high temperature in a prior step which may eliminate non-sporulating cells
 - Total bioburden of sporulating organisms was very low (<100 CFU per sterilization unit)
  - 4 unique sporulating organisms identified
    - *Lysinibacillus sphaericus*
    - *Bacillus cereus*
    - *Brevibacillus choshinensis*
    - *Bacillus subtilis*

Hold Times

- **Product was compounded and held at the point prior to sterilization for an extended period of time. Bioburden, turbidity, pH were monitored hourly**
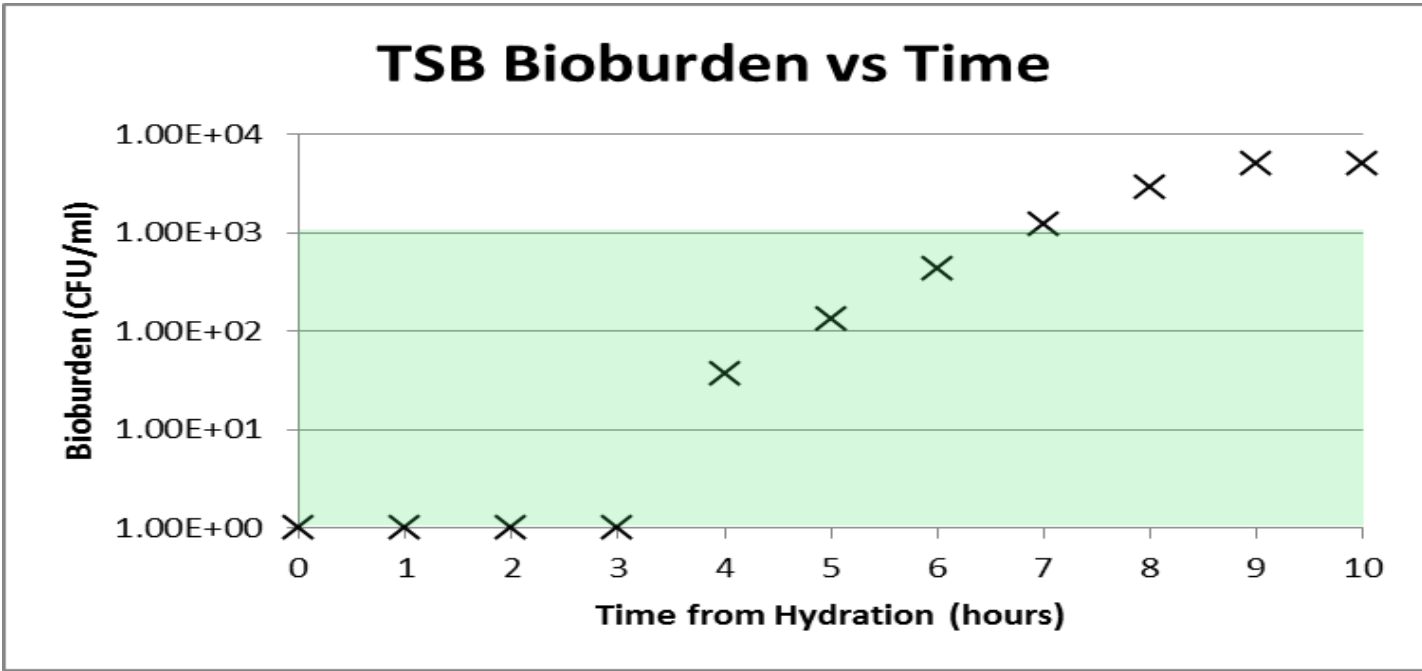
-Acceptable Range is highlighted

- It has previously been shown that counts higher than 10^4 CFU/ml prior to sterilization may impact product performance, appearance, or impact the SAL of the product

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- **Bioburden Study indicated low total bioburden of potentially heat-resistant organisms (spore-forming organisms)**
  - The 4 spore-forming isolates will be sent for D-value analysis by an outside lab
- **Hold Time Study indicated that hold times >6 hours may have an impact on the performance or SAL of the product**
  - Note: bioMerieux Lombard uses a target hold time of <4 hours

- **How is the data reflected in SAL?**
  - Products have different target Fo values. Lowest is ~12.0.
  - At a 4 hour hold time, bioburden approximately 10^2
  - Maximum bioburden of spore-formers also indicates bioburden of 10^2 (max. Not this high in routine production)

Log SAL = (-F / D) + Log N	Log SAL = (-F / D) + Log N
Log SAL = (-12.0/1.0) + Log (10^2)	Log SAL = (-12.0/1.5) + Log (10^2)
Log SAL = -12.0 + 2	Log SAL = -8.0 + 2
Log SAL = -10.0	Log SAL = -6.0
SAL = 10^{-10}	SAL = 10^{-6}
PNSU: 1 in 10 billion	PNSU: 1 in 1 million

Summary

- **Sterility Assurance is a probability, not an absolute and is influenced by the bioburden of product and resistance of that bioburden to the sterilization agent**

- **Gathering microbiological data to support SAL is critical and may look very different depending on the manufacturing process**
  - Careful consideration must be given to factors which may impact microbial growth

- **Microbiological media presents a unique look at how SAL can be affected by deviations to the process such as increasing hold times**

Questions?