STERILITY ASSURANCE-GATHERING
MICROBIOLOGICAL DATA

BIOMERIEUX CASE STUDY

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Presentation Outline

- 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
How do we measure Sterility?

- **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
Defining the variables (Steam sterilization)

- **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 to 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 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 121.1°C. The resulting $F_o$ was found to be 10.0.

What is the SAL?

Log SAL = \(-\frac{F}{D}\) + Log N

Log SAL = \(-\frac{10.0}{1.0}\) + 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.
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?

$$\text{Log SAL} = \frac{-F}{D} + \text{Log N}$$

$$\text{Log SAL} = \frac{-10.0}{1.0} + \text{Log (10^4)}$$

$$\text{Log SAL} = -10.0 + 4$$

$$\text{Log SAL} = -6.0$$

$$\text{SAL} = 10^{-6}$$

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
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.
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.
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.
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 = (-12.0/1.0) + Log ($10^2$)
Log SAL = -12.0 + 2
Log SAL = -10.0
SAL = $10^{-10}$
PNSU: 1 in 10 billion

Log SAL = (-F / D) + Log N
Log SAL = (-12.0/1.5) + Log ($10^2$)
Log SAL = -8.0 + 2
Log SAL = -6.0
SAL = $10^{-6}$
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?