CLEANING AND DISINFECTION TECHNIQUES

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4 October 2017
Cleaning and Disinfection Agenda

1. Cleanroom classification and definition
2. Sources of Contamination:
3. Cleaning and disinfection products
4. Liquid disinfection procedures
   • GRADE A
   • GRADE B/C/D
5. Validation
1. Cleanroom Classification

EX 1 MANUFACTURE OF STERILE MEDICAL PRODUCTS
Airborn particulate classification

<table>
<thead>
<tr>
<th>Grade</th>
<th>At rest</th>
<th>In operations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum permitted number of particles in /m³ equal to or above</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0,5 µ</td>
<td>5 µ</td>
</tr>
<tr>
<td>A</td>
<td>3.520</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0,5 µ</td>
<td>5 µ</td>
</tr>
<tr>
<td>B</td>
<td>3.520</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>352.000</td>
<td>2.900</td>
</tr>
<tr>
<td>C</td>
<td>352.000</td>
<td>2.900</td>
</tr>
<tr>
<td></td>
<td>3.520.000</td>
<td>29.000</td>
</tr>
<tr>
<td>D</td>
<td>3.520.000</td>
<td>29.000</td>
</tr>
<tr>
<td></td>
<td>Not defined</td>
<td>Not defined</td>
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</tbody>
</table>

Non viable particles
Powder, dust, paper, packaging, crystals
# Cleanroom Classification

## RECOMMENDED LIMITS FOR MICROBIOLOGICAL MONITORING OF CLEAN AREAS DURING OPERATIONS

<table>
<thead>
<tr>
<th>Grade</th>
<th>Air sample (cfu/m³)</th>
<th>Settle plates (diam. 90 mm) (cfu/4 hours)</th>
<th>Contact plates (diam. 55 mm) (cfu/plate)</th>
<th>Glove print (5 fingers) (cfu/glove)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>200</td>
<td>100</td>
<td>50</td>
<td>-</td>
</tr>
</tbody>
</table>
What does contamination mean?

Pharmaceutical Definition

The entry of unwanted particles and micro-organisms into an air controlled environment that can “contaminate” the room and products.
Types of contamination

There are two types of contamination

- Non – viable : Particulate
- Viable : Microbial
Non- Viable, particles or particulates are:

Tiny amounts of non-living matter or material which may be seen without a microscope, they can float in the air or be suspended in a liquid.

- Powder
- Dust
- Skin
- Paper particles
- Dirt
Viable, micro-organisms are:

Living organisms or germs which we can only see under a microscope

– Bacteria
– Fungi (spores)
– Yeast, Moulds
– Viruses
– Bacterial spores
Microbial Contamination

Vegetative bacteria

Bacterial Spores

Fungi and moulds
Where is contamination coming from?

- Personnel:

- Materials:
2. Sources of Contamination

- Personnel
- **Transfer of materials**
  - Raw materials
  - Equipment
  - Packaging
  - Cleaning and disinfection products
Personnel
Where does it come from?

YOU!
Bacteria on people

Bacterial Skin Population

CFU per contact plate

Series1

Forehead
Temple
Under Eye
Nose
Upper Lip
Cheek
Angle of Jaw
Behind Ear
Under Chin
Back of Neck
Neck
Personnel

Hygiene Issues

- Good basic hygiene (washing hands)
- No make-up, no nail varnish etc...
- No jewellery
- Be careful if you own pets
- Some illnesses may exclude you
Cleanroom Clothing –Annexe 1 GMP

• **Grade D**
  – Hair and beard covered
  – Protective suit
  – Appropriate shoes or overshoes

• **Grade C – as above plus:**
  – Trouser suit gathered at wrists, high neck
  – Non shedding materials
Cleanroom Clothing – Annexe 1 GMP

• **Grade A/B** – as previous slide plus:
  – Headgear should totally enclose hair and be tucked into neck of suit
  – Face mask / Sterile gloves and footwear
  – Trouser bottoms tucked in
  – No shedding materials and should retain shed by the body
Gowning Checklist

- Cover Hair
- Remove make-up
- Remove jewellery
- Put on under suits (if worn)
- Wash hands
- Apply hand-cream/sanitiser
- Put on changing gloves (if worn)
- Headwear
- Mask
- Gown – suit or coat
- Footwear
- Check in mirror
- Discard any packaging
- Remove changing gloves
- Put on work gloves
- Eye protection
- Clean gloves
Materials

Bioburden of consumables

Needles syringes, swabs etc....

! 60% contaminated with bacteria
! 40% contaminated with bacterial spores

Hospital Pharmacist 2001
Demand for additional wiping

Validation of user methods of liquid disinfection

<table>
<thead>
<tr>
<th>% Reduction of organisms</th>
<th>Spray 70% Denatured Ethanol</th>
<th>Wipe 70% IPA</th>
<th>Spray and Wipe</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>99,8</td>
<td>99,6</td>
<td>100,0</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>27,6</td>
<td>80,6</td>
<td>93,9</td>
</tr>
</tbody>
</table>
Definitions

• **Cleaning**
  The removal of contamination by physical means or by suitable agents from surface to render it visibly clean.

• **Disinfections**
  The process of reduction in the number of micro-organisms in or on an inanimate matrix, by the action of an agent on their structure or metabolism to level judged to be appropriate for a specified defined purpose.
Quote 2

What is the most important of both below?

- Cleaning :
- Disinfection :
Key Issues - Reproducibility

• Disinfectants are inhibited by “dirt”

• Disinfection processes done on a dirty surface will be inhibited, either partially or fully, depending on the amount of dirt.

• Therefore the results of the disinfection process will be variable and dependent on the amount of dirt on the surface
Key Issues - Reproducibility

Therefore:

Industry “Best Practice” is to first clean to remove dirt, residu, biofilm... and then disinfect.

This maximises reproducibility of results
Residues

• Left-overs of products like detergents and biocides

• Accumulating layers forming a sticky or slippery surface

• Environmental Monitoring not reliable anymore
Biofilms

• Micro-organisms working together to create a microclimate which is more resistant to the environmental forces and will deliver them all the food and optimal conditions they need to survive.

• 90 % of all bacteria in nature are living in biofilms.
Biofilms

Bacteria packed in glyccalyx

Arrows = microcolonies of bacteria
* = microcolonies on inner wall

Biofilm formation:
Attachment | Colonization | Growth

Bulk Fluid
Surface
3. Cleaning and disinfection

• Cleaning Products – Detergents

• Disinfection Products - Biocides
Definitions

• **Detergent**
  A cleaning agent with wetting and emulsifying properties used to aid in the removal of residues from a surface leaving it visibly clean.

• **Biocide**
  A biocide is a disinfectant. A chemical that is capable of killing micro-organisms. It may not be 100% effective but is able to reduce the numbers of microbes to a specified level.
  A disinfectant is not totally effective against all types of microbial contamination.
<table>
<thead>
<tr>
<th>DISINFECTANT</th>
<th>Bactericidal</th>
<th>Fungicidal</th>
<th>Virucidal</th>
<th>Sporicidal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols 70%</td>
<td>Good</td>
<td>Fair</td>
<td>Good</td>
<td>None</td>
</tr>
<tr>
<td>Amphoteric Surfactant</td>
<td>Good</td>
<td>Fair</td>
<td>Fair</td>
<td>None</td>
</tr>
<tr>
<td>Biguanide</td>
<td>Good</td>
<td>Fair</td>
<td>Good</td>
<td>None</td>
</tr>
<tr>
<td>Quaternary Ammonium Compounds</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>None</td>
</tr>
<tr>
<td>Glucoprotamine</td>
<td>Good</td>
<td>Fair</td>
<td>Fair</td>
<td>None</td>
</tr>
<tr>
<td>Phenolic Compounds</td>
<td>Good</td>
<td>Good</td>
<td>Fair</td>
<td>None</td>
</tr>
<tr>
<td>Hydrogen peroxide 6 %</td>
<td>Good</td>
<td>Fair</td>
<td>Good</td>
<td>Fair</td>
</tr>
<tr>
<td>Chlorine Dioxide/Quat blend</td>
<td>Good</td>
<td>Fair</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>H Peroxide / Paracetic blend</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Hypochlorite 0,5 %</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
</tbody>
</table>
## Properties of Disinfectants

<table>
<thead>
<tr>
<th>DISINFECTANT</th>
<th>PROPERTIES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irritation</td>
</tr>
<tr>
<td>Alcohols 70%</td>
<td>Medium</td>
</tr>
<tr>
<td>Amphoteric Surfactant</td>
<td>Low</td>
</tr>
<tr>
<td>Biguanide</td>
<td>Low</td>
</tr>
<tr>
<td>Quaternary Ammonium Compounds</td>
<td>Low</td>
</tr>
<tr>
<td>Glucoprotamine</td>
<td>Low</td>
</tr>
<tr>
<td>Phenolic Compounds</td>
<td>High</td>
</tr>
<tr>
<td>Hydrogen peroxide 6 %</td>
<td>Low</td>
</tr>
<tr>
<td>Chlorine Dioxide/Quat blend</td>
<td>Low</td>
</tr>
<tr>
<td>H Peroxide / Paracetic blend</td>
<td>High</td>
</tr>
<tr>
<td>Hypochlorite 0,5 %</td>
<td>High</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Very High</td>
</tr>
</tbody>
</table>
Regulations

• Some disinfectants in use are not manufactured for use in a GMP environment.
• Is there a cert of analysis for batch to batch consistency?
• Validated efficacy for appropriate application?
• Are they supported by appropriate change control?
• Will they be supported by the BPR?
Quote 3

How many products can I use in a regime?

- Only one:
- Two or more:
Rotation of biocides

• Eudralex Annex 1 states:
  “Where disinfectants are used more than one type should be employed.”

• USP Chapter <1072> states:
  “The rotation of an effective disinfectant with a sporicide is to be encouraged. It is prudent to augment the daily use of a bactericidal disinfectant with weekly (or monthly) use of a sporicidal agent”.
4. Liquid disinfection procedures

Decontamination Programme:

- Cleaning
- Disinfection
- Validation
Decontamination Programme

- Visibly clean state
- Validated SOP’s
- Trained personnel
- After every session
Formats of disinfection products

• **Protected trigger sprays**
  – Eliminate the potential for airborne contamination by closed spray system.

• **Aerosols**
  – Eliminate the potential for airborne contamination by over pressure.

• **Pre-saturated wipes**
  – Impregnated with detergent, alcohols or biocides.
  – Standardized volume.

• **Unit dose and concentrates**
  – Ideal for large surface areas – walls, floors, ceilings
Cleaning and disinfection methods

- **Wipe from cleanest to dirtiest areas**
  - Top of wall to bottom.
  - From back of horizontal surface to front
  - Away from filter housings

- **Use linear parallel overlapping strokes 10 – 25 %**
  - Avoid circular wiping pattern.

- **Use folded wipe**
  - Change to clean surface regularly.

- **Low particulate or 100% polyester wipes**
Cleaning and disinfection methods

• Carry out preferably at a natural break.

• By trained operators – preferably who work in the room.

• Disinfection
  – Apply uniform pressure
  – Leave visible film of disinfectant on surface

• Residues $\rightarrow$ rinse down

• Both cleaning and disinfection should always be completed SLOWLY to minimise the generation of particles
4A. Grade A zones

Typical cleanroom routine for isolators and LAF:

- **During sessions:**
  Constant spray and wipe with sterile IPA or Denatured Ethanol

- **Every Day:**
  Daily spraying with biocides (rotation) – contact time.
  Use ICT for areas difficult to reach.

**ALL DISINFECTANTS USED IN GRADE A AND B AREAS SHOULD BE STERILE PRIOR TO USE**
Grade A zones
Grade A zones

Typical cleanroom routine for isolators

• Consider special tools for awkward places.

• Procedures should be in place to ensure a tool is used correctly and does not introduce contamination.

• Cleaning can be carried out with an isolator “open”, disinfection or disinfection combined with cleaning should be carried out with the isolator “closed”

• Be careful of areas which are scratched or chipped
4B. Grades B,C and D

- Daily wiping of floors with sterile rotational biocides.
- Rinse down afterwards if necessary
- Daily disinfectant horizontal surfaces
- Weekly surface disinfection of chairs, lab benches, frames, etc...
- Weekly/monthly disinfection of walls and ceiling
- For large areas the use of sterile concentrates should be considered
- Consider flat mop heads with sterile disposable pads made of low particulate material or 100 % polyester.
Frequency of cleaning/disinfection

• Dependent on grade
• Decide from validation, monitoring
  – Contact plates or swabs
  – Settle plates
• Dependent on workload
• Critical and horizontal surfaces most frequently
5. Validation of Biocides

• To establish:
  – Efficacy: hard surface test EN 13697 or EN16615
  – In use concentration
  – On in house micro-organisms
  – Compatibility with cleanroom material
  – Compatibility with equipment
  – Personnel instructions, SOP’s
  – Residues, comfort ....
Questions