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Debunking Myths on Cleaning and Disinfection

A few myths exist about using biocidal products that are an essential part of the cleaning and disinfection program. The aim here is to debunk those myths that can lead to an ineffective bioburden control program and a potentially higher risk of microbial contamination in cleanrooms.

Pathogenic microorganisms can harm patients taking the medicines, be it vaccines, sterile injectables, oral solid dose in tablets, ointments, and creams. Medical devices and even cosmetics are equally affected by the presence of objectionable microorganisms.

Annex 1 Draft (2020)

4.36 The disinfection of cleanrooms is particularly important. They should be cleaned and disinfected thoroughly in accordance with a written programme.[(1)]

It is a regulatory expectation that GMP manufacturing facilities have an effective cleaning and disinfection program to minimize the risk of microbial contamination.

The following six myths are discussed and explained.

Myth #1 – Effectiveness of Alcohol

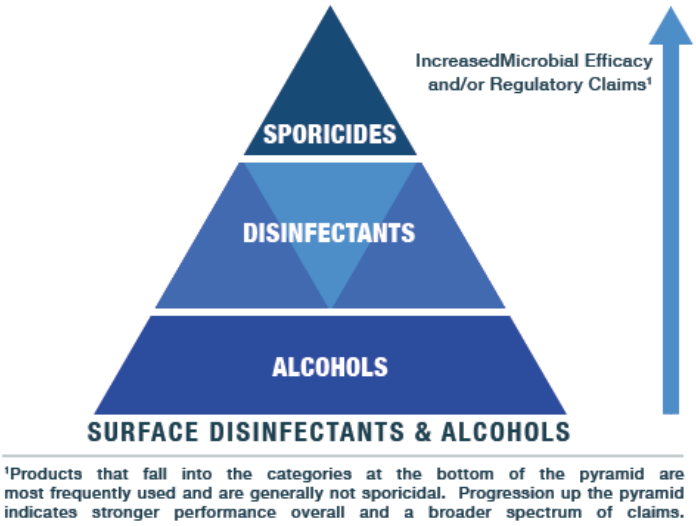
One misconception is that alcohol alone can be used for bioburden control in cleanrooms, including control of fungal and bacterial spores. Alcohols such as 70% Isopropyl alcohol (IPA) or 70% Ethanol are generally effective against vegetative microorganisms and enveloped viruses. However, they may not be effective against the hard to kill bacterial and some fungal spores as alcohol is not a sporicidal agent. Bacterial spores such as *Bacillus* have exosporium and spore coat, which enable them to be extremely tolerant against various stresses such as heat, chemicals, and harsh physical conditions, and typically require strong oxidizing sporicidal agents such as hydrogen peroxide and peracetic acid blend. Based on the above, alcohol alone is not effective in the bioburden control in cleanrooms, as it is not effective against bacterial spores. An effective cleaning and disinfection program typically requires the use of a sanitizing agent (example, 70% IPA or 70% Ethanol), one or two disinfectants (example, phenolics or Quaternary Ammonium Compound), and a sporicidal agent (example, Hydrogen peroxide/peracetic acid blend) to control a broad spectrum of microorganisms including the hard to kill bacterial spores.

Myth #2 – Disinfectant Rotation & Microbial Resistance

There is considerable debate and misconception about the rationale for disinfectant rotation and microorganisms developing resistance to disinfectants. Is the rationale of disinfectant rotation to prevent microorganisms from developing resistance to a disinfectant? There is documented evidence that bacteria can develop resistance to antibiotics[(2)], which leads to whether bacteria can also develop resistance to biocidal agents.

To date, there is no conclusive published data to suggest that microorganisms can develop resistance to disinfectants used in the cleaning and disinfection program[(3)]. As stated in USP 1072 'Disinfectants and Antiseptics' and PDA Technical Report 70 'Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities,' microbial resistance is less likely to occur in cleanroom settings, as the population of microorganisms is very low, and the biocidal agent used is potent compared to the antibiotic. The biocidal agent typically has numerous effects on several aspects of cellular physiology. Therefore, it is highly unlikely the microorganisms can develop resistance in a short contact time to the chemical agent used in cleanroom settings.

Diagram 1: Usage Pyramid



Since microorganisms are unlikely to develop resistance to disinfectants, the rationale for disinfectant rotation is actually to control the broad spectrum of microorganisms, including the hard to kill bacterial spores. From diagram 1, it is recommended to include a sporicidal agent such as sodium hypochlorite or hydrogen peroxide/peracetic acid blend in the cleaning and disinfection program.

A typical bioburden control program includes using a sanitizing agent, one or two disinfectants, and a sporicidal agent.

- 70% IPA or 70% ethanol can be used daily to sanitize pass-through items, parts, and surfaces such as glass and stainless steel.
- Disinfectants such as phenols or Quaternary Ammonium Compounds (Quats) are commonly used daily to control the cleanrooom's bioburden level. However, these are not effective against bacterial spores, such as *Bacillus cereus*, *Bacillus subtilis*, etc. Efficacy against mold spores such as *Aspergillus brasiliensis*, *Penicillium chrysogenum*, etc., would depend on the concentration and contact time of disinfectants used.
- Sporicidal agents such as sodium hypochlorite or hydrogen peroxide/peracetic acid blend need to be used periodically such as weekly or monthly, to control the fungal and bacterial spores. The usage frequency would depend on the environmental monitoring results. Frequent isolation of spore formers will trigger an investigation into the root cause, and an increase in the usage frequency of a sporicidal agent may be required depending on the outcome of the investigation.

Myth #3 – Volume of Disinfectants Used

Does a higher volume of disinfectant use equate to better efficacy? Not necessarily. Ultimately, efficacy is determined by the type of antimicrobial actives, the formulation of the disinfectant, the surface temperature, the concentration of disinfectant, the type of cleanroom surface, the wet contact time, and the type of microorganisms. Applying a greater volume of disinfectants can increase the contact time, and leave behind more residues on the surfaces if the disinfectants contain non-volatile compounds and are known to have residues. It can also lead to safety issues such as sticky/slippery floors and disinfectant wastage.

It is typically sufficient to maintain the wet contact time determined in the coupon study during the disinfectant coupon testing.

Myth #4 - Cleaning and disinfection: One or two-steps?[4]

Cleaning is the removal of residues or contaminants from the surfaces, whereas disinfection is the inactivation or killing of microorganisms. Should there be a cleaning step first before the disinfection step? The answer to this question depends on the following factors:

- Type of disinfectants used - If the disinfectants contain surfactants that facilitate the removal of residues from the surfaces. Since this type of disinfectant is formulated to combine cleaning and disinfection into a single step typically, no pre-cleaning is required unless there is a high level of visible residues/soils on the surfaces.

  According to the US Environmental Protection Agency (EPA), "an antimicrobial agent identified as a 'one-step' cleaner-disinfectant, cleaner-sanitizer, or one intended to be effective in the presence of organic soil, must be tested for efficacy by the appropriate method(s), which have been modified to include a representative organic soil such as 5% blood serum".

For disinfectant registration in US, US EPA specifies the AOAC International test methods to generate the disinfectant's efficacy data. The testing conditions may include the dirty conditions in the presence of 5% serum[5]. The test conditions related to the temperature, contact time, use dilution, and clean/dirty conditions are typically reflected on the disinfectants' labels.

For disinfectants without surfactants in the formulation or not effective as a cleaner, a pre-cleaning step is normally recommended to remove visible soils before disinfection.

- Amount of residue on the surface – Certain manufacturing processes such as those related to active pharmaceutical ingredients (API), tableting, powder filling process, etc., and with inadequate containment systems may generate high amounts of visible residues on the cleanroom surfaces. In this case, pre-cleaning is typically required before the disinfection step. A pre-cleaning step can involve the use of HEPA filtered vacuum cleaner, water, or a detergent. Manufacturing processes with an adequate containment system generate very little residues on surfaces. In this case, there is no need to have a pre-cleaning step before disinfection.
- Interaction of residue with disinfectants. Pre-cleaning needs to be performed if there is evidence of adverse interaction of residues with the disinfectants, which can reduce the disinfectants' efficacy.
- Application method – Mechanical wiping action facilitates cleaning and disinfection. For spraying or fumigation (even with disinfectants that contain surfactants with cleaning ability) to be effective, the surfaces generally need to be pre-cleaned to remove any high level of residues.

A risk assessment to justify a one or two step cleaning and disinfection procedure needs to be conducted based on the above considerations to determine whether a pre-cleaning step is required before disinfection[4].

Myth #5 – Is Fumigation Alone Sufficient ?

There are various ways to apply disinfectants onto cleanroom surfaces. Below are some of the application methods.

- Mopping
- Wiping
- Fumigation
- Spraying

Understanding type of application methods' advantages and disadvantages will enable us to use the appropriate method suited to each facility's specific needs and processes.

The most common method of application is the manual wiping and mopping method. This method can remove the microbial residues and other contaminants from the cleanroom surfaces, equipment, and other non-product contact surfaces through the mechanical wiping action. However, the limitation is not able to reach hard to reach cleanroom areas.

Fumigation has good coverage, but it lacks the mechanical action to remove the microbial contamination and soil from the surfaces. Because of this reason, fumigation alone is not recommended as there may be residues left on the surfaces, and the surfaces generally need to be pre-cleaned before fumigation as residues left on the surfaces may affect the efficacy of fumigation. Fumigation can be used periodically to complement manual wiping and mopping methods. This combination can ensure the removal of microbial contamination and soiling/particles from the surfaces, and at the same time, provide good coverage. Fumigation can also be used to quickly bring down bioburden levels in cleanrooms during a microbial excursion or new facility startup.

Spraying is more commonly used in the material transfer process, where the sanitizer/sporicide is sprayed onto the material's packaging or the part's surfaces. Wiping may not be necessary if the packaging is discarded after the material is used. Wiping with IPA may be used after the contact time to remove any residues and dry the surfaces.

Myth #6 – Application Technique

The root cause for recurring fungal and bacterial spore contaminations in cleanrooms can often be related to the improper techniques for wiping and mopping in cleanrooms.[6] There also needs to be a consistent way of wiping. If wiping in a non-controlled circular motion, the result is contaminating the surface (instead of cleaning), as seen in diagram 2. The correct wiping method is the pull and lift method, with about 20 to 30% (or 2 inches) overlapping strokes (refer to diagrams 3 and 4). The contaminants are removed more effectively using consistent long overlapping strokes compared to the method using a circular motion. It is important to have training conducted to the personnel involved in the cleaning and disinfection process to use the correct method.

Diagram 2 - Result of Wiping in Non-controlled Circular Motion[6]

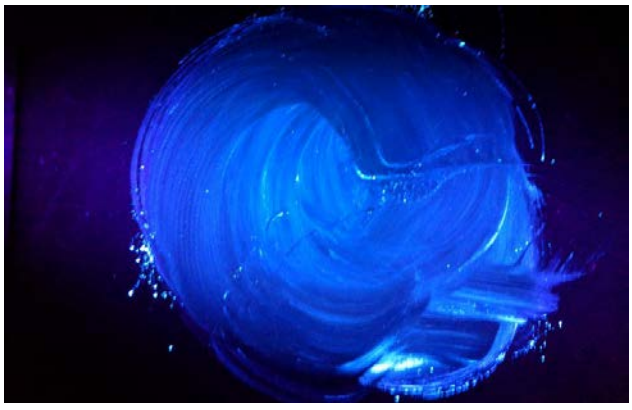


Diagram 3 – Single Pass Stroke[6]

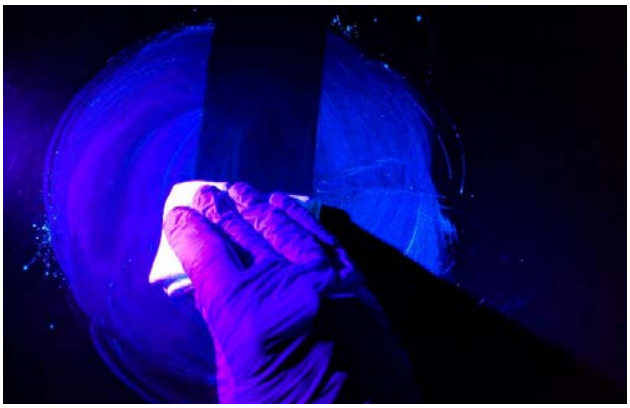


Diagram 4 – Importance of Overlapping Stroke[6]



Cleaning and disinfection procedures should incorporate the cleaning method and technique on wiping and mopping clearly. Strict adherence to systematic and methodical technique is required to achieve consistency in the bioburden control effectiveness.

Conclusion

Designing a robust cleaning and disinfection program in a GMP facility requires the understanding of various key elements related to cleaning / disinfecting agents. These key elements include, but not limited to, the efficacy of various chemical agents, the rotation strategy, determination of the requirement of a pre-cleaning step before disinfection through risk assessment, and appropriate application method and technique. This will ensure the development and implementation of a robust cleaning and disinfection program that can consistently achieve successful regulatory inspections by any regulatory agency.

References

1) Annex 1 Draft ‘Manufacture of Sterile Medicinal Products’ (2020).

2) United States Pharmacopeia 43 (USP) <1072> –Disinfectants and Antiseptics. The United States Pharmacopeia Convention/National Formulary, Rockville, MD.

3) PDA Technical Report No. 70 (2015). Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities. Available from Parenteral Drug Association, Inc.

4) Walid El Azab (Mar 2019) Justified Process for Cleaning and Disinfection. Cleanroom Technology.

5) Environmental Protection Agency, Pesticides: science and policy: efficacy data requirements supplemental recommendations, access on October 18,2018: https://archive.epa.gov/pesticides/oppad001/web/html/dis-02.html

6) Jim Polarine JR., Joe McCall (Sep 2018) Cleaning and Disinfecting Cleanrooms: Critical Employee Training. Controlled Environments