Microbiology and Cleaning Validation

-From a Microbiologist point of view

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Agenda

1. Short presentation
2. Microbiological Requirements
3. Microbiological methods used in CV
4. Objectional organisms
5. Microbiological aspects of a CV strategy
6. Questions
Microbiological Requirements

• Why

Patient safety:
Microorganisms and microorganisms by-products such as toxins and pyrogens can be harmful to patients

Product quality:
Presence of Microorganisms and microorganisms by-products can alter and degrade the product affecting the drug effectiveness and stability
Microbiological Requirements

Cleaning validation must demonstrate that harmful residues or organisms are properly removed during cleaning to predetermined safety levels, thus eliminating contamination of manufacturing equipment.

- Cleaning validation should be performed in order to confirm the effectiveness of a cleaning procedure (Eudralex, volume 4)

- Equipment cleaning/sanitization studies should address microbiological and endotoxin contamination for those processes where there is a need to reduce total microbiological count or endotoxins in the API, or other processes where such contamination could be of concern (ICH Q7)

- The data should support a conclusion that microorganisms and residues have been reduced to an ‘acceptable’ level (FDA)
Microbiological Methods used in CV

- Bioburden
- Microbiological swabs
- Microbiological ID
- Test for Endotoxin
Bioburden

- Indirect method, result reported in CFU/volume
- Membrane filtration of rinse samples
- Filter transferred to growth medium
- Incubation 30-35 degrees C for ≥3 days
- Limits refer to the type water used to rinse the equipment
Bioburden – CV issues

Advantages:
• Sample very large areas

Issues:
• Incubation time  = waiting time
• Short shelf live of samples
• Contaminations are rarely evenly distributed
• Method suitability test required
• Rinse samples not always clean, resulting in false negative results
Microbiological Swabs

• Direct sampling method
• Requires surface challenge test on used surfaces

Disadvantages:
• Assumes evenly distributed contamination
• Low recovery
• Low reproducibility
• Covers small area

• Only for investigational purposes of areas with high concentration of microorganisms
Microbiological ID

- Identification of isolated organisms from membrane filtration or swabs
- Different methods: Eg. Morphological, Classical, Sequencing, Typing
- For investigational purposes to find source of contamination
Endotoxin

- Endotoxins are large molecules consisting of a lipid and a polysaccharide – they are also called LPS’s
- Found in the outer membrane of Gram negative bacteria
- Release require cell lysis
- Elicit strong immune response in animals, fever, diarrhea ,....
Endotoxin testing (LAL)

- Limulus amebocyte lysate (LAL) is an aqueous extract of blood cells (amoebocytes) from the horseshoe crab, *Limulus polyphemus*.
- LAL reacts with bacterial endotoxin. This reaction is the basis of the LAL-tests that are used to detect and quantification of endotoxin

  - Four basic LAL-test methodologies: Gel-clot, colormetric, turbidimetric, and chromogenic

  - All sensitive assays to inhibition and enhancement affecting recovery pH, residues, product, solvents in samples

Challenges in cleaning validation. Samples suppose to be water, but......

Can indicate deviations in the cleaning process
Objectable Organisms/Organisms of Concern

- Issue in the production of non sterile products

- Organisms that are not wanted in the product or in the equipment

- The meaning of objectable has several facets that needs to be evaluates on a case to case basis of each manufacturer.
Objectable Organisms/Organisms of Concern

Two types:

- Microorganisms that represents a potential health hazard to patients or able to grow in the product *Eg. S. aureus, E. coli, Salmonella spp*

- Microorganisms that can harm the products effect or stability. *Eg. Bacillus spp*
Microbiological aspects of a CV strategy

- Analyse risk of contamination
- Equipment should be stored dry
- Consider equipment hold time

- Analytical method used to generate data
- Sampling method
- The sample transport and storage conditions should be defined

- Water vs rinse water
- Keep the communication line open
Questions?
Delta-Endotoxin

- Produced by the Gram positive bacteria *Bacillus thuringensis*
- Poreforming toxin produced during spore formation
- Not an LPS endotoxin
- LAL-test only detects LPS