Kurt McCauley & Stephanie Haag

Mesa Labs

Work Experience

- Product and Application Engineer at Mesa Labs
- Microbiologist and Production Manager at Mesa Labs
- Electrician's Mate and Metrologist in US Navy

Education

- BS Microbiology- Montana State University

Hobbies

- Hiking, fishing, rafting, snowmobiling

Work Experience

- Research and Development Scientist at Mesa Labs
- Process Engineer at Micron Technology

Education

- BS Chemical Engineering- Montana State University
- BS Biological Engineering- Montana State University
- PhD Chemical Engineering- University of Idaho

Hobbies

- Traveling, hiking, kayaking, painting, and experimenting with new recipes

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Surrogate Spore Calibration

- Biological Indicators in the Medical Device and Pharmaceutical Industries
- Collaboration with Pepsi Co in the Development of a Liquid Hydrogen Peroxide BI and Resistometer

Calibration of devices

Calibration

» A comparison between a device under test and an established standard.

Established standards – NIST (National Institute for Standards and Technology)

» Time, temperature, pressure, mass, volume, length

» Example:

> The meter (m) is defined by taking the fixed numerical value of the speed of light in vacuum c to be 299,792,458 when expressed in the unit m s^{-1}, where the second is defined in terms of Δv_{Cs}.

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Calibration of devices containing microorganisms

- "There are no standard entities in nature be the man, monkey, or microorganism…not even bacterial spores"—Dr. Pflug
- How then can biological indicators containing viable spores be "calibrated"?

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Surrogate microorganisms

- Harmless microorganisms which have equal or greater resistance to a sterilization process than the naturally occurring bioburden
- Used as substitutes for the bioburden during process development and validation of sterilization and decontamination processes
- Limited to several well characterized spore forming species
- A component used in biological indicator systems

History of Surrogate Organisms

- Microorganisms are responsible for communicable diseases, infection and food spoilage
- Tyndall, Pasteur, Koch, and Lister were among the early pioneers in developing tools to study and combat problematic organisms
- Koch (1880s) successfully isolated spore-forming bacteria and recognized that if a sterilization process can inactivate hard-to-kill spores, then it will likely kill other forms of microorganisms

History of Surrogate Organisms

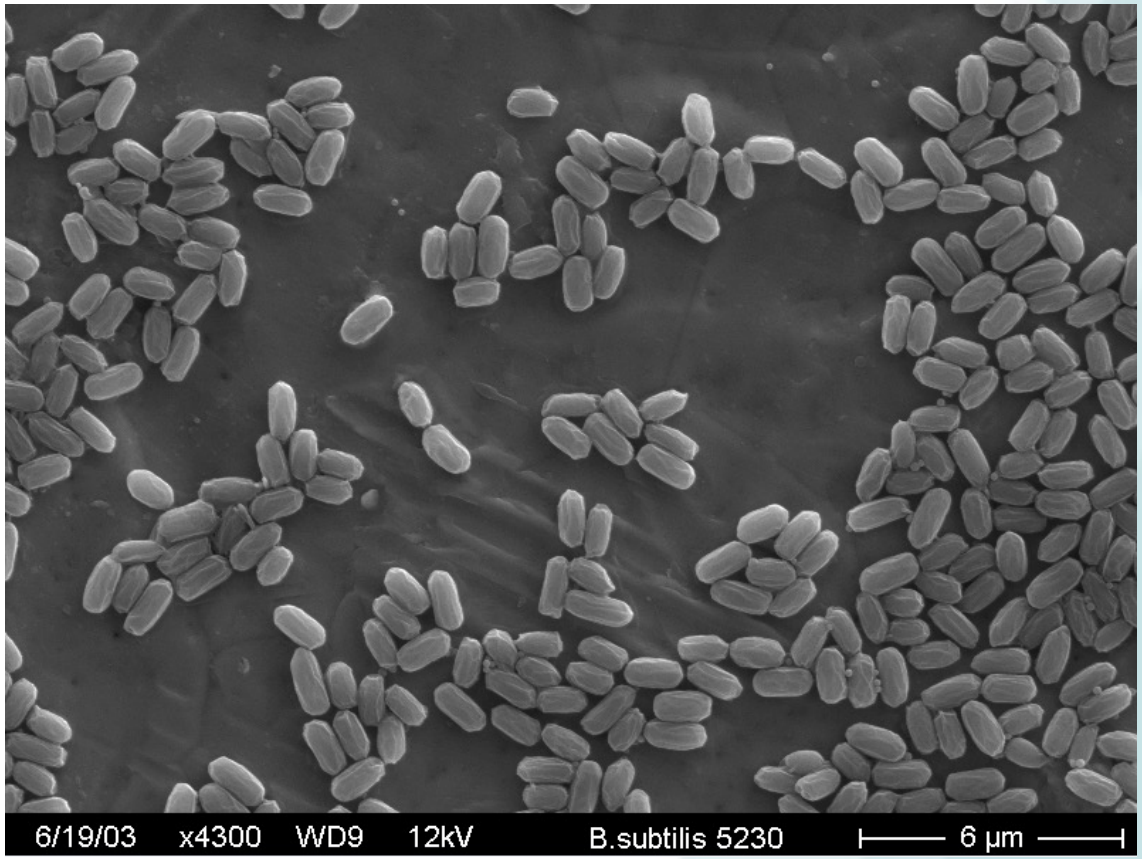
- Packaged surrogate organisms used to judge the success of an industrial steam sterilization processes (Kilmer, 1890s)
- *Geobacillus stearothermophilus* isolated from spoiled canned corn treated at 118 °C for 75 minutes (Donk, 1917).
- *G. stearothermophilus* spores and other spore formers are widely used as surrogates for multiple sterilization/decontamination processes.

Surrogate Organisms

Microorganism	Modality
Geobacillus stearothermophilus	Thermal—moist heat Chemical—Hydrogen Peroxide, Peracetic Acid, Nitrogen Dioxide
Bacillus atrophaeus	Thermal—dry heat Chemical—Ethylene Oxide, Hydrogen Peroxide, Peracetic Acid
Clostridium sporogenes	Thermal—moist heat (lower lethality cycles)
Bacillus subtilis “5230”	

Scanning Electron Micrograph of Bacterial Spores

G. stearothermophilus (left) and *B. subtilis* (right)



Biological indicator manufacturing controls and calibration

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Biological Indicators

- Test system containing viable microorganisms providing a defined resistance to a specified sterilization process
- The only sterilization monitoring device that provides a direct measure of process lethality
- **“Calibration” occurs throughout entire manufacturing process**
- Tight control of manufacturing components/processes essential for reliable performance

Biological Indicators

Purpose of Manufacturing and Calibration Control

- Provide meaningful tool for process lethality measurement
- Minimize BI performance variation within a lot and from lot to lot
- Provide consistent BI lot performance over time
- Provide consistent and repeatable performance under defined conditions

- Survival/kill times
- Spore Count
- D-value
- z-value

- Steam 121 °C
- Dry heat 160 °C
- Hydrogen Peroxide Vapor 2.3 mg/l, 35 °C, 30-50% RH
- Ethylene Oxide 600mg/l, 54 °C, 50-70% RH

Biological Indicator Standards

AAMI/ANSI/ISO - Sterilization of health care products –Biological Indicators

- 11138-1 – General requirements
- 11138-2 – Ethylene Oxide
- 11138-3 – Moist Heat
- 11138-4 – Dry Heat
- 11138-5 – Low-temperature steam and formaldehyde
- 11138-7 – Guidance for the selection, use and interpretation of results
- 11138-8 – Method for validation of a reduced incubation time
- 18472 – Biological and chemical indicators – test equipment

United States Pharmacopeia

- 1229.5 – Biological Indicators for sterilization
- 55 – Biological Indicators – resistance performance tests

Manufacturing Controls

- The BI is a system comprised of several parts and each has an influence on BI performance
- BI Components
  - Surrogate organism
  - Carrier material
  - Primary packaging
  - Growth media

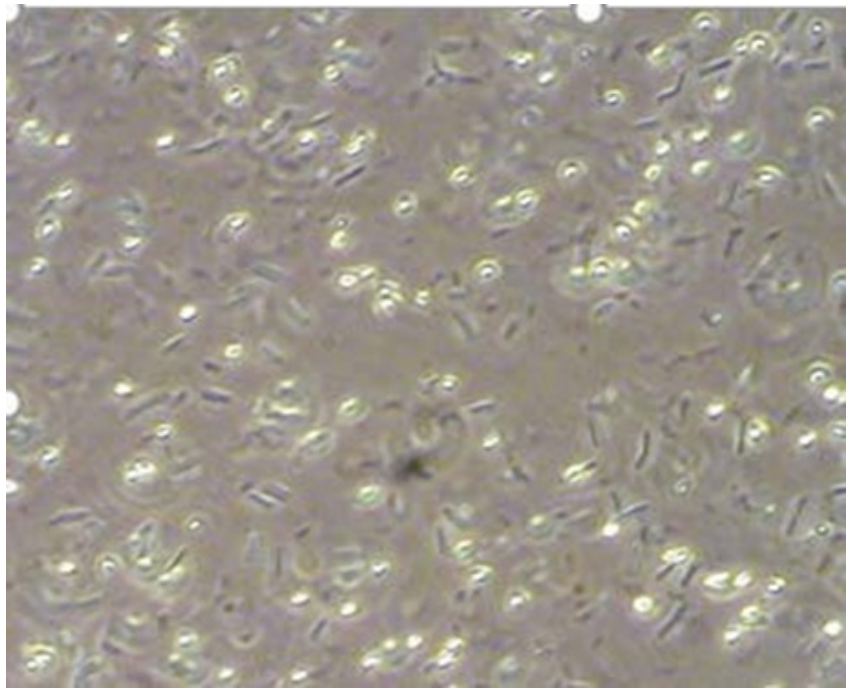
Manufacturing Controls

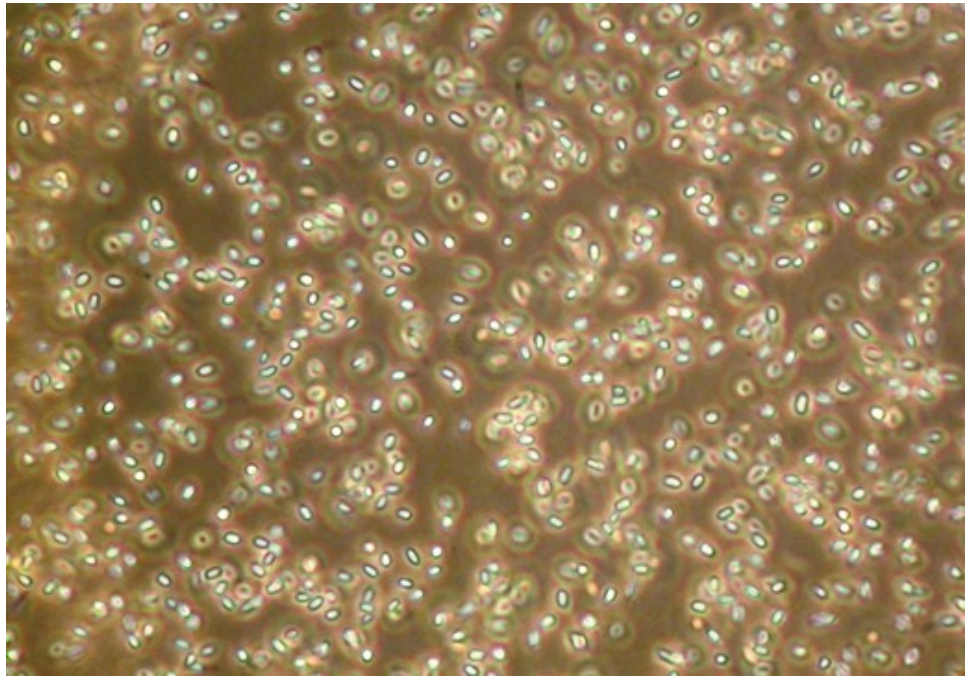
Surrogate organisms

- Must demonstrate higher resistance than the target organism in the product bioburden
- Must be traceable to a recognized culture collection
- Must not be pathogenic or require special handling practices or containment equipment
- Must be a pure culture and ideally free of clumps and debris
- Ideally is produced in sufficient crop sizes to allow the manufacture of large BI lots

Manufacturing Controls

Spores after harvesting (left) and after cleaning (right)





Phase contrast microscope X1000

Manufacturing Controls

Carrier Material

- Must not react with or degrade sterilizing agent
- Must not impact spore viability
- Must not retain residual sterilizing agent

Paper strips

Stainless steel discs or ribbons

Liquid Media

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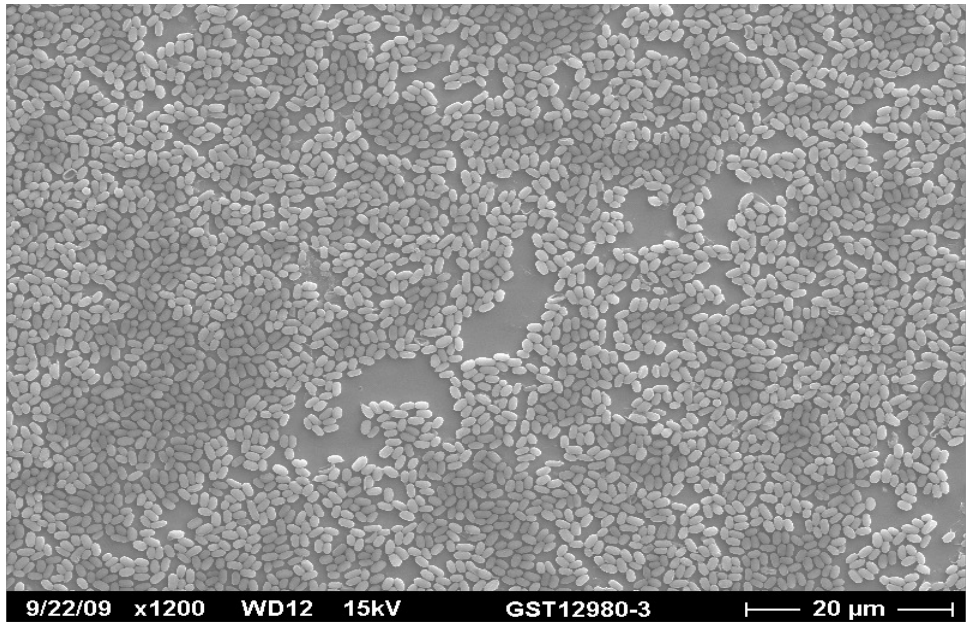
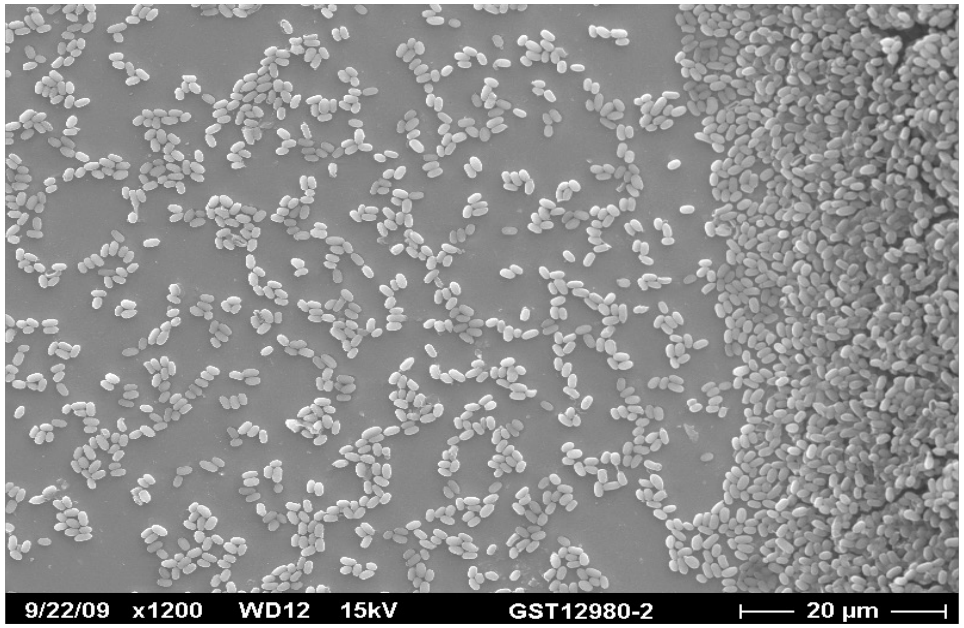
Manufacturing Controls

Spore presentation on solid carrier, monolayer and edge effect





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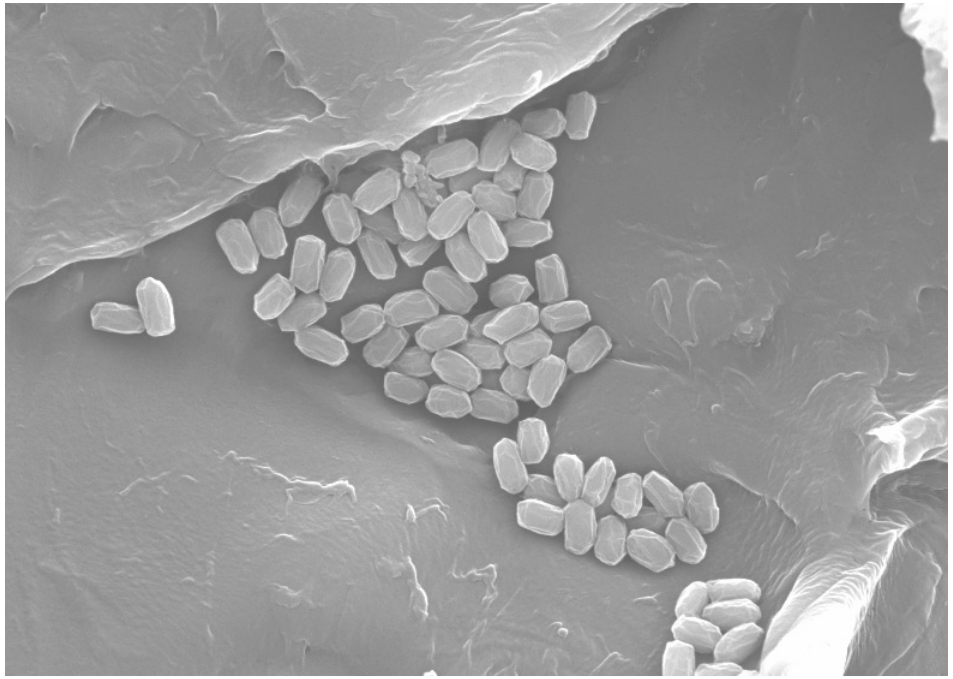
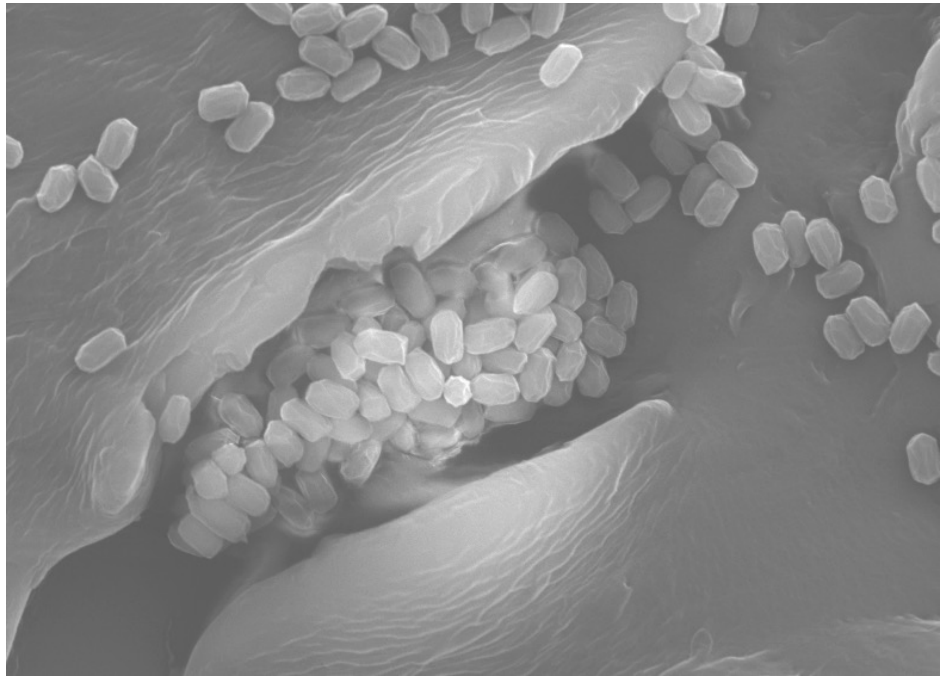
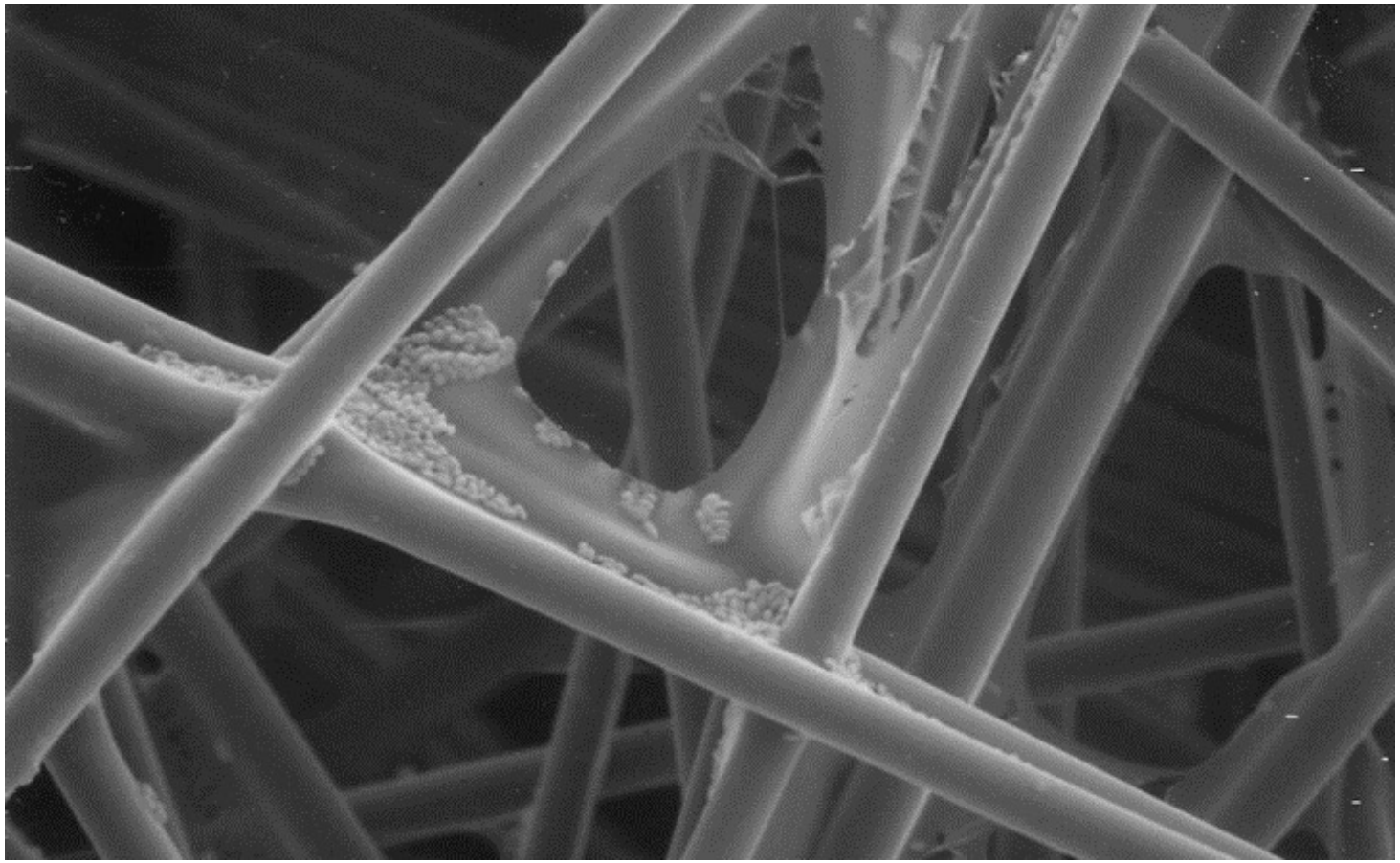
B. subtilis inoculated on juice box





Magnification X5000

G. stearothermophilus spores on glass fiber



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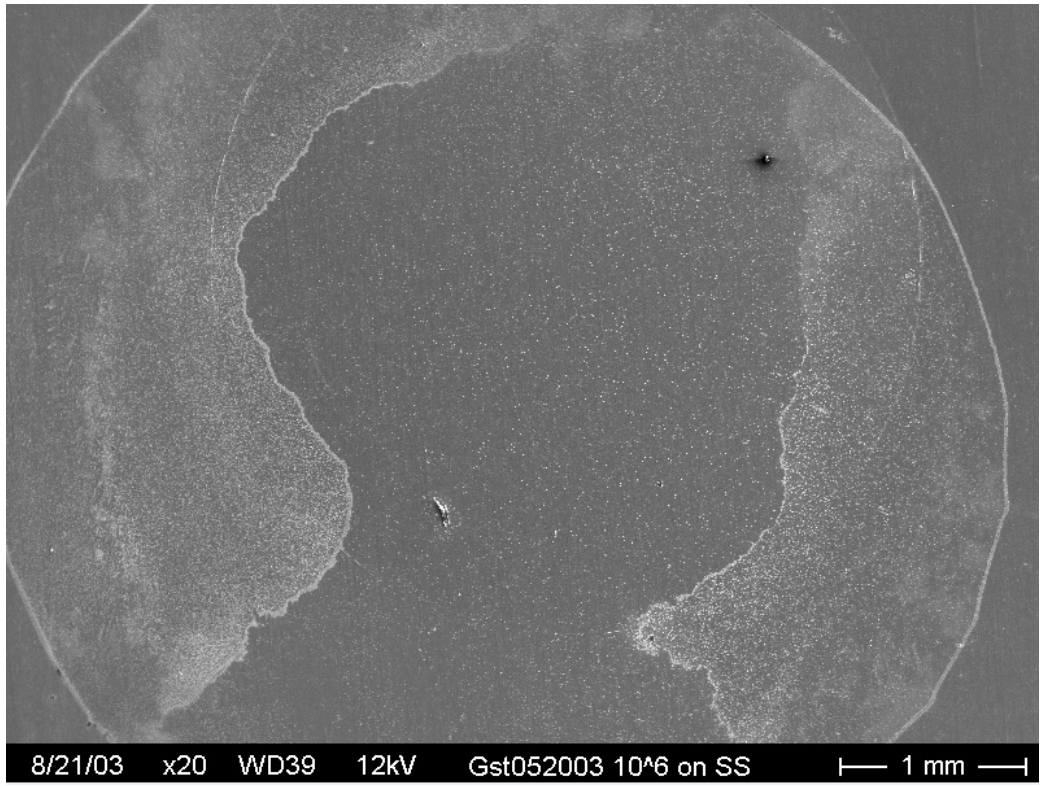
Manufacturing Controls

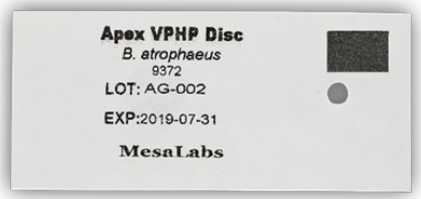
Image indicates poor cleaning stainless steel carrier



Manufacturing Controls

Primary Packaging

- Must allow the penetration of sterilizing agent
- Must not react with or degrade sterilizing agent
- Must not impact spore viability
- Must not retain residual sterilizing agent



Tyvek envelope

Glassine Paper

Glass ampoule

Manufacturing Controls

Growth Media

- Must be able to recover low numbers of injured organisms
- Must give obvious indication of results

SCBI Media

SCBI media

Tubed media

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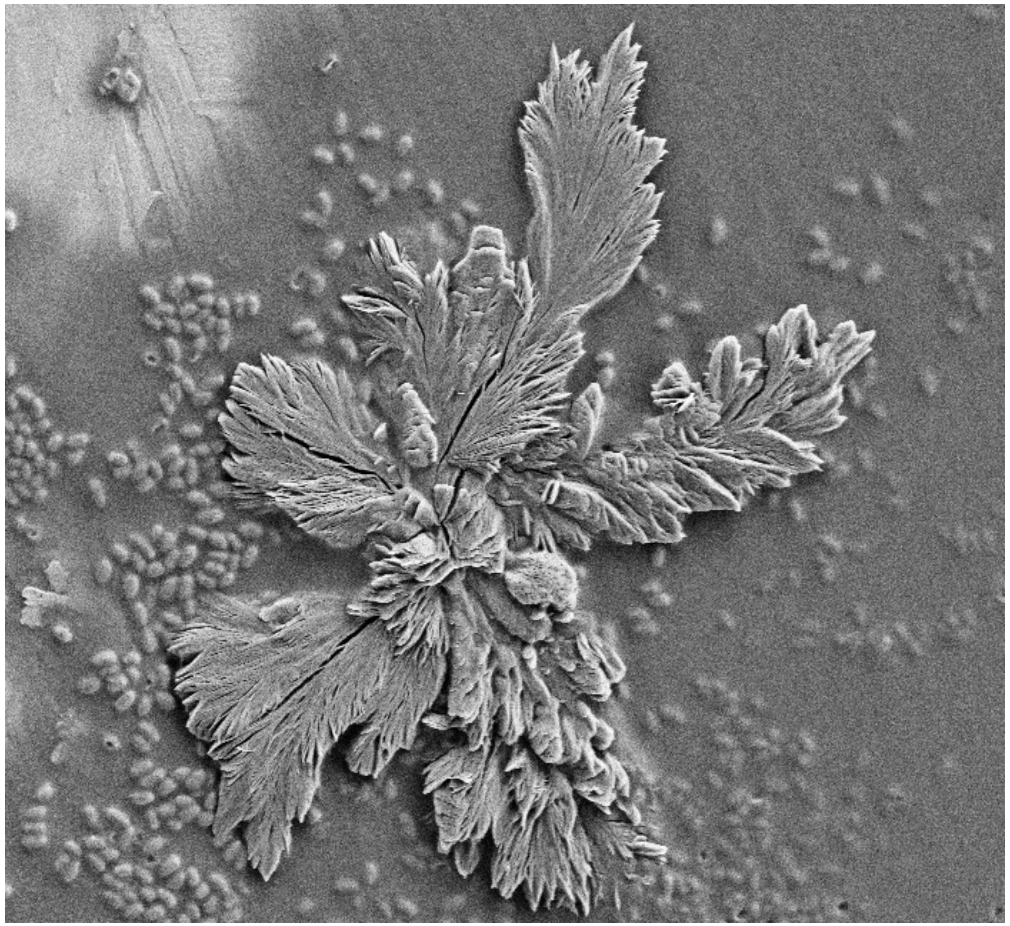
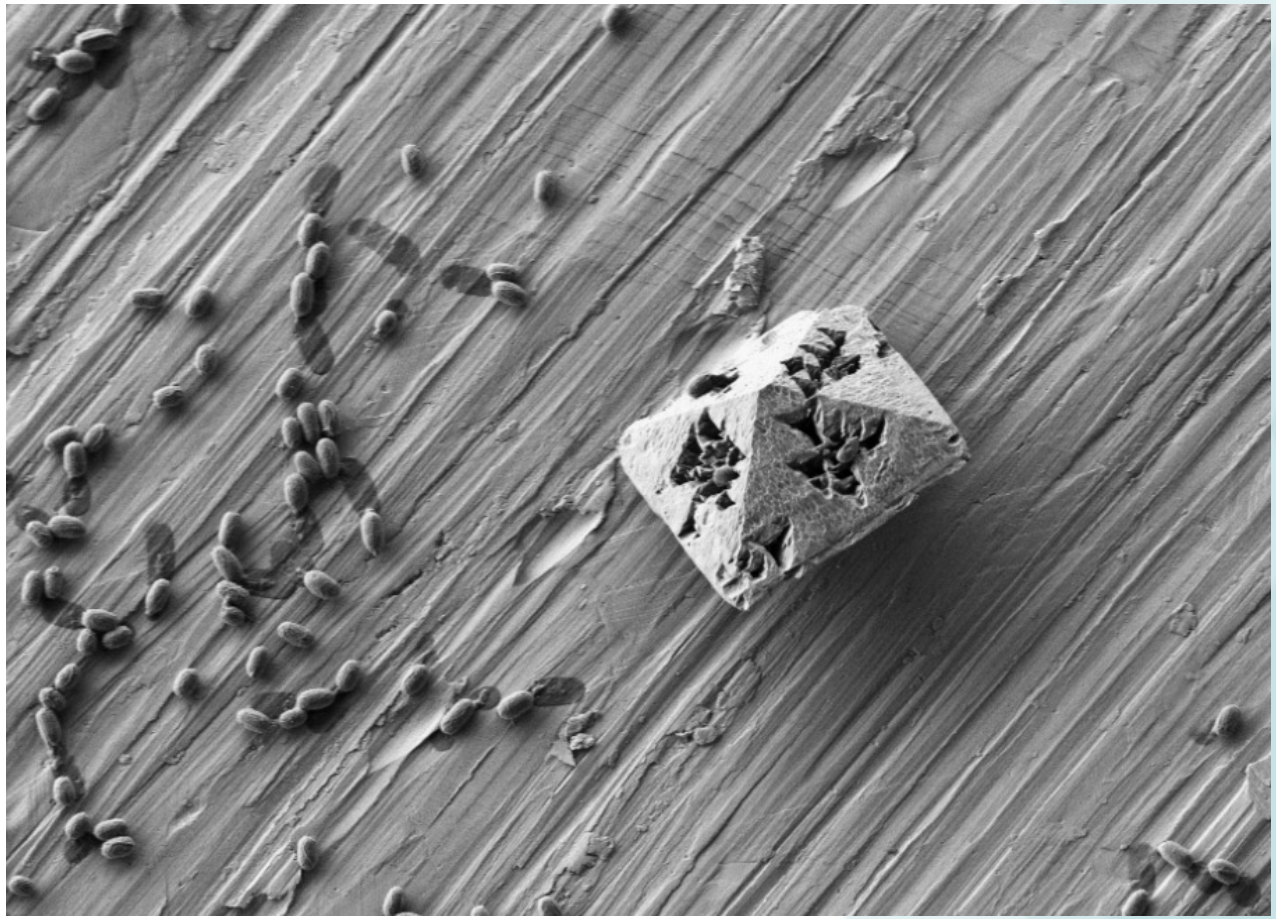
Beautiful Examples of Poor Manufacturing Controls





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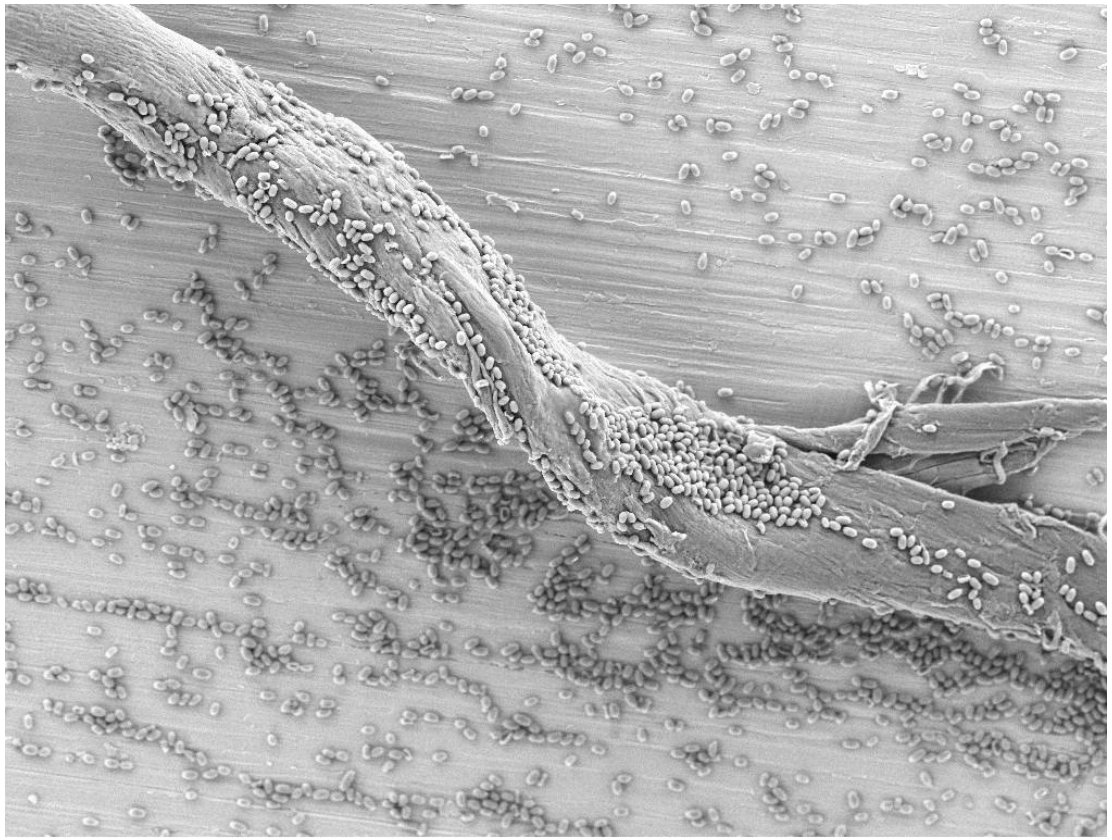
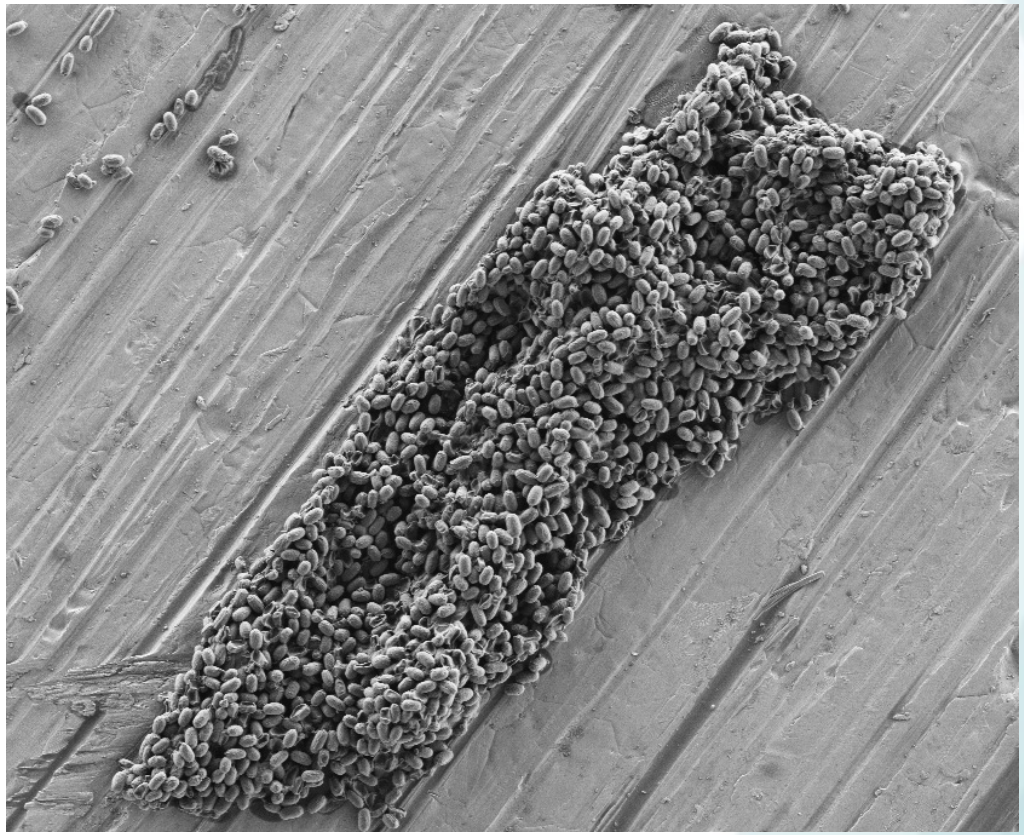
Foreign material associated with spores on stainless carrier





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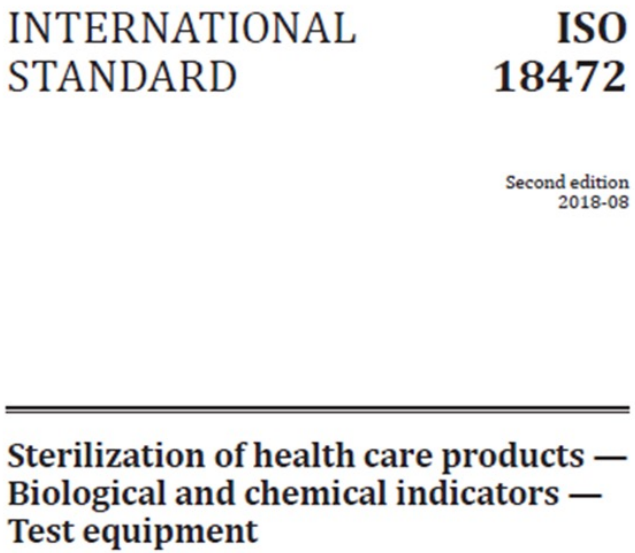
Resistance Testing

Conducted in Resistometer


INTERNATIONAL STANDARD

ISO 18472

Second edition
2018-08

Sterilization of health care products — Biological and chemical indicators — Test equipment

"Resistometers constitute test equipment designed to create precise and repeatable sterilizing environments…"

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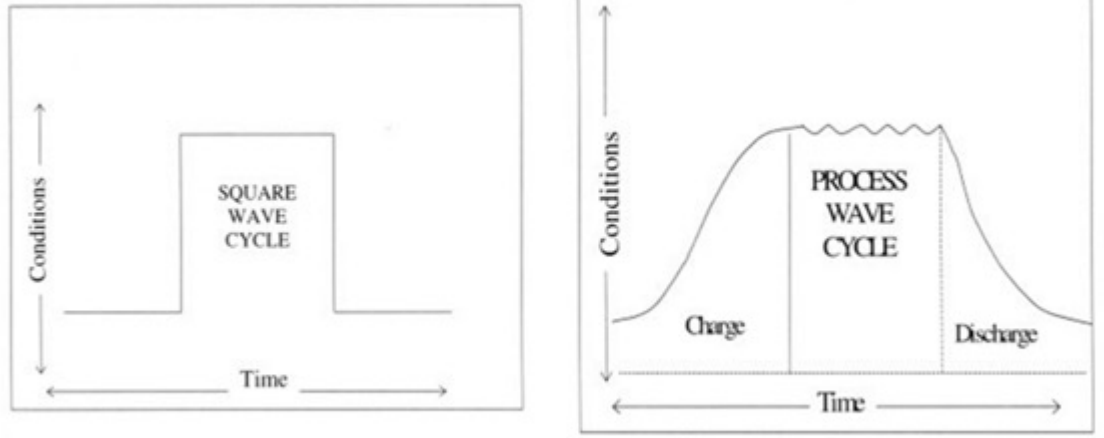
Resistometer



Resistometer square wave vs process cycle

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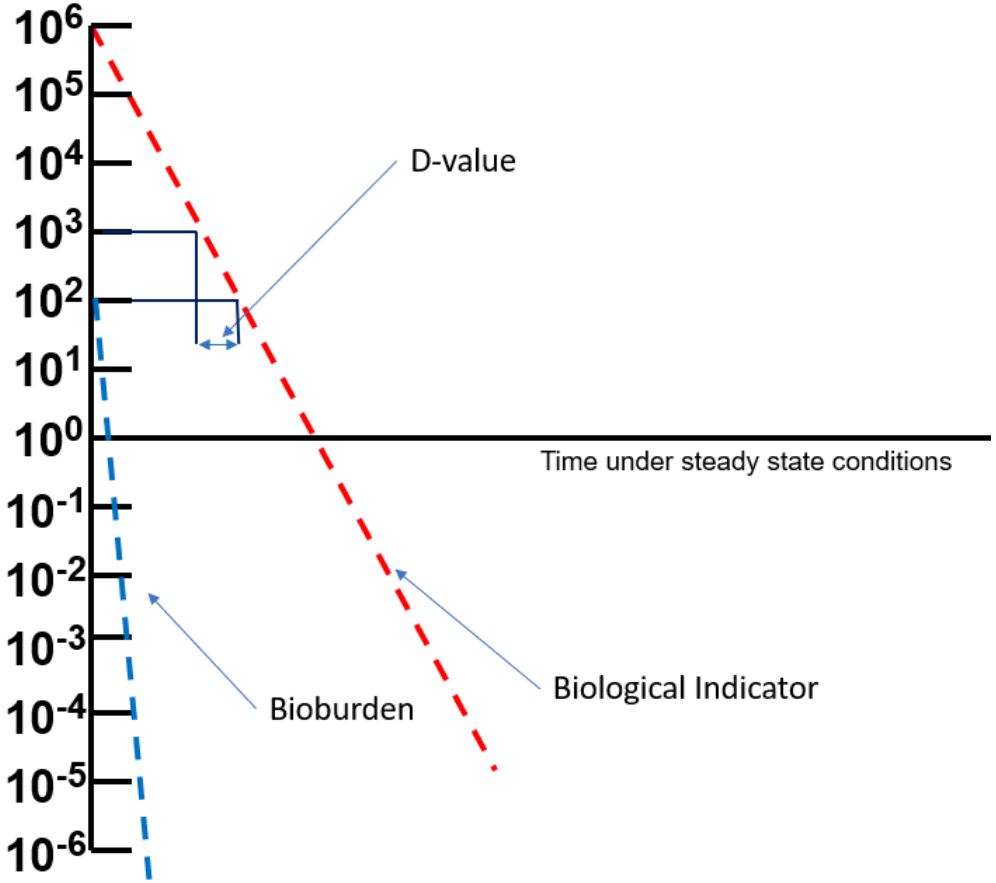
Methods of Resistance Testing

- Survivor Curve (direct enumeration) method used to demonstrate log linear kill kinetics
  - The r^2 for the linearity of the curve shall not be less than 0.8
  - **Key characteristic for BI calibration**

- Fraction Negative methods targets end point where both growth positive and growth negative results are achieved with replicate BIs. Methods for D-value calculation:
  - Stumbo Murphy Cochran
  - Holcolmb Spearman Karber
  - Limited Holcolmb Spearman Karber
  - Maximum Likelihood

Survivor Curve demonstrating log linear inactivation



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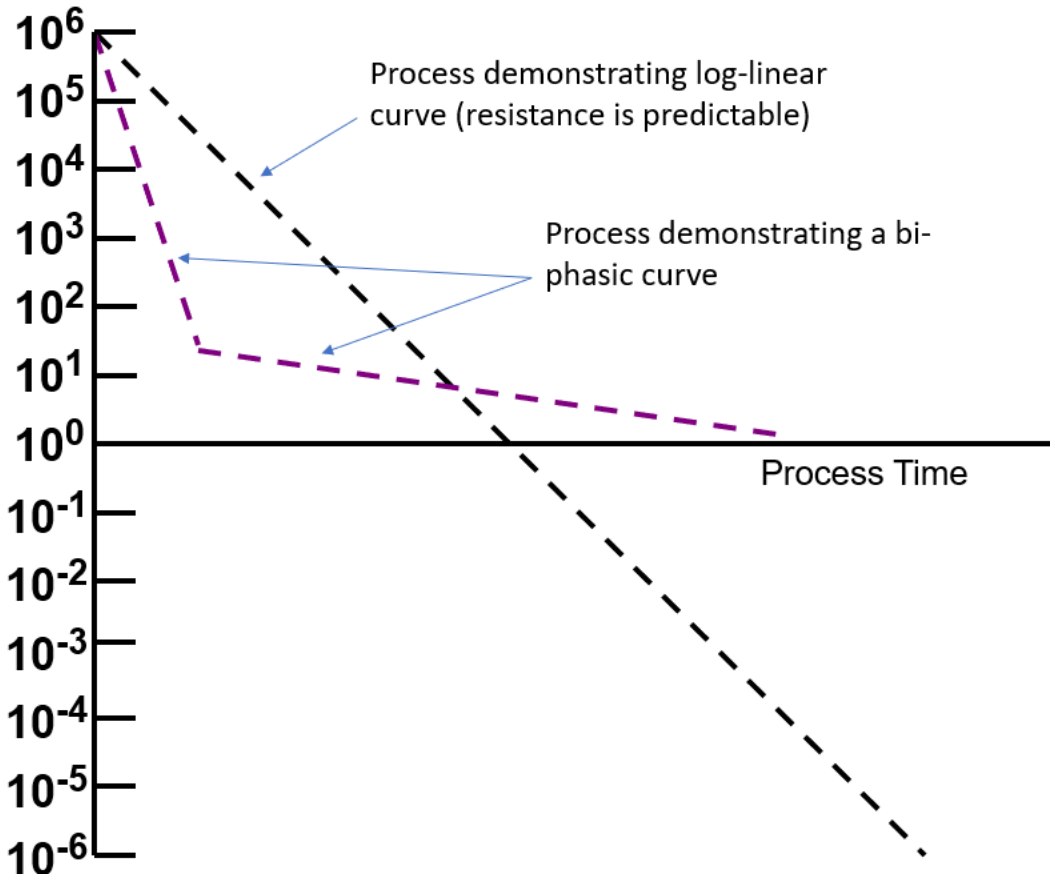
Survivor Curves – log linear vs biphasic



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BI Labeling

Certificate of Analysis

Apex Biological Indicator for
Gaseous Hydrogen Peroxide
INDUSTRIAL USE ONLY

Reorder #: HMV-091

Geobacillus stearothermophilus 12980[1]

Stainless Steel Biological Indicator used for Gaseous Hydrogen Peroxide.

Culture: Incubate at 55-60C for 7 days. The recommended growth medium is Soybean Casein Digest Medium (SCDM), Tryptic Soy Broth (TSB) or Mesa Releasat Medium (PM/100).

Storage: 2 - 8°C; less than 50% RH; move to ambient conditions ≥ 1 hour before use.

Disposal: Treat as non-pathogenic material and sterilize (steam, EtO, etc) or incinerate before discarding.

Shipping conditions: Ambient temperatures; cold pack and desiccant may be used to moderate conditions during shipping.

Lot #: **AH-182** Manufacture Date: 2023-01-25

Expiration: 2023-10-31

Mean Population: 2.5×10^6 CFU per stainless steel carrier [2]

Resistance Characteristics:

D-value [3]: 0.7 minutes in 2.0 mg/L gaseous H_2O_2

Units are manufactured in compliance with Mesa Labs, Bozeman Manufacturing Facility's quality standards and ISO 11138 guidelines and all appropriate subsections.

[1] Culture is traceable to a recognized culture collection identified in USP and ISO 11138.
[2] Heat shock population determined at 95-100°C for 15 minutes.
[3] Resistance calculated using Fraction Negative method. The D-value is reproducible only when exposed and cultured under the exact conditions used to obtain results reported here.

Certified by: _____ 07FEB2023
Quality Representative Date

HMV-091
BIOLOGICAL INDICATOR

INSTRUCTIONS FOR USE

PROCESS EVALUATION:

1. Place biological indicators in locations previously determined to be the most difficult to sterilize. Areas experiencing minimal gas flow or poor gas distribution include enclosure corners, areas in and around equipment, and locations among disposable materials to be used in the enclosure. Note that the inoculated side of the carrier faces the printed label on the Tyvek pouch, therefore the printed side should face outward during a process cycle.
2. Validation and mapping processes generally require multiple indicators at numerous sites in an enclosure.
3. Conduct the sterilization and aeration cycle.
4. Remove the indicators and deliver them, plus one or more unexposed control indicators, to the laboratory for sterility testing. Culturing of exposed indicators should be conducted as soon as possible following removal from the enclosure being tested.

CULTURING PROCEDURES:

1. Culture in a laminar flow hood. Observe strict aseptic technique at all times. Minimally, sterile gloves should be worn. Include donning hoods, masks, and gowns as appropriate for the facility and circumstances.
2. Aseptically open the Tyvek pouch by cutting with sterile scissors or peeling apart at the end with the package offset.
3. Using sterile forceps, withdraw the carrier and place in a tube containing sterile Soybean Casein Digest Medium (SCDM), Tryptic Soy Broth (TSB) or Mesa Releasat Medium (PM/100).
4. Aseptically culture the control carrier(s) last.
5. Select one or more tubes of the same lot of culture medium to serve as negative controls.
6. Incubate test and control tubes for 7 days at 55-60°C. Observe daily for evidence of growth (turbidity).

INTERPRETATION:

1. Turbidity:
   a. For test indicators, turbidity suggests that the sterilization was incomplete and that at least one spore survived the process.
   b. For positive control indicators, turbidity indicates that viable spores were present and capable of outgrowth in the culture medium used.
   c. In negative control tubes, turbidity indicates that viable organisms may have been present in the growth medium. Contact your supplier.
2. No turbidity:
   a. For test indicators, lack of turbidity indicates sterilization was complete and no spores survived the process.
   b. In negative control tubes, lack of turbidity indicates no other viable organisms were present in the culture medium.
   c. For positive control indicator, no turbidity suggests no viable organisms were present on the carrier or that the media may be inhibiting the outgrowth of the test organism. Contact your supplier.

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BI Development and Manufacturing Controls

Summary of BI “calibration”

» Occurs throughout the manufacturing process:

- Surrogate organism (spore former) selection
- Spore crop preparation (media recipe, growth temperature, etc.)
- Control of spore count
- Control of components, manufacturing and testing processes
- Resistance testing (i.e., D-value, z-value)

» BI performance must meet criteria per appropriate standard(s)

- Example: Moist heat $D_{(121)}$-value ≥ 1.5 min, Spore count ≥ 1.0×10^5

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Development of a biological indicator and resistometer for use in decontamination processes using liquid hydrogen peroxide

Collaboration Project with Pepsi Co.

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Project Objectives

- Design BI based on needs of the industry
- Design and build resistometer to evaluate BI performance

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BI Design

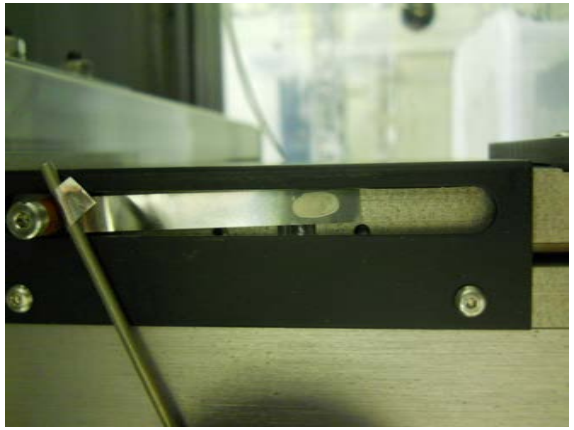
Performance characteristics

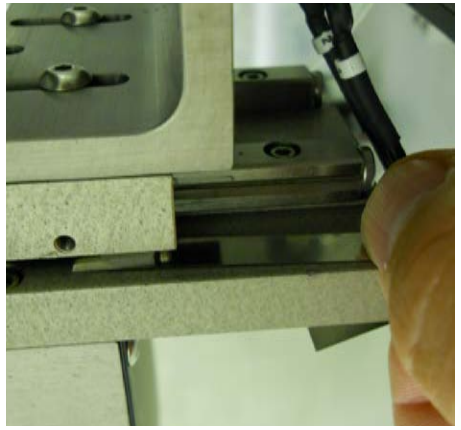
- Must have greater or equal resistance to target organisms
- Spore presentation on carrier must be as uniform as possible
- Carrier must be flexible to allow spore delivery into challenging locations
- Inactivation curve should be log-linear
- Must be user friendly
- Interpretation of results must be clear and readily apparent

BI Design

BI Components

- Surrogate organism: *B. atrophaeus* (10^5 - 10^6 spores/unit)
  Recognized in standards as an appropriate surrogate organism for chemical process
  Is more resistant than target organism (*C. botulinum*) to hydrogen peroxide treatment (Toledo 1973)
- Carrier material: flexible stainless-steel ribbon (6.5mm x 70mm)
- Primary packaging: none
- Growth media: optimization in progress





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Toledo RT, Escher FE, Ayres JC. Sporicidal properties of hydrogen peroxide against food spoilage organisms. Appl Microbiol. 1973 Oct;26(4):592-7. doi: 10.1128/am.26.4.592-597.1973. PMID: 16349974; PMCID: PMC379855.

Resistometer Design

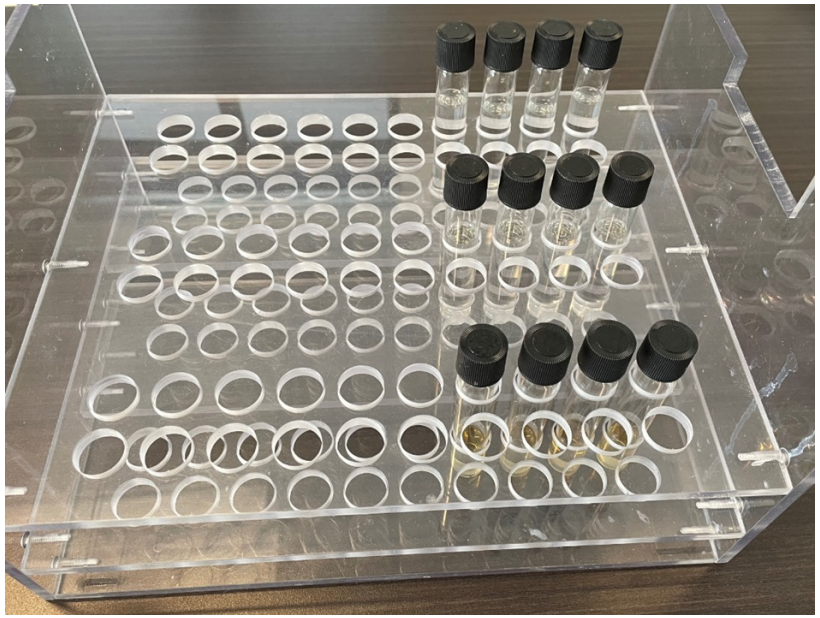
Performance characteristics

- Must deliver square wave cycles under stated conditions
- Must be flexible to allow testing at multiple conditions based on food aseptic industry standards
  - Hydrogen peroxide concentration 35%
  - Temperature range 140°F-180°F
- Must allow for the evaluation of no less than 20 BI units/test

Manual Testing Equipment

Can use for survivor curve or fraction negative testing

Water bath heated to 160°F



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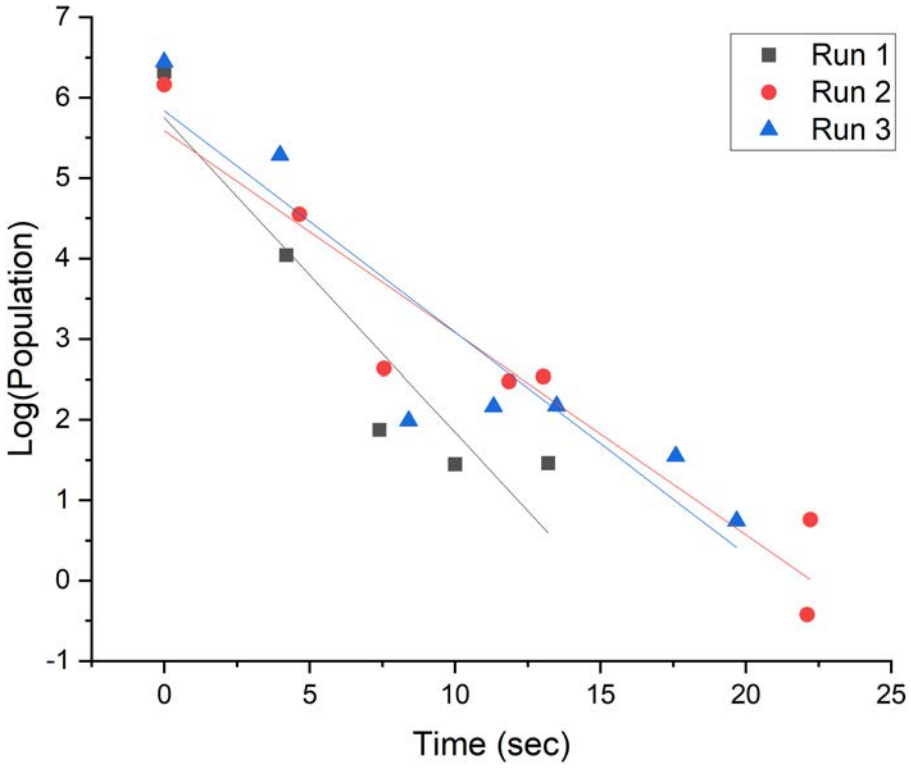
Initial Biological Testing

Survivor Curves

- Initial manual dipping and timing
- Variation run to run



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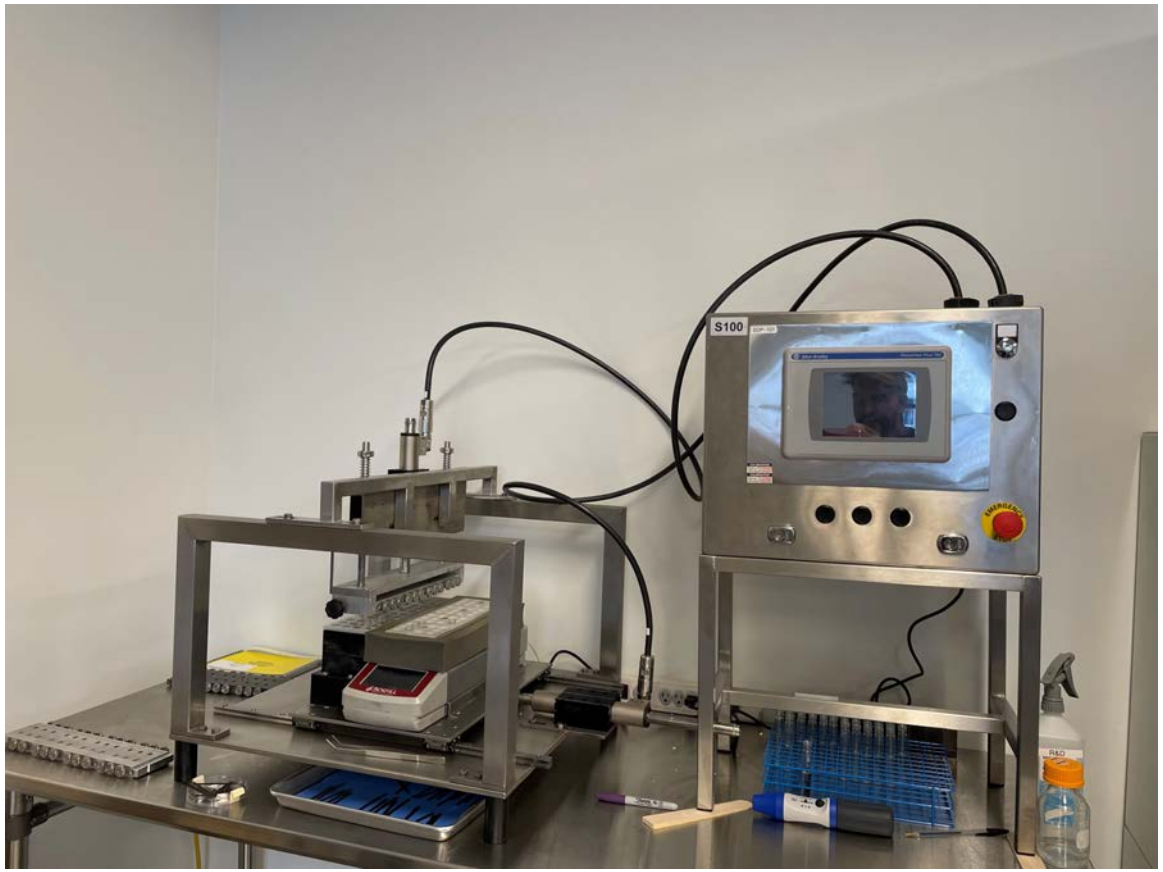
Automate Testing- Robotic Arm

Can use for survivor curve or fraction negative testing



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Automate Testing- Robotic Arm

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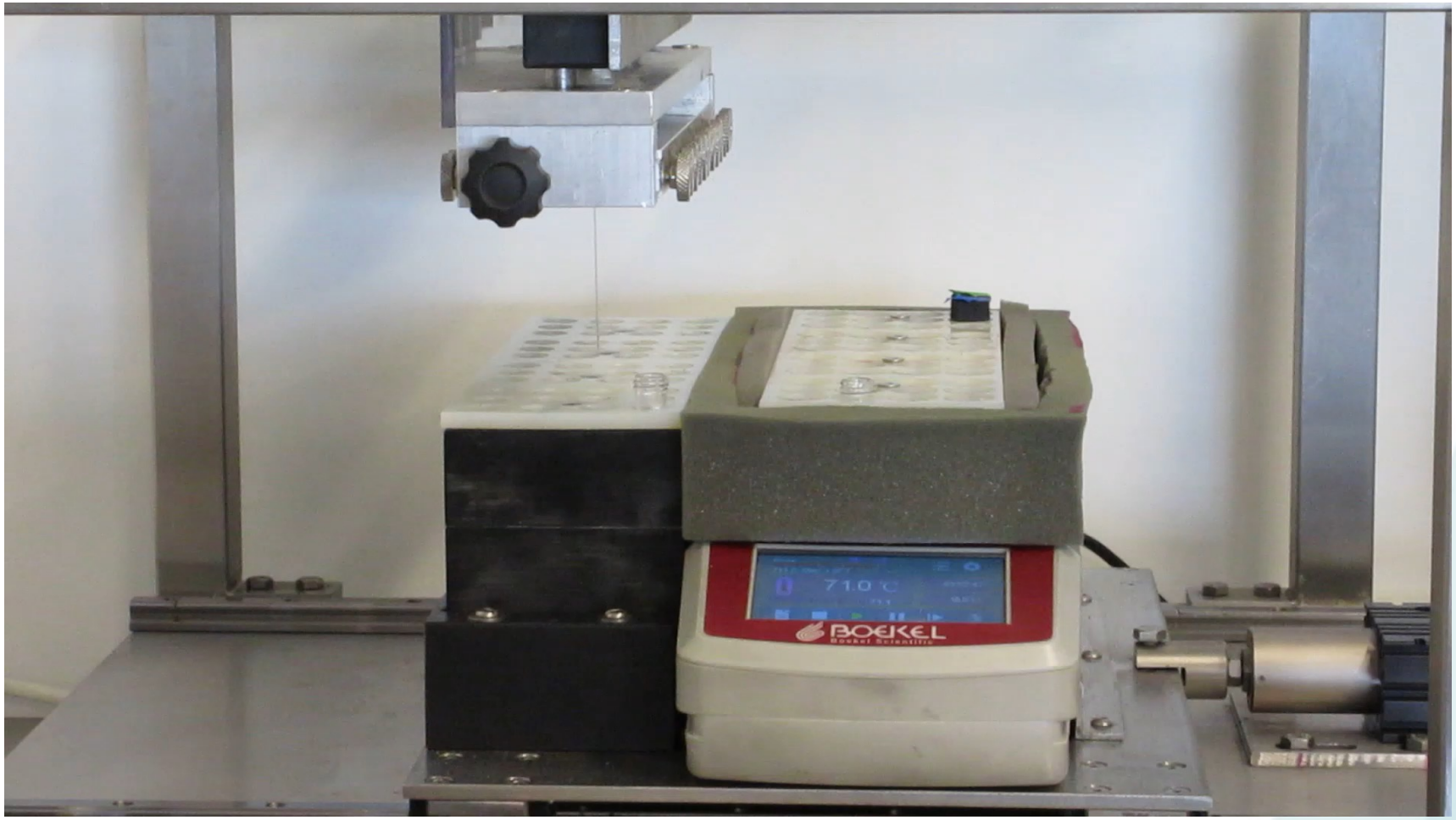

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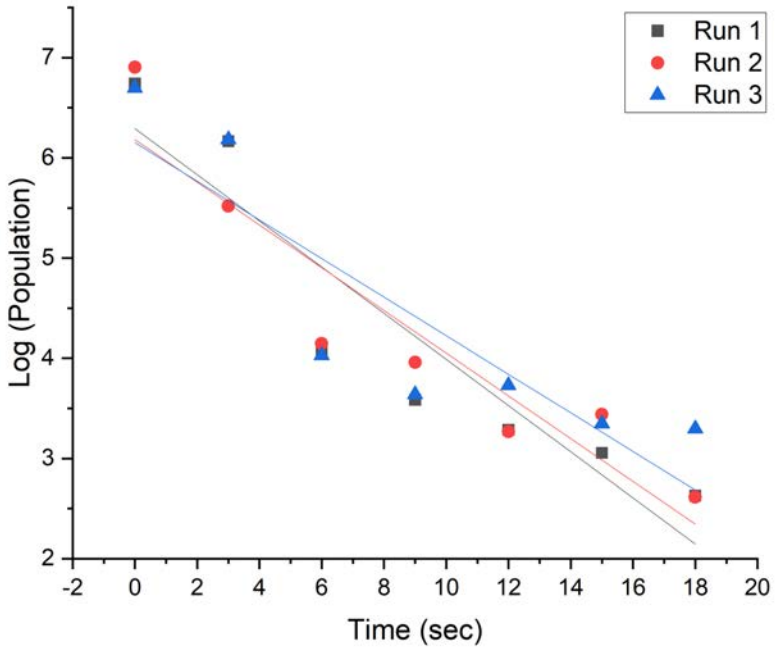
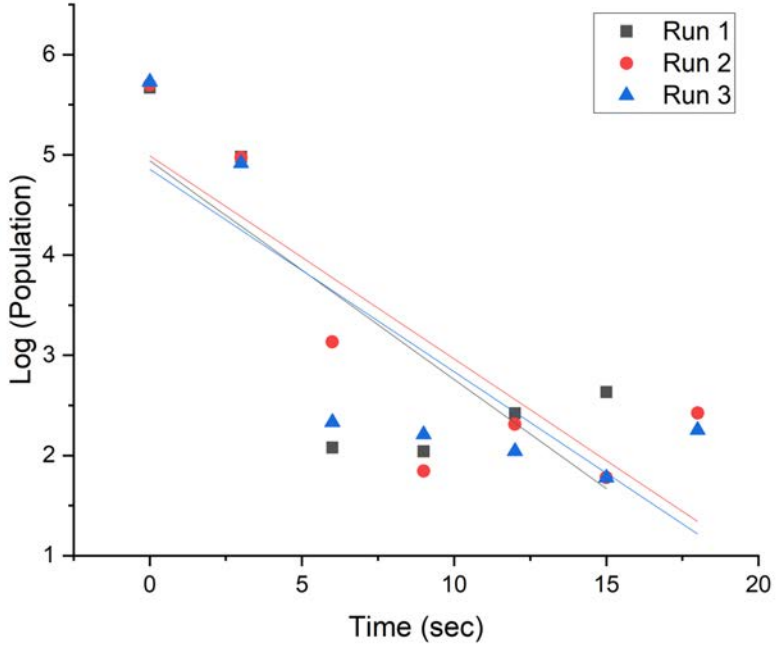
Biological Testing

Initial survivor curves-automated testing





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Biological Testing

Understanding Kill kinetics

- Observed bi-phasic curve
  - Reality vs artifact of test system or BI
- Lower population (E5)
- Larger inoculation volume
- Analyze lethality in peroxide vs residual peroxide on ribbon
- Reprogrammed equipment to minimize travel time
- Reprogrammed arm to help remove bubble formation

Biological Testing

Kill kinetics

- Bubbles form on surface during exposure to H_2O_2
- Shaking does not remove bubbles efficiently
- Quick removal from peroxide appears to remove bubbles

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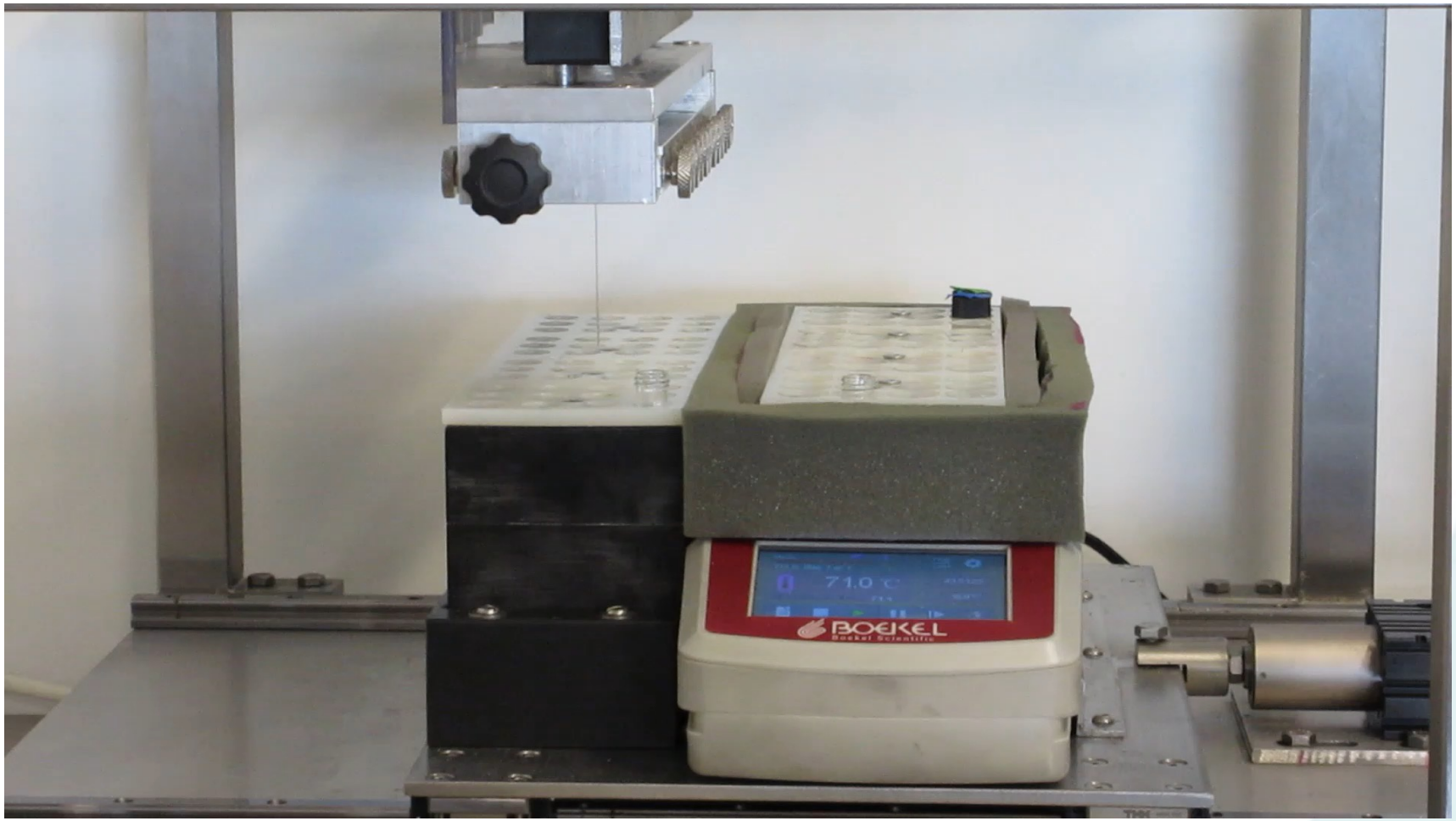
Automate Testing- Robotic Arm

Reprogrammed



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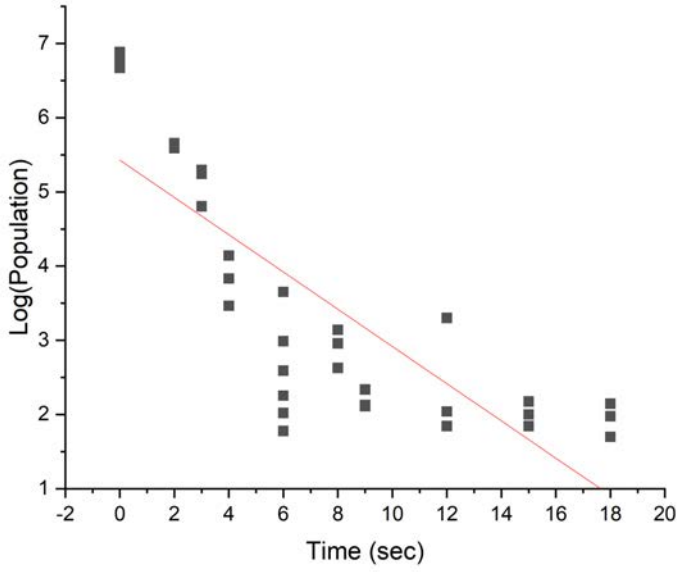
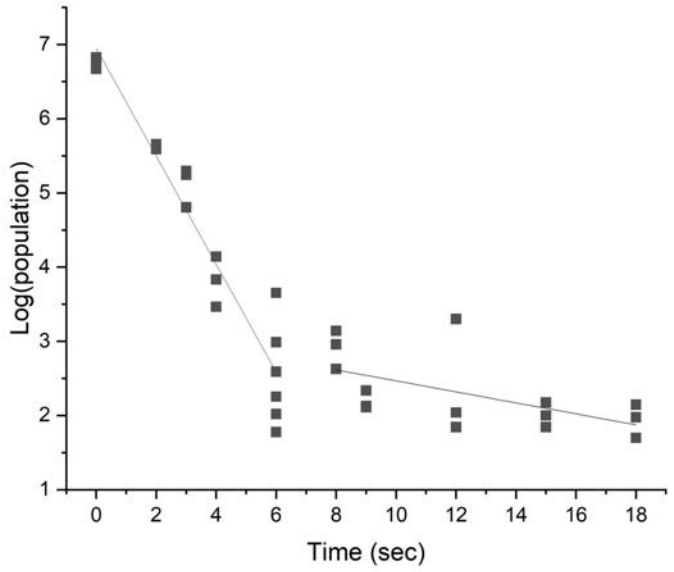
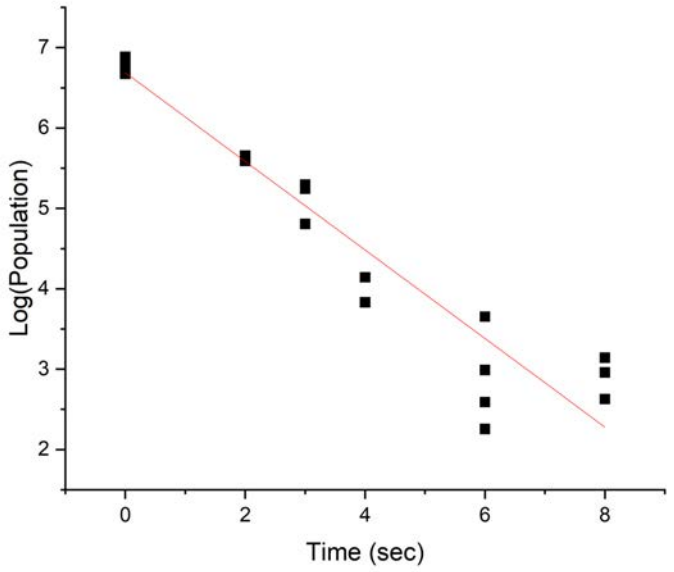
Biological Testing

Survivor curves-automated testing

- All Data
  - D-value: 3.98 sec
  - R^2: 0.71
- Only counts 30-300
  - D-value: 1.81 sec
  - R^2: 0.92







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Biological Testing

Neutralization

5 sec dwell

	0 sec exposure	3 sec exposure
Water	6.48E6	5.5E1
Catalase	5.88E6	1.34E4
TSB	5.85E6	Too low
DTB	5.4E6	Too low

20 sec dwell

	0 sec exposure	3 sec exposure
Water	5.58E6	4.05E2
Catalase	6.75E6	1.05E4
TSB	5.73E6	Too low
DTB	4.95E6	6.5E1

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Biological Testing

Next Steps

- Neutralization for square wave cycle
- Media Optimization
- Fraction Negative Testing

Thank You!

Please contact your Mesa Representative or bi-support@mesalabs.com for any further information on Mesa Products.

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Protecting the Vulnerable™

Supplementary Information

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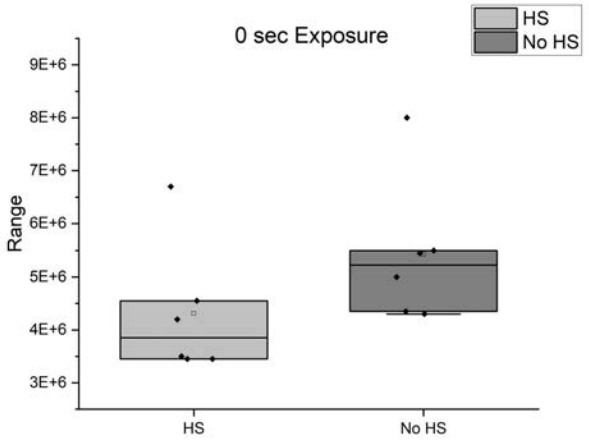
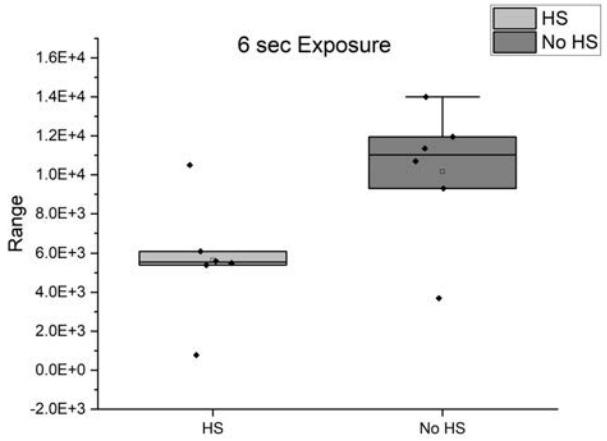
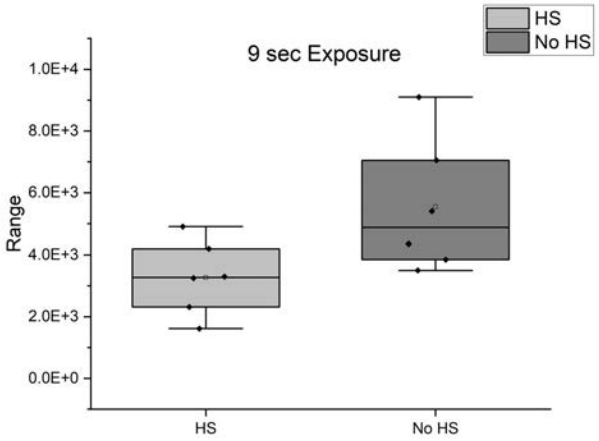
Biological Testing

Impact of Heat Shock

- Unexposed samples
  - No statistical difference with and without heat shock
- Partial kill cycles (6 sec and 9 sec)
  - Heat shocking results in population statistically lower than without heat shock
- Conclusion
  - Decision moving forward is to not use heat shock for any further tests







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