Bioburden estimation of reusable and single use medical devices – methodology and evaluation

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Sterilization is a special process

The effectiveness of the process can not be fully verified by subsequent inspection and testing of the product
Bioburden

Population of viable microorganisms on or in product and/or a package
Origins of bioburden

- raw materials
- manufacturing of components
- assembly process
- manufacturing environment
- assembly/manufacturing aids
- cleaning process
- packaging of finished products
Use of bioburden determination

- Validation and revalidation of sterilization processes
- Routine monitoring of:
  - raw materials
  - components
  - packaging
  - manufacturing processes
- Assessment of the efficiency of cleaning processes
- Environmental monitoring
Sterilization in industry

Bioburden determination common as part of sterile production
Sterilization in hospitals

Bioburden determination **not** common

- In validation of sterile production
- For routine monitoring

- **Medical devices**
  - Multiple use
  - Single use
Microorganisms on surgical instruments

Modified from Nyström B. J Hosp Infect 1981;2:363
Bioburden on surgical instruments before sterilization

- 0-10 CFU: 70% of instruments
- 11-100 CFU: 10% of instruments
- > 100 CFU: 20% of instruments

Modified from Rutala WA, et al. AJIC 1998;26:143
Bioburden on surgical instruments after use and after washing

Modified from Chu NS, et al. AJIC 1999;27:315
Change of bioburden on surgical instruments during washing

Modified from Chu NS, et al. AJIC 1999;27:315
Bioburden determination and sterilization in hospitals

- Non-medical devices
  - Multiple use
  - Single use
ISO 11737
Sterilization of health care products
- Microbiological methods

Part 1: Determination of a population of microorganisms on product

Part 2: Tests of sterility performed in the validation of a sterilization process

Part 3: Guidance on evaluation and the determination of bioburden data
Selection of product

- Representative of the batch
- Whole product, if feasible
- If not: As large a portion as possible
Selection of an appropriate method

- Removal of microorganisms
- Culturing
- Enumeration
- Characterization
TECHNIQUES FOR REMOVAL OF MICROORGANISMS FROM PRODUCT
Adhesion of microorganisms to surfaces

- Surface characteristics
- Microorganisms involved
- Origin of contamination
- Presence of other substances
Removal techniques

- Stomaching
- Ultrasonication
- Shaking (mechanical or manual)
- Vortex mixing
- Flushing
- Blending (disintegration)
- Swabbing
Stomacher
Removal techniques

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Eluents
Diluents
Transport media

No proliferation or inactivation of microorganisms
Eluents and diluents

- Sodium chloride 0.25 – 0.9 %
- Ringer
- Buffered peptone water
- Phosphate buffered saline
- Thiosulphate Ringer
Additives to eluents and diluents

- Surfactant
  - Polysorbate (Tween) 80
- Neutralizers for microbicidal and microbistatic substances
Release of substances from product

Substances released into suspending fluid should neither promote nor inhibit the growth of microorganisms

➢ bacteriostasis test
Methods where removal of microorganisms is not employed

- Contact plating
- AgarOverlaying
Contact plate
Contact plate
-- after incubation
Medical device?
TRANSFER TO CULTURE MEDIUM
Membrane filtration

- Filtration of an eluent followed by incubation of the filter on appropriate growth medium
- Plates are incubated
Membrane filter
Growth of moulds on filter
Growth of bacteria on filter
Membrane filtration

Particularly useful for:

- Suspensions with low concentrations of microorganisms
- Suspensions with microbicidal or microbistatic substances
Pour plating

- Defined aliquots of suspension mixed with molten agar medium at 45 °C

- Agar allowed to solidify in the plate

- Plates are incubated
Spread plating

- An aliquot of defined volume is spread on the surface of a nutrient medium
- Plates are incubated
Spiral plating

- A defined volume spread in a spiral track on a rotating agar plate
- Plates are incubated
Media and incubation conditions

Several combinations of media and incubation conditions are usually required

- Bacteria
- Yeasts and moulds
- Anaerobic bacteria

- 30 - 35 °C
- 20 - 25 °C
ENUMERATION
Enumeration procedures

- Detecting small colonies
- Counting and reporting unusual colonies e.g. spreaders
- Enumerating and reporting crowded plates
- Reporting counts from multiple dilutions
CHARACTERIZATION
Characterization

- staining properties
- cell morphology
- colony morphology
- use of selective culturing
- biochemical or other means of identification
- genetic analysis
Spore forming bacteria
Validation of method for removing microorganisms

- Repetitive recovery
- Inoculating a known number of microorganisms onto product
### Repetitive recovery

<table>
<thead>
<tr>
<th>Treatment no.</th>
<th>Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>94</strong></td>
</tr>
</tbody>
</table>

Removal by first treatment  80,8%
Correction factor  1,24
Routine monitoring of bioburden

- Sample size
- Sampling frequency
- Acceptable limits and actions taken if a limit is exceeded
- Trend analysis
Bioburden in air/water channel of flexible endoscopes after disinfection

Median: 0 CFU
Mean: 39 CFU

Ashurst L, Kjos B, Lingaas E. 2004
Evaluation and corrective action
Does bioburden determination tell the truth??

Sampling deficiency?

- Adhesion
- Biofilm
- Incomplete dispersion
Does bioburden determination tell the truth??

Lack of growth?

- Germination of spores
- "Dormant" bacteria
- Small colony variants

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- Raw materials
- Cleaning/lubrication/manufacturing liquid
- Transport/holding containers
- Work surfaces
- Personell attire/hygiene practices
- Handling/assembly
- Packaging materials and procedures
- Storage conditions
- Characterization of organisms recovered
But what is the relevance?
Can the problem be solved simply by adding another minute to the sterilization cycle?
Bioburden, inactivation factor and sterility assurance level (SAL)

Number of organisms

Exposure to inactivation process

Inactivation required:
- \(10^{12}\)
- \(10^9\)
- \(10^6\)

SAL \(10^{-6}\)
Dead microorganisms can also create problems

- Endotoxin
- Proteins
- Teichoic acid
- ---
What is the maximum allowable number of dead bacteria on a sterile device?

- 0
- 10
- 100
- 1000
- 10000
- 10,000
- 100,000
- 1,000,000
- 10,000,000
- 100,000,000