USE OF MICROBIAL SURROGATES IN VALIDATION OF LOW-ACID ASEPTIC FILLERS (LAF)

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AGENDA

- What is Aseptic Processing and Packaging?
- Regulatory Requirements – Low Acid Canned Foods (LACF)
- The concept of Validation
  - Why? When? How?
  - Need for microbial surrogates
- Selection and Characterization of Surrogate
  - Identifying the microbial target
  - Selecting the surrogate
  - Case study: Peracetic Acid (PAA) Based Sterilants

FIGURE 2. Validation and verification of the food safety management system

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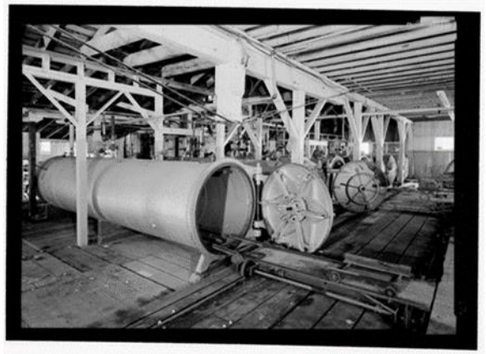
What is Aseptic Processing?

Aseptic processing is a form of canning

Conventional Canning:

Product

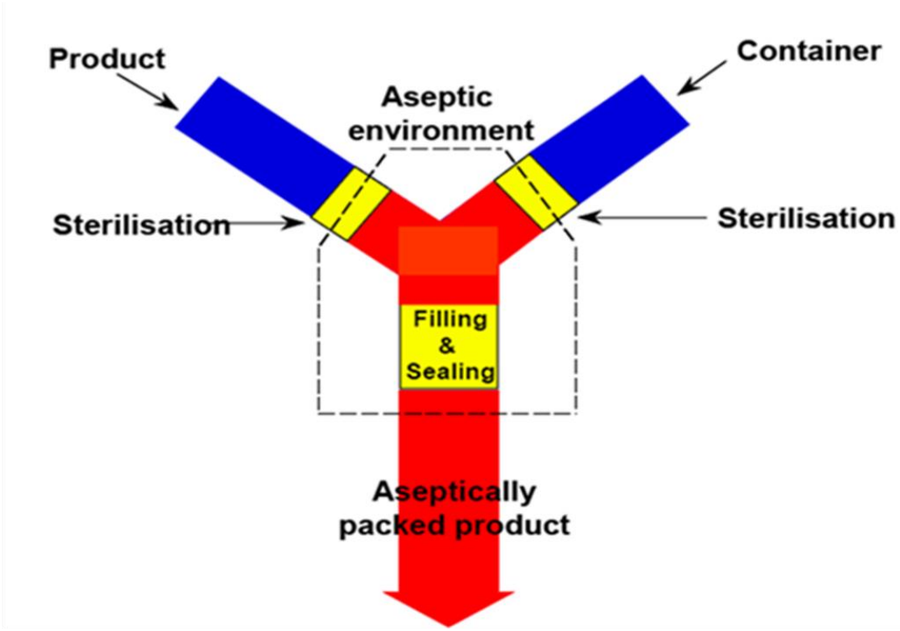
+ Retort Heating



= Shelf-stable product

Aseptic:

Sterile product + Sterile Container + Sterile Environment = Shelf-Stable Product



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BASIC PREMISES – ASEPTIC LINE

Achieve Sterility

- Product
- Package
- Product Contact Surfaces
- Aseptic Zone
- Other Media (air, nitrogen, water)

} Environment

Maintain Sterility

- Product
- Product Contact Surfaces
- Aseptic Zone

Hermetic Seal

- Produce, Maintain and Verify Seal Integrity

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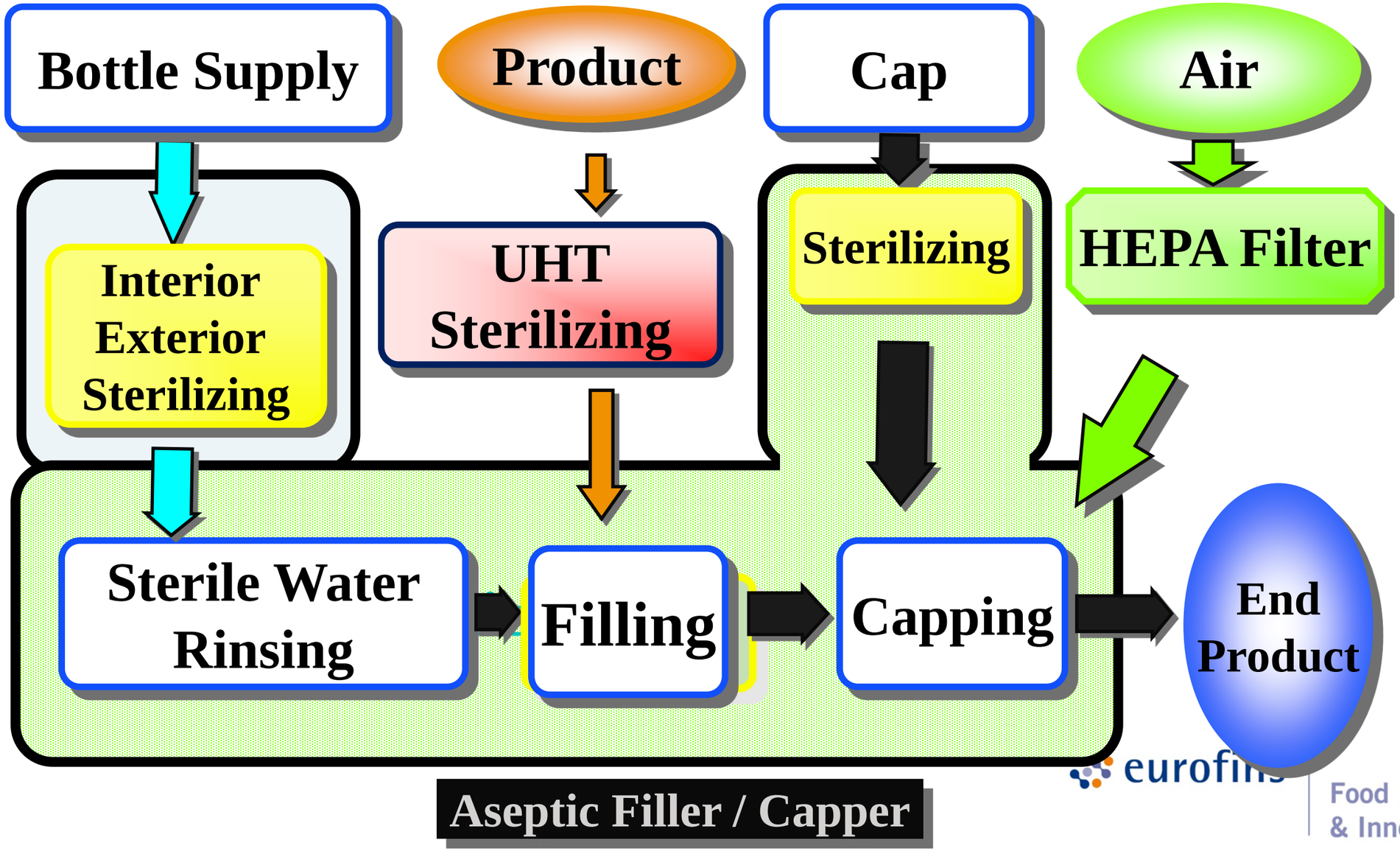
PET Bottle Aseptic Filling



Validation and FDA Filings of Aseptic Fillers

DEFINITIONS, REGULATIONS AND *CLOSTRIDIUM BOTULINUM*

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FDA Classification of Foods

Shelf-Stable (LACF)

Refrigerated, Acid and Low-moisture

- ***Low-acid foods (LACF)***: Any foods, other than alcoholic beverages, with a finished equilibrium pH greater than 4.6 and a water activity (a_w) greater than 0.85. (21CFR113.3n) *Example: Milk and non-dairy, plant-based "milk"*
- ***Acidified foods***: Low-acid foods to which acid(s) or acid foods are added…They have a_w greater than 0.85 and a finished equilibrium pH of 4.6 or below. (21CFR114b) *Example: Acidified teas and juices*

- 117 cGMP Hazard Analysis and Risk-Based Preventive Controls of Human Food
  - ✓ ***Acid Foods***: Foods that have a natural pH of 4.6 or below (21CFR114.3a) *Example: orange, apple and grapefruit juices.*
  - ✓ ***Low-moisture products –*** Water activity < 0.86 (21CFR117) *Example: dried milk, flour, cookies, etc.*
- Juice HACCP (21CFR120)
- PMO (State Level)

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The Concept of Commercial Sterility (21CFR113)

Absolute Sterility (microbiology) – "...free of living microorganisms)

Commercial sterility (**food**) – "...means the condition achieved (i) By the application of heat which renders the food free of (a) microorganisms capable of reproducing in the food under normal non-refrigerated conditions of storage and distribution and (b) viable mo's (including spores) of public health significance….." (21CFR113.3e.1)

Commercial sterility (**equipment and containers used for aseptic**) – "...means the condition achieved (i) By the application of heat, chemical stereilant(s), or other appropriate treatment that renders the equipment and containers free of viable micoorganisms having public health significance, as well as microorganisms of non-health significance capable of reproducing in the food…" (21CFR113.3e.2)

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The Processing Authority

LACF regulations require a processing authority

- **21CFR113.83 States:** *"…process for low-acid foods **shall** be established by qualified persons having knowledge of thermal processing requirements for low-acid foods in hermetically sealed containers and having **adequate facilities** for making such determinations"*

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Why The Regulatory Concern About Low-acid Canned Foods?

- Anaerobic conditions
- Ambient temperature storage
- Neutral pH
- High moisture/water activity
- Rich nutritional environment
- Absence of preservatives
- A faulty process can still inactivate competitive organisms (vegetative cells)

Perfect conditions for growth and toxin production by *Clostridium botulinum*

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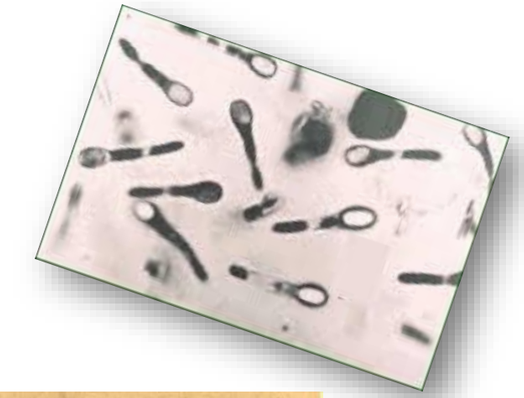
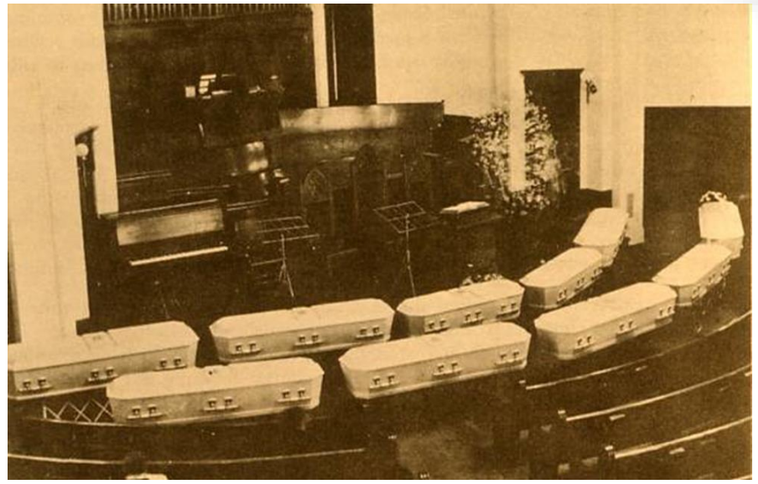
Why All The Concern About Botulism?



- ***Clostridium botulinum***
  - ✓ Produces most potent biotoxin known – lethal dose just a few nanograms.
  - ✓ Lethal if untreated
  - ✓ Toxin is heat labile
  - ✓ Spores are highly heat resistant
  - ✓ Anaerobic organism
  - ✓ Mesophilic (likes body temperature)
  - ✓ Gram positive rod
  - ✓ Sub-terminal spores
  - ✓ Infant botulism (sudden infant death syndrome)
  - ✓ Truly the main reason for the canning regulations



1924 (Albany, Oregon) – Funeral of the Gerber family, all 12 died of botulism (Type A toxin) from home canned green beans

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Validation and FDA Filings of Aseptic Fillers

THE CONCEPT OF VALIDATION

WHY VALIDATE?

- To demonstrate and document that sterilization parameters (critical factors)are sufficient to provide a microbiologically safe and commercially sterile (stable) low-acid, shelf-stable product
- Satisfy regulatory requirements
  - ✓ 21CFR113/108.25 for LACF
  - ✓ FSMA, PC Rule (21CFR117) for other FDA regulated products

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What do we validate?

- Control Measures
  - Aseptic Zone Pre-sterilization
  - Container Sterilization
  - Closure Sterilization
  - Maintenance of Sterility in Aseptic Chamber
- Control Measures Consist of **Process Parameters** (Critical Factors, CCP's)
  - Temperature, Time, Concentration, volume, etc.
- Process Parameters Contain **Critical Limits**
  - Minimum Temperature = 121°C/250°F
  - Minimum time = 3 min

Note: Validation test parameters **must** represent "worst case" condition.

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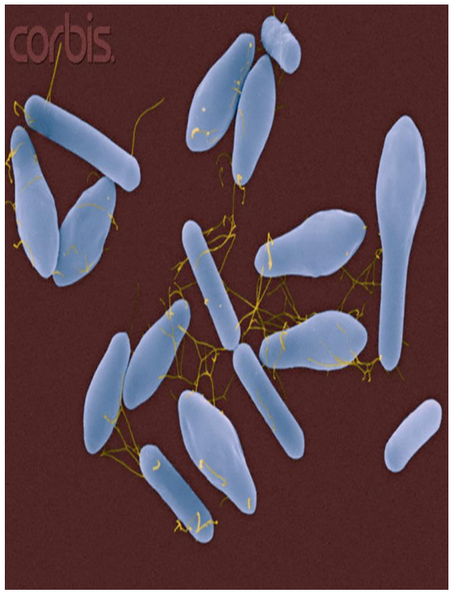
Validation of Low-acid Aseptic Fillers

SELECTION AND CHARACTERIZATION OF ADEQUATE SURROGATE

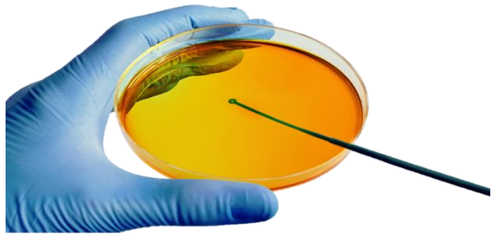
WHY ARE MICROBIAL SURROGATES NEEDED?

- Validation of low-acid aseptic fillers require demonstration of efficacy against **microbial pathogen(s)**
- Microbial pathogens must not be purposely introduced into a food production environment
- Use of physical and/or chemical measurements is not possible (i.e., temperature, time, chemical concentration, etc.) at all points of application



CHARACTERISTICS OF IDEAL MICROBIAL SURROGATE

- Non-pathogenic
- Resistance is quantitatively correlated to target organism (destruction kinetics – D and z values)
- Correlation is established for each sterilant
- Correlation is valid through range of treatment
- Correlation to target allows for practical use
- Can be prepared as a stable suspension
- Every crop is calibrated prior to use
- Possess distinct metabolic or morphological characteristic(s)



SURROGATE SELECTION STRATEGY

Step 1: ID Microbial Target

Step 2: Quantify Resistance of Target

Step 3: Define Performance Criteria

Step 4: ID and Screen Potential Surrogates

Step 5: Final Correlation to Target

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SURROGATE SELECTION
STEP 1: IDENTIFY THE MICROBIAL TARGET

- **Target Organism**
  - Most resistant to sterilization strategy
  - Pertinent to public health and/or microbial stability of product
  - The sterilization process must deliver a lethality level sufficient to inactivate the Target Organism

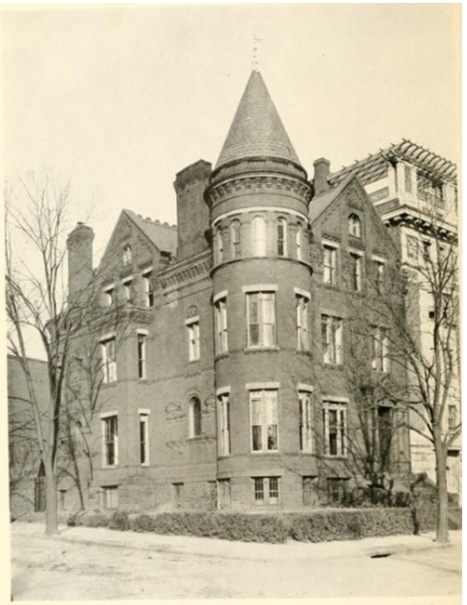
SURROGATE SELECTION
STEP 1: IDENTIFY THE MICROBIAL TARGET

Sterilization Agent	Microbial Target : Food Safety / Quality
H2O2	*C. botulinum / C. botulinum*
Vapor H2O2	*C. botulinum / C. botulinum*
Saturated Steam	*C. botulinum / C. sporogenes*
Dry heat	*C. botulinum / G. stearothermophilus*
Peracetic Acid (PAA)	***Bacillus cereus***
Electron Beam/Gamma Radiation/ UV	*C. botulinum / B. pumilus*
Filtration	*Brevundimonas diminuta*

SURROGATE SELECTION
STEP 2: QUANTIFY RESISTANCE OF TARGET MO'S

Hydrogen Peroxide Sterilization - Low-Acid Fillers

- Work done at National Canners Association (1974):
  - Sterilant: 35% Hydrogen peroxide
  - Target Organism: *Clostridium botulinum*
  - 15 strains (A, B and E) of *C. botulinum* screened against 35% H_2O_2 @ 85°F
  - Strains of *C. botulinum* type B were most resistant
- Strain 169-B was most resistant overall and was selected for further characterization at additional temperatures



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TIN PLATE LABORATORY.

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SURROGATE SELECTION
STEP 2: QUANTIFY RESISTANCE OF TARGET MO'S

Peracetic Acid (PAA)-Based Sterilization - Low-Acid Fillers

- Work done at Eurofins (The NFL, 2004 - 2008):
  - Various PAA formulas were tested (with and without adjuvants added)
  - Sterilant: PAA @ 4,100 ppm + Adjuvant @ 1,200 ppm
  - Target Organism: *Clostridium botulinum* (12 strains screened) and *Bacillus cereus* (10 strains screened)
  - Most resistant strain was *B. cereus* N1127 (more resistant than N1009 reported by Barbara Blackistone, et al. 1996)

STEP 2: QUANTIFY RESISTANCE OF TARGET MO'S (PAA)

Organism	D value (sec)		Organism	D value (sec)
C. botulinum			***B. cereus***	
56A (proteolytic)	<1.42		**N1127**	**4.82**
62A	<2.24		N1051	2.32
69A	<2.39		N1009	2.26
77A	<2.48		N1012	2.13
90A	<2.07		N1028	2.11
4B	<1.44		N2101	1.47
53B	<2.33		ATCC 10876	<2.19
113B	<2.20		ATCC 4579	<2.06
213B	<2.09		N2103	<2.0
Lamanna B	<2.16		N2100	<1.31
Kapchuka B	<2.44			
2129B	<2.14			

PAA - 4100 ppm

Adjuvant – 1200 ppm

Temperature 58°C

Inoculum level 10^6

STEP 3: DEFINE PERFORMANCE CRITERIA (PC) FOR STERILIZATION

- First Step in selecting a PC is to:

  Define the desired Food Safety Objective (FSO)

- A proposed FSO for sterilization of low-acid foods against *B. cereus*:
  - Achieve Probability of Non-sterile Unit (PNSU) of 1×10^{-6} for **Target Microorganism (*B. cereus*)** in the Food (1 contaminated unit in 10^6 units)
- A performance criteria (i.e., **log cycle reduction**, LCR or lethality value (Y) of target organism) can then be established to achieve desired FSO.

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STEP 3: DEFINE PERFORMANCE CRITERIA FOR STERILIZATION CONT.

- FSO = PNSU of 1 in 10^6 containers (for *B. cereus* N1127)
- Used a conservative a contamination level of **1 spore of BC N1127 per 100 packages (10^{-2})**
- Calculate a target lethality (Y):

$$Y = \log N_o - \log N_F$$

Where:

N_o = Initial spore load; and N_F = desired number of survivors

  - $Y = \log 10^{-2} - \log 10^{-6}$
  - **$Y = -2 - (-6) = 4$**

Thus, a 4-log reduction of *B. cereus* is a reasonable PC to achieve the desired FSO

- This would be equivalent to >8-log reduction of most PAA resistant strain of *C. botulinum*

STEP 4: IDENTIFY AND CHARACTERIZE SURROGATES

SCREENING POTENTIAL SURROGATES

- PAA - 4100 ppm
- Adjuvant – 1200 ppm
- Temperature 58/67ºC
- Inoculum level 10^6
- Target Organism:
  - *B. cereus* N1127
  - D_{58C} = 7.24 sec ; D_{67C}= 3.09 sec

Isolate #	$D_{58°C}$ (sec)	$D_{67°C}$ (sec)
1	3.72	2.51
2	<3.51	<2.11
3	<3.49	<2.10
4	<3.44	<2.07
5	<3.39	<2.03
6	3.87	<2.32
7	<3.53	<2.12
8	<3.49	<2.09
9	<3.64	<2.19
10	<3.72	<2.23
11	<3.54	<2.14
12	<4.33	<2.60
13	<3.88	<2.33
14	<4.06	2.43
15	<3.46	<2.08
16	>8.15	>4.53

Isolate #	$D_{58°C}$ (sec)	$D_{67°C}$ (sec)
17	<4.21	<2.53
18	3.70	<2.22
19	<3.81	<2.29
20	4.20	2.47
21	5.01	2.72
22	3.62	<2.17
23	<3.61	<2.17
24	<6.78*	<4.07*
25	<4.43	<2.66
26	<3.56	<2.14
27	<3.63	<2.18
28	<3.47	<2.07
29	<3.55	<2.15
30	<3.54	<2.12
31	<3.50	<2.10
32	<3.44	<2.08
B. CEREUS N1127	7.24	3.09

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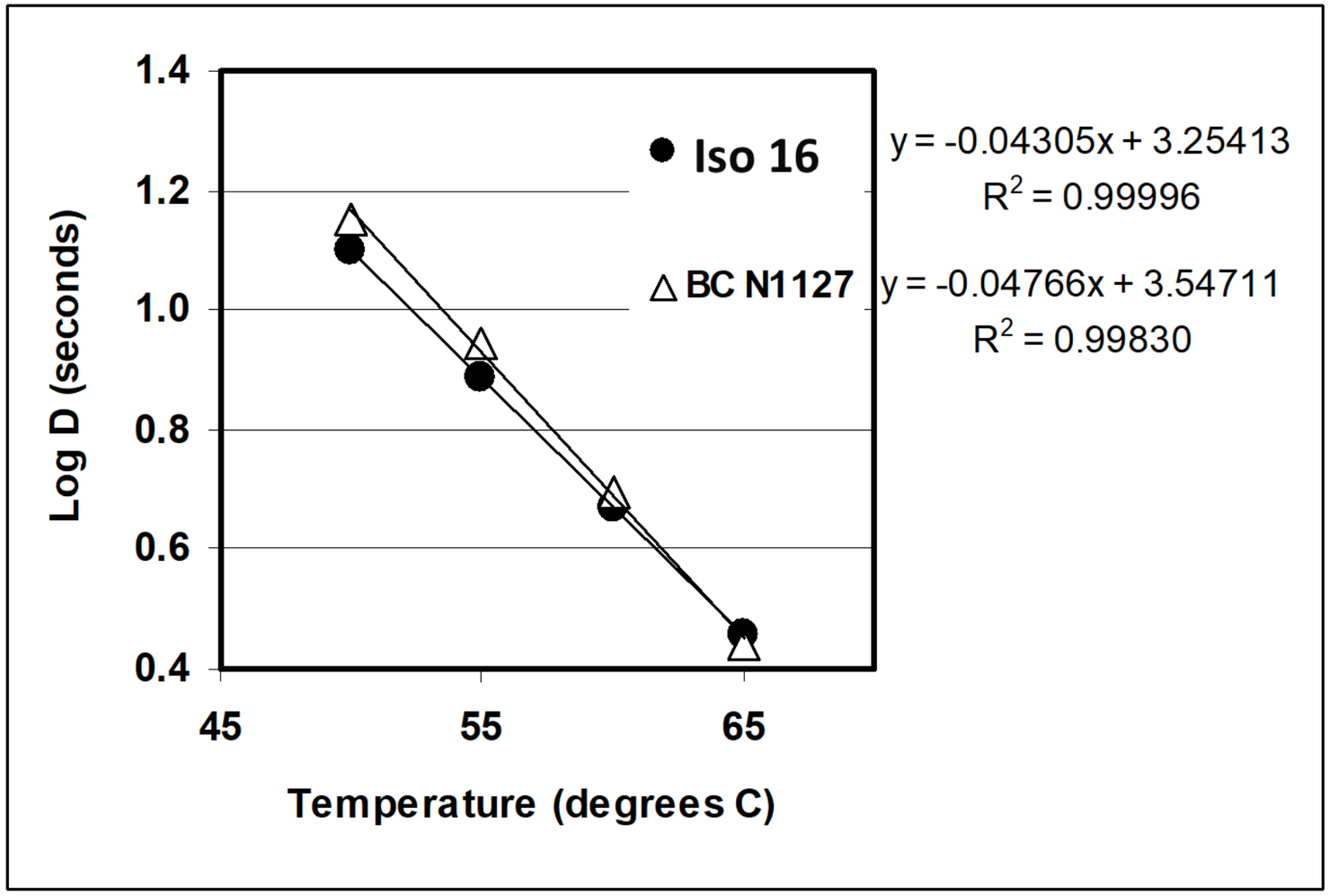
STEP 5: FINAL KINETICS RESULTS – TARGET VERSUS SURROGATE

Temperature (C)	Organism	Measured D-value (sec)	Kill Ratio Surrogate:Target
50	BC NC1127	14.2	0.89
	Isolate #16	**12.6**	
55	BC NC1127	8.7	0.89
	Isolate #16	**7.7**	
60	BC NC1127	4.9	0.96
	Isolate #16	**4.7**	
65	BC NC1127	2.8	1.04
	Isolate #16	**2.9**	

IDENTIFY AND CHARACTERIZE SURROGATES



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Validation and FDA Filings of Aseptic Fillers

MICROBIAL CHALLENGE TESTS

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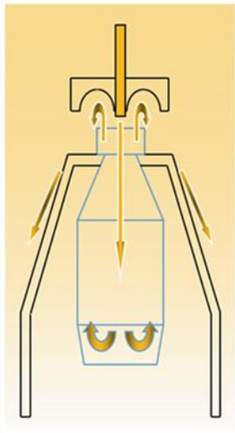
Microbial Challenge – Bottles/Caps

- Bottle and closures must be brought to a condition of commercial sterility
- Bottle interior (product contact) must be sterilized prior to entering aseptic zone
- Bottle exterior surfaces that break the plane of aseptic zone must be sterilized prior to entry
- All closure (cap) surfaces enter the aseptic zone, so must sterilize all surfaces of closure
- **Bottle/closure sterilization tests using microbial surrogates serve to demonstrate the system sterilization ability**



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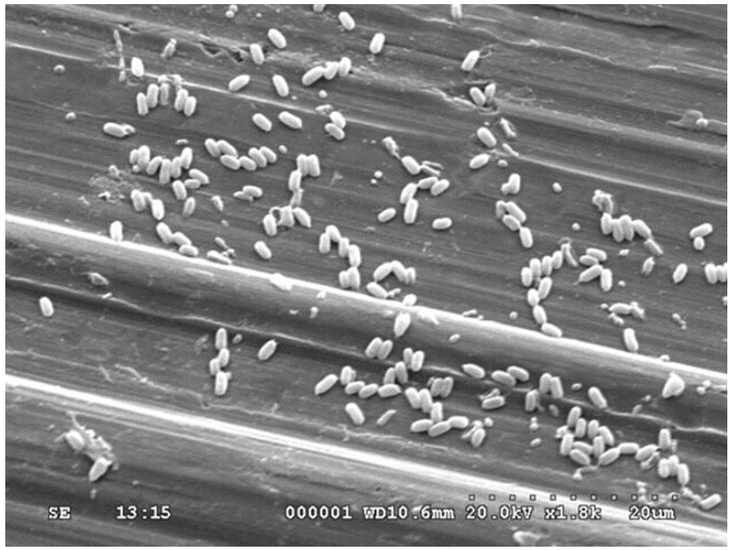
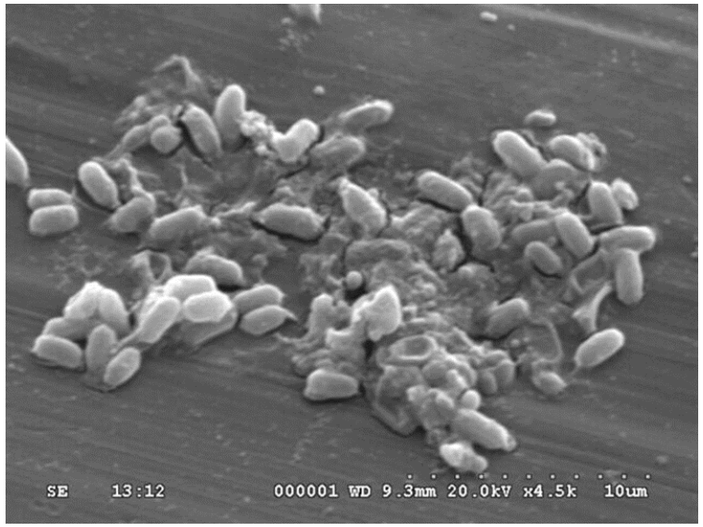
Bottle/Cap Inoculation–Spot Inoculation Method

- Spot inoculation
  - 10 µl precise spot
  - Accurate
  - Precise
  - Single point on bottle surface
  - Multiple inoculation points
  - Spore layering
  - Potential flake off of inoculum





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Expose inoculated bottles to treatment

- Adjust all parameters to “worst case”
  - ID target critical limits and adjust accordingly
  - Inactivation of alarms and/or interlocks often needed
- Experiment is normally repeated 2-3 times
- All parameters must be restored to operational mode after testing

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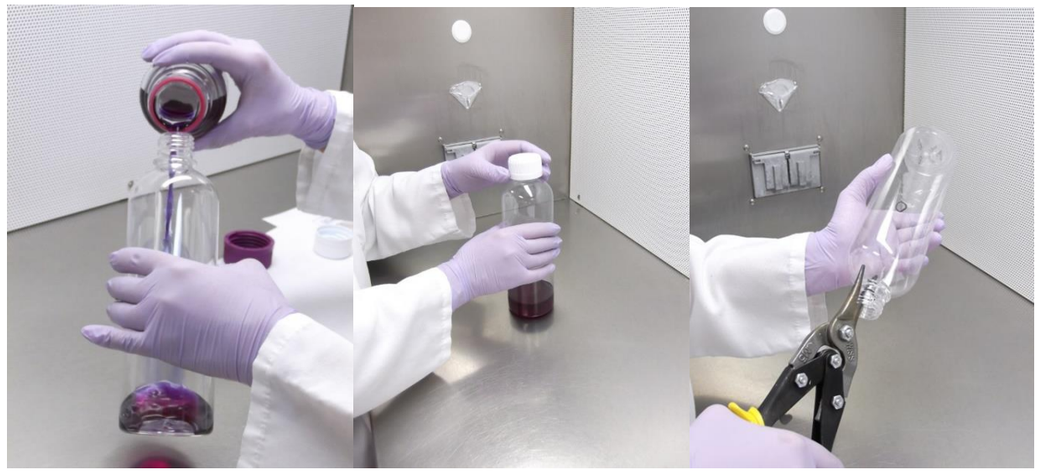
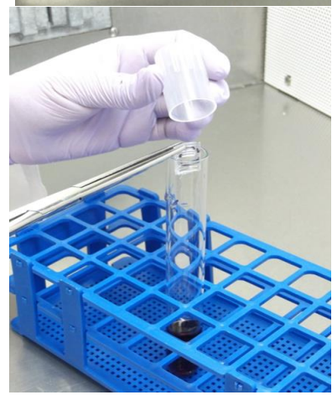
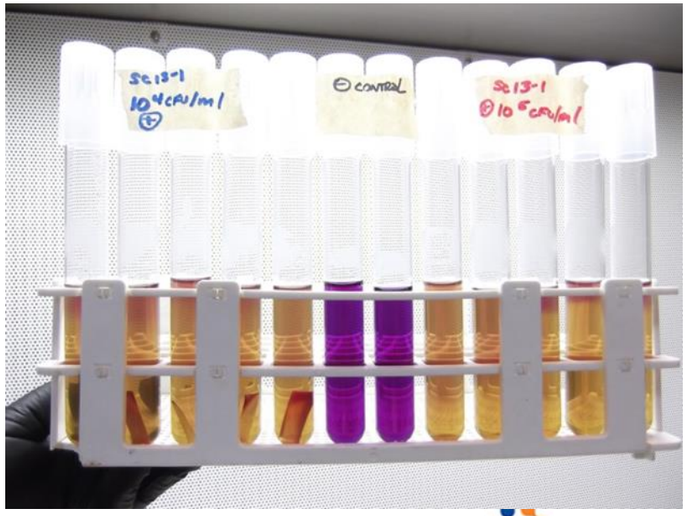
Recover Surviving Spores

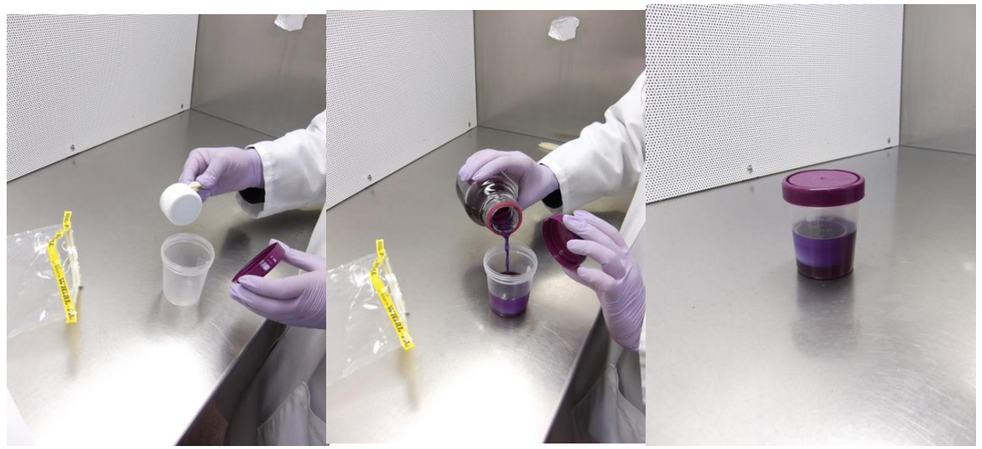
End-point method

1. Addition of sterile non-selective sterile media to bottle/closures
2. Incubate samples
3. Determine if growth occur in media.
4. Confirm growth as test organism
5. Calculate Average Number of Survivors









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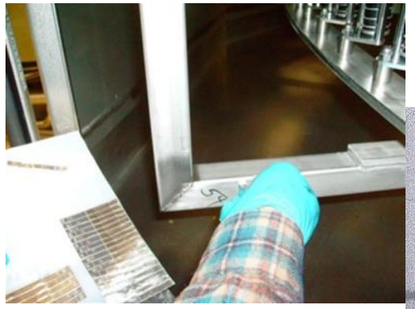
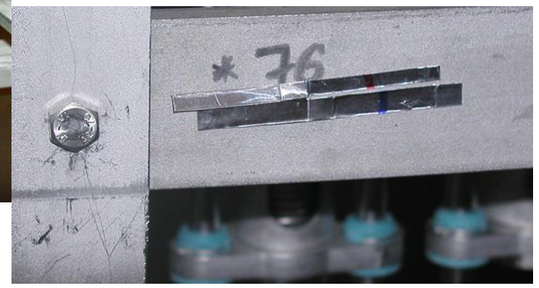
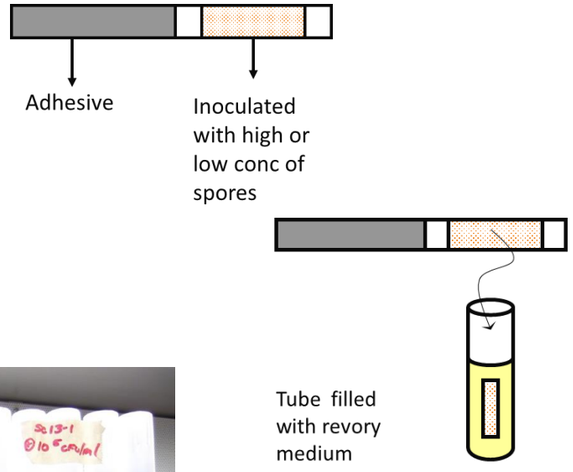
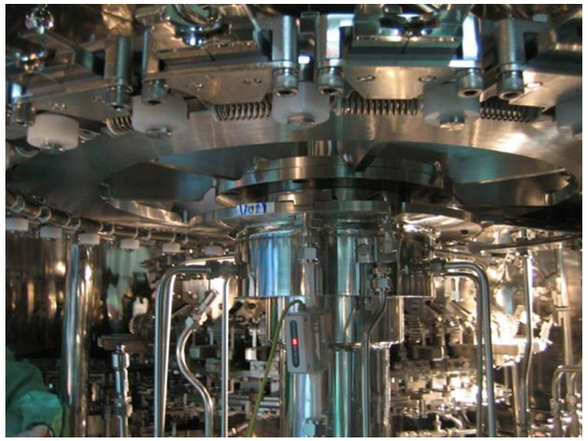
Filler Pre-sterilization Test

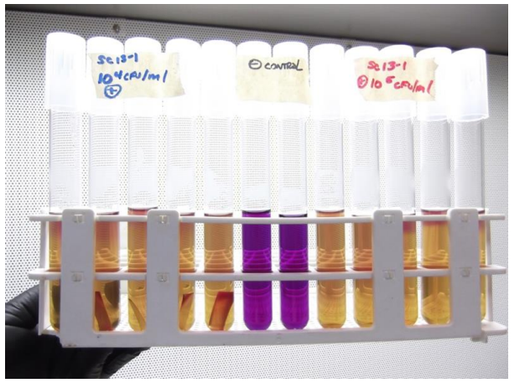
- Prior to production all surfaces within aseptic zone must be brought to a condition of commercial sterility
- Tests consist of adhering spore strips on machine and recovering after treatment.
- Select “worst case locations”
  - Furthest away from sterilant source
  - “Shadowed” surfaces
  - Hot surfaces (may denature sterilant)
  - Sterile air drafts during sterilization may blow sterilant away
  - Water accumulation may dilute sterilant











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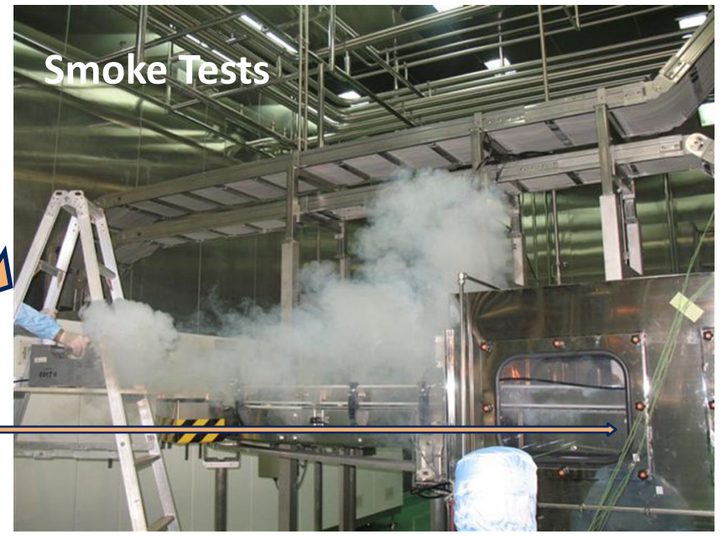
FINAL NOTES

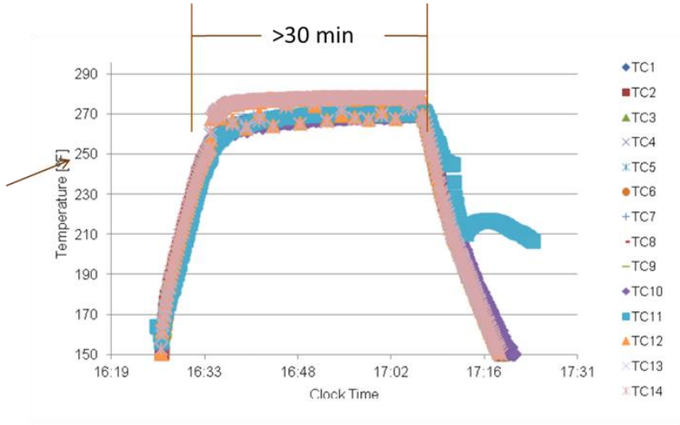
- There might not be a perfect surrogate but…
- …properly characterized surrogates provide a “measure stick” which have served to compare the performance of low-acid aseptic fillers across decades of technical developments
- In a validation, the genus and species of the surrogate is of secondary importance as compared to the characterization of the specific spore crop used in the validation

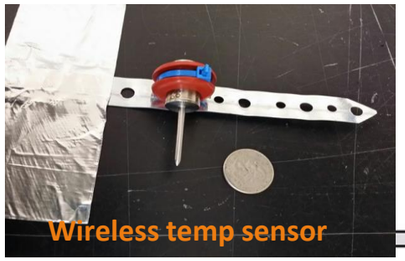
Other Validation Tests

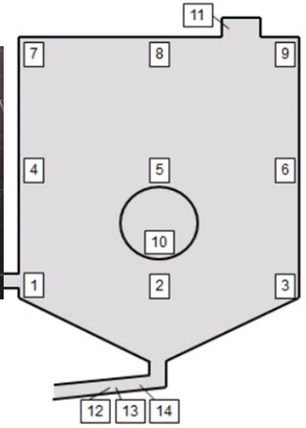
- In addition to microbial challenge testing the validation for an aseptic line may include the following tests:
  - Maintenance of sterility testing for aseptic zone in filler (smoke tests/air quality measurements)
  - Temperature distribution tests for sterilization of large aseptic surge tanks



Surge tanks temp distribution tests







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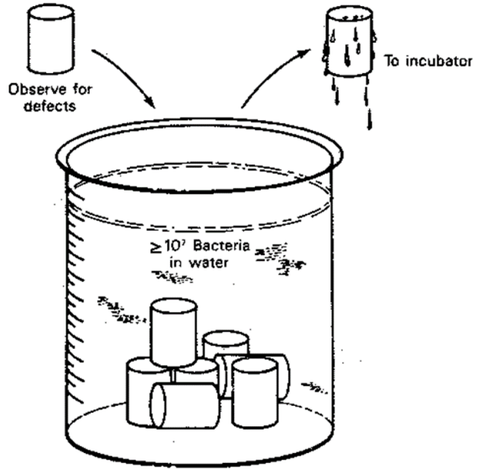
Other Validation Tests

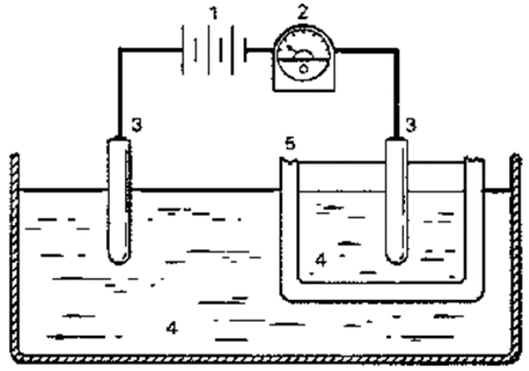
- In addition to microbial challenge testing the validation for an aseptic line may include the following tests:
  - Dye/Riboflavin challenge of machine cleaning cycle (CIP)
  - Media fill Tests – Shipping Tests
  - Hermetic seal integrity testing

CIP Test



Bioimmersion

Vacuum immersion



Electroconductivity

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