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Post Sterilization Lab Contamination

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Overview

- Ethylene Oxide sterilization utilizes Process Challenge Device (PCD) to support lot release
- PCDs are biological indicators that are tested for sterility
- Testing lab was identified to have a high failure rate – prompted onsite audit
- Observations of laboratory, operator technique, cleaning/EM practices lead to identifying alternative labs

Ethylene Oxide Sterilization

- EtO is a carcinogenic, explosive gas used in medical device sterilization
  - Utilized in food industry for treatment of spices
- Alkylating agent that disrupts the DNA of microorganisms – requires heat and moisture
  - Cycles run up to 55°C
- Material limitations when utilizing EtO
  - Gas will not penetrate most plastics/glass – require use of Tyvek patches on packaging
  - Material of construction limitations
    - Cannot be used for neoprene, silicone, polyisoprene

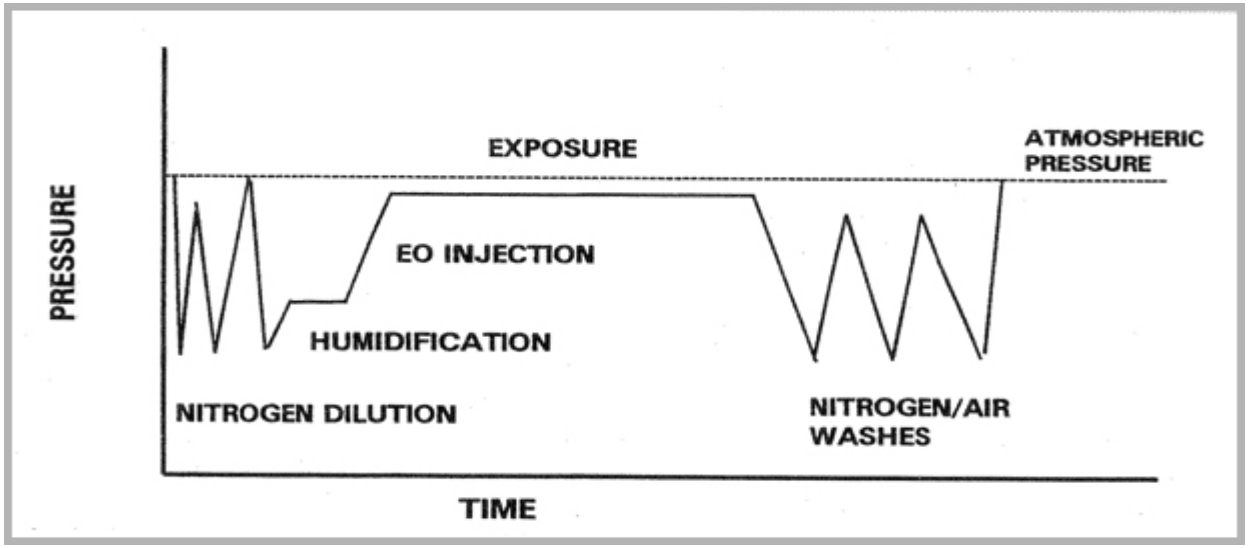
Ethylene Oxide Sterilization

3 stages of process:

1. Preconditioning & Humidification

- Takes place in sterilizer chamber or separate room

2. Sterilization Cycle



3. Aeration

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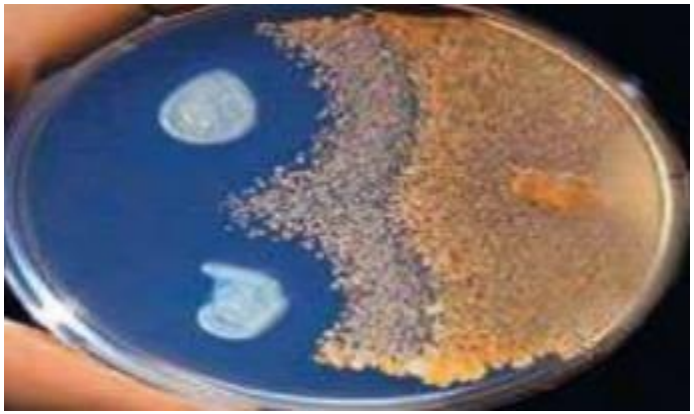
Process Challenge Device (PCDs)

- Spore strip with 10^6 CFU of *Bacillus atropheus* (*B. subtilis var niger*)
  - Spore-forming organism, growth has distinctive orange color
  - Analog for *B. athracis* in US bioweapons program
- Internal PCDs placed inside challenge product in load in most difficult to sterilize location
  - Create a challenge for migration of gas into product
- External
  - Placed on outside of product load
  - More resistant to sterilization than internal PCDs



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Sterility Testing of PCDs

- Necessary for product release when not utilizing parametric release
- Remove External PCD from product prior to aeration
  - Ensure worst-case in terms of exposure to EtO
- Aseptically transfer to a culture media (Tryptic Soy Broth)
- Incubate 30 - 35°C for seven days

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Aseptic Fill/Test Suite and USP <71> Sterility Analogs

Aseptic Fill/Test Suite	USP <71> Sterility
Environment	
ISO 5 Cleanroom	Biological Safety Cabinet
Test Article	
Sterile filtered product filled in a sterile container	Sterile product dropped or injected in sterile media
Process Controls	
Supported by robust Environmental Monitoring program with investigation of Over Alert and Over Action events	Supported by robust Environmental Monitoring program with investigation of Over Alert and Over Action events
Operator Gowning	
Full sterile tyvek bunny suit or sterile reusable gown, sterile gloves	Sterile sleeves, hairnet, facemask, sterile gloves
Material/Personnel Flow	
Unidirectional flow from low to high classification areas and regown to reenter	Operators enter the testing hood and regown if they leave
Successful Execution	
Zero growth on media fills	No growth

Inputs to Aseptic Areas & USP <71> Sterility Investigations

- Environmental/Engineering Controls
  - Control of surrounding environment – HEPA filtration, Temperature, Humidity
    - Any conditions outside normal operating conditions
      - Humidity too high ⇒ mold growth
      - Temperature too high ⇒ increase in operator perspiration
    - Loss of differential pressure between different areas
      - Flow of air from lower classification to higher
  - Materials in manufacturing/testing areas
    - Cardboard ⇒ Gram positive bacillus
- Cleaning of affected areas
  - Proper cleaning agents, including sporicide in ISO 5, utilized/rotated
    - Frequency of cleaning documented and followed
      - Prevents bioburden from developing resistance
  - Use of UV light (time on and time off requirements/documented)
    - Effective against Gram positive cocci – human sourced organisms

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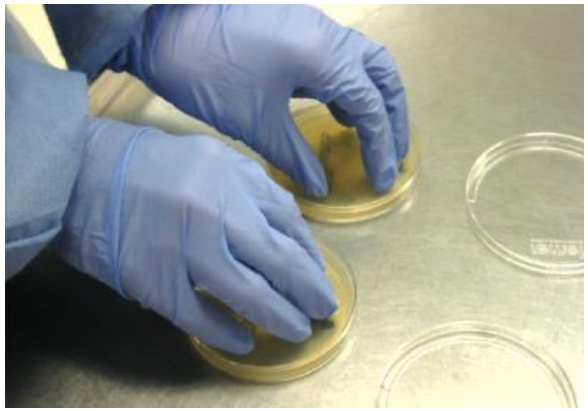
Inputs to Aseptic Areas & USP <71> Sterility Investigations

- Environmental Monitoring - not acceptance criteria for product
  - Surface
    - RODAC plates – informs if cleaning/sanitization program is effective
  - Air
    - Nonviable air informs if engineering controls are functioning
    - Viable air informs if organisms present in air
    - Active monitoring via a sampler or passive via settle plates
  - Personnel
    - Sample operator gowns and hands
    - Effectiveness of gowning
    - Operator training
  - Resample at affected sites
  - Identify trends



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Sterility Failures – 15 June 2015

> Lab investigation examined sterilization cycle – all parameters met

> Root cause analysis

– Gram stain results: Gram positive bacillus

Colonial Morphology	Colony morphology of PCD # 4 was white, circular and wrinkled.  Figure N°1: Colonial morphology of PCD #4 isolate. Positive control´s colony morphology was orange, circular and flat.  Figure N°2: Colonial morphology of Positive control isolate.	Probable identification of target microorganism. Damage caused by the EO process can inhibit microorganism´s capacity of developing the orange pigment.

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Sterility Failures – 15 June 2015

> Vitek analysis: 93% *Bacillus vallimortis*

Biochemical Analysis	Vitek results were available on 29-Jun-15 and yielded identification with 93% of likehood of *Bacillus vallismortis* (see attachment 4).	Probable identification of target microorganism. Damage caused by the EO process can affect normal cell metabolism and replication, causing slightly different behavior during biochemical analysis. *B. vallismortis* differs from *B. atrophaeus* only in its oxidase activity, for all major phenotypic tests. (Roberts, Nakamura, & Cohan, 1996)

3. *Conclusion*

Upon review of the abovementioned data within [redacted] control, no assignable causes to this event could be identified. No current History of previous BI failures associated with this cycle.

The PCD # 4 can be considered as a true positive result. It is probable that a spore (or very few) of *Bacillus atrophaeus* survived the cycle. The spore(s) was(were) likely injured and it was possible that it lost the ability to produce the orange pigment normally associated with healthy *Bacillus atrophaeus* spores (potential mutations) and affected the biochemical response of the strain, result of an alkylation process as a consequence of exposure with EO.

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Sterility Failures

> 03-Aug-15 – Genotypic identification: 99.01% *Bacillus circulans*

3. *Conclusion*

Upon review of the abovementioned data within [redacted] control, no assignable causes to this event could be identified.

The PCD # 9 was identified as *Bacillus circulans*; based on genetic sequencing of 16S rRNA. Phylogenetic information of the sequence of *Bacillus circulans* shows that the organism is not closely related with *Bacillus atrophaeus* therefore it cannot be considered as the indicator microorganism. Considering the source of this microorganism, a laboratory contaminant is discarded. It is s probable that the spore crop used for the BI preparation was contaminated with this microorganism. However, it could not be verified. Additionally the fact that the EPCD #9 did not grow upon subsequent attempts to culture it, suggests that the microorganism got so damaged after the EO cycle that got impaired replication capability.

Based on this information, for PCD # 9 it cannot be concluded whether the growth should be considered as a true positive result.

Sterility Failures

> 05-Oct-18: Genotypic analysis: *Bacillus megaterium*

> 22-Oct-15: No identification of positive organism

Description of Evaluation Activities and Findings
EPCD's were processed on 13-Oct-15 and placed in incubation at 30-35°C as per recommended conditions by the manufacturer. The EPDC's were inspected at day 1-3 and 6 of incubation, all EPCDs were negative. On day seven all PCD's were negative, except EPCD #2 that showed turbidity of the culture media. Gram Staining Isolates of the growth were reported as Gram positive rods (same microscopic morphology as the positive control). Biochemical identification of the sample is pending.

Correction(s) Made?:	No

> Common threads:

- Sources of identified organisms: soil, feces, environment
- Lab claim that damage to biological indicator caused loss of pigmentation
- Lab claimed potential contamination of BI spore crop

> **Lab experienced a 6% rate of failure across all testing for a 12 log reduction overkill cycle**

> **The same PCDs had 100% inactivation during half cycles**

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Next Steps

- Discontinued testing at that lab – transferred to alternate lab on Approved Supplier List
  - Fully gowned Operators in an ISO clean room
- Challenged the claim that damage to the BI could cause loss of pigmentation
- Challenged claim of contamination of the spore crop
- Scheduled onsite audit of testing lab
  - Audit of Quality system by dedicated Supplier Quality Auditor
  - Audit of Sterilization activities by Sterility Assurance Director
  - Audit of Microbiology laboratory by Sterility Assurance Microbiology Manager
- Identified local alternative labs to save shipping/time

For Cause Audit of Testing Lab

- Quality System and Sterilization were excellent
- Microbiology Lab had multiple possible vectors of contamination
  - Laboratory
    - Discolored ceiling tiles above testing hood
    - Cardboard stored under and around testing hood
    - Positive controls and test articles not segregated
    - No environmental controls outside hood (HEPA filters, temperature, humidity)
  - Testing hood
    - No UV light
    - No use of sporicide for cleaning and no rotation of cleaning agents
    - No environmental monitoring of hood before or after testing
    - No control of background area to the hood
  - Gowning
    - Lab coats worn during testing not routinely cleaned, stored in lab
    - Nonsterile gloves utilized and stored unprotected in lab
    - No hair net/face mask utilized
    - No operator monitoring

For Cause Audit of Testing Lab

- Micro Lab issues (cont.)
  - Performance of the Testing/Training
    - Operators passed hands and arms over exposed media used for testing
    - No requirement for operators to wash hands prior to testing
    - Operators' hands and materials passed into and out of hood during testing
    - Personnel performing investigation: QA Technician with no micro background
  - Perceptions
    - Underlying attitude of TS environment rather than aseptic environment
    - Laboratory part of a contract sterilization organization
    - "Why is this important? We have never had to do this before"
    - Conclusions of investigations never looked at the test itself, deflecting root cause to BI spore crop or chemical action of EtO on the organisms

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Alternative Testing Labs

- Determined to cease testing with original lab and transfer to an alternative
- Performed onsite audits of two local labs
- Both Corporate and Manufacturing site Microbiologists reviewed documentation and observed testing
  - Supplier Quality reviewed Quality system
- Identified a prioritized list of requirements:
  1. Operator training and technique
  2. Controls inside the testing hood
  3. Environmental controls outside the hood
- Some requirements identified in SQA, others in procedural changes in lab's QMS

Requirements at Alternate Testing Lab

- Operator Training & Gowning Updated
  - Concept of first air – avoiding passing hands over test articles is stressed
  - Sterile gloves and sterile sleeves utilized
  - Operators hands remaining in hood during testing
  - Microbiologist on staff
- Environmental Monitoring
  - Operator hands, surfaces after testing before cleaning, settle plates for passive air
  - Trending performed on EM results
  - Hood cleaned with a rotation of sporicide and IPA
- Testing performed in a BSC in a controlled/unclassified background environment
  - Cleaning program in lab in place. No corrugate storage in background
  - All materials wiped with IPA and in hood prior to testing
  - UV light exposure on materials in BSC or 20 minutes prior to testing
- **The result: No sterility failures since utilizing the new testing lab**

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References

> http://informahealthcare.com/bty

Bacillus atrophaeus: main characteristics and biotechnological applications – a review

Sandra R. B. R. Sella, Luciana P. S. Vandenberghe, and Carlos Ricardo Soccol

> Image credits:

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