Microorganism Contaminants – Unique, Insidious and Dangerous©
By Lisa Strickland

It might not be too dramatic to state that bacteria, viruses, fungi and spores represent a unique, insidious and dangerous class of contaminants – unique in that under the right conditions the contaminant population can multiply over time - insidious in that these organisms can penetrate into small openings and crevices that can persist for long periods of time– and dangerous in that they can affect human health. So these contaminants must be destroyed and/or removed to achieve the desired cleanliness and aseptic condition. That's the role of disinfectants.

If there were only one chemical agent – i.e. one disinfectant – available to accomplish our objective, life would be simple. Unfortunately, there are many products to select from. So how to choose? Do we base our decision on chemical structure? Personnel protection issues? Type of organisms to be destroyed? In this paper, we'll focus our attention on these questions and others.

Chemical agents that destroy microorganisms can be termed "biocides"; it is useful to categorize them in terms of potency. **Sanitizers**, such as alcohols, can reduce microbial contamination by as much as 99.999% (known as a 5-log reduction), but are ineffective against spores. **Disinfectants**, such as phenolics and quaternary ammonium compounds provide 100% kill of vegetative bacteria, some fungi and viruses but are also ineffective against spores. To destroy spores and achieve 100% kill of all microorganisms requires **sterilants** – aldehydes or strong oxidants such as bleach or hydrogen peroxide.[*]

Finding the optimal chemistry for each environment is critical to removing these creative and complex microorganisms.

**The great bug hunt.**
Every facility must determine the resident micro flora unique to each environment. The United States Pharmacopeia (USP) provides guidance on microbial control and testing. Specifically, USP <1072>[1],"Disinfectants and Antiseptics", and USP <797>[2], "Pharmaceutical Compounding—Sterile Preparations" provide guidance on identifying key organisms in critical areas. It is crucial in testing to determine the organisms down to the genus and species level. Identifying the type of microorganisms and the number will provide the framework on which to build your microbial control program.

**Now what**?
Once the microorganisms have been identified, you can select the proper biocide solution for your environmental isolates and surface materials. Things to consider:

- Did you discover any spores in your testing? If so, it is critical that a surface sterilant be employed.
- Personnel safety. Many biocides are eye and skin irritants, unpleasant to use, and toxic. It is very important when choosing the application mode (fogging, spraying, wiping, mopping or

[*]These 3 classes conform, respectively, to low-level, intermediate-level and high-level disinfectants as categorized by the Center for Disease Control (CDC). The reader is cautioned not to confuse the meaning of the word "disinfectant". In this paper we will use it as defined above, not as a CDC descriptor.

immersion), to be aware that these applications can create situations that are hazardous to personnel.
- Surface contact time and material compatability. Dwell times can vary significantly depending on the particular biocide and the specific isolate to be destroyed. The effective contact time can be determined by following the biocide's label recommended claims or performing an in situ sanitization validation.
- Several formats of chemical disinfectants are available for your convience; ready to use, concentrates, and presaturated wipes. Sterile solutions of biocides are commercially available as well. These solutions are aseptically sterile-filtered, and/or gamma-irradiated to provide the requisite Sterility Assurance Level (SAL).

**Selection**
There are three important components to chemical selection; chemical effectiveness, compatibility with substrates, and safety to personnel. There are numerous biocides available that can offer a broad spectra of activity to kill susceptible pathogenic species. Arranged alphabetically, and described more fully below, are some of the most commonly used biocides found in critical environments.[3] [4]

**Alcohols** are sanitizers commonly used as a skin antiseptic. Of the available alcohols, isopropyl alcohol (IPA) is most often employed. Typical IPA concentrations vary between 60 – 85%. Most commonly used is 70% IPA, because there is enough water in the solution to allow it to effectively penetrate the pathogenic cell. A minimum contact time of 10 minutes is recommended when using IPA. The quick evaporation of IPA is a major disadvantage as a biocide, because the concentration diminishes before the recommended dwell time can be met. Conversely, because of the volatility, IPA is an exellent option to clean and dry equipment without leaving a residue. [5] [6]

**Aldehydes** are powerful and aggressive disinfectants that can be used effectivly as a sterilant. In concentration aldehydes are highly toxic to personnel, and require a long contact times for sporicidal claims. A typical aldehyde, gluteraldehyde, can require up to 10 hours of exposure at a concentration of 2% to kill *Bacillus subtilis*. Aldehydes are ideal for use on equipment that can be submerged for periods of time, under conditions which can keep irritating vapors at a minimum. Some countries have banned or restricted the use of aldehydes because of their safety profile as carcinogens.[5] [6]

Inorganic **chlorine & chlorine compounds** solutions are broad-spectrum biocides that can be used as a disinfectant or a sterilant. Chlorine chemistries are inexpensive, readily available, and relatively fast acting. However, these chlorine solutions are corrosive, unstable over time and rapidly lose activity in the presence of heavy metals found in the environment. Chlorine solutions have a high toxicity profile and should be used in well-ventilated areas. Sodium hypochlorite (NaOCl), the most commonly available chlorine solution, can be found in a range of concentrations from 1 to 35%. Typically concentrations for sodium hypochlorite are 1 to 5%. A 1% solution provides approximately 10,000 ppm of free chlorine. As little as 5 ppm will kill vegetative bacteria. Unfortunately to kill spores the concentration must be 10-1000 times greater.[5]

**Hydrogen peroxide** is a potent biocide that is environmentally friendly because it degrades to water and oxygen. Peroxides are rendered ineffective in the presence of organic and inorganic soils, so pre-cleaning is required to achieve the desired reduction in the microbial population. You can also achieve disinfection with lower concentrations of peroxides.[6] In concentrations as low as 0.5%, hydrogen peroxide can be combined with other ingredients to dramatically increase its germicidal potency and cleaning performance. These chemistries are effective with short contact times an offer an excellent health and safety profile.[11] Sterilization can be achieved with hydrogen peroxide concentrations of 35-50%. Peroxides can also be used in the vapor form, vaporized hydrogen peroxide (VHP), and are very effective in sporicidal cleaning.[6]

Hydrogen peroxide blended with **Peracetic acid** (PAA) is very effective at low concentrations and degrades to acetic acid and water. It is more effective than peroxide alone because it is not inactivated in the presence of soils. When high concentrations of this biocide are present, adequate ventilation is required. The combination of hydrogen peroxide and peracetic acid is an unstable solution; therefore, concentration testing must be performed prior to application.[6]

**Phenolics** are broad range disinfectants that are used on environmental surfaces. Substituted phenolics (e.g. p- t – amylphenol) are employed because they reduce the corrosive, toxic and carcinogenic characteristics of the parent phenol molecule. Standard concentrations are 2 to 5% with contact times of 5 to 10 minutes. These biocides are commercially available in low pH, high pH, and buffered solutions with added detergents to provide one-step cleaning and disinfecting.[5]

**Quaternary Ammonium Compounds,** commonly called quats, are effective at concentrations of 0.1% to 2% as disinfectants to clean counters, floors, and walls. While quats are non-irritating and non-corrosive to surfaces, most are not effective in removing biofilms, and have poor biodegradability. Typical quat solutions require 10 minutes of contact time to kill microorganisms, and leave surfaces with a residue that must be removed after disinfection.[6]

Below is a generalized chart that summarizes the effectiveness of various chemistries against the different classifications of microbes. Concentration and contact times may vary to obtain sporicidal claims.[5] [6]

**Biocide Activity**

| | Alcohols ex. Isopropyl Alcohol (IPA) | Aldehydes ex. Glutaraldehyde | Chlorine and Chloride Compounds ex. Sodium hypochlorite | Hydrogen Peroxide | Hydrogen Peroxide / Peracetic Acid | Phenolics ex. p- t – amylphenol | Quaternary Ammonium Compounds ex. Benzalkonium chloride |
|---|---|---|---|---|---|---|---|
| Bactericidal | Good | Good | Good | Good | Good | Good | Good |
| Fungicidal | Good | Good | Good | Good | Good | Good | Good |
| Virucidal | Good | Good | Good | Good | Good | Good | Fair |
| Sporicidal | None | Good | Good | Good | Good | None | None |
| Fast Acting | No | No | Yes | Yes | Yes | No | No |
| Residues | No | Yes | Yes | No | No | Yes | Yes |

**Surfaces**
Surface characteristics such as texture, porosity and durability can affect the biocide that is chosen. Most biocides are safe for cleanroom surfaces but can become corrosive if the biocide residue is not removed in a timely manner. Typical surfaces that are seen in critical environments include stainless steel, glass, vinyl (curtains), Plexiglas®, epoxy-coated gypsum (walls and ceilings), Fiberglass-reinforced plastic (wall paneling), Tyvek®, and terrazzo floors. The effects of the biocides will vary depending on concentration and frequency of use. Stainless steel can pit and rust with the use of alcohols, aldehydes, chlorine compounds, and hydrogen peroxide/peracetic acid blends. The detioriation of the stainless steel may lead to “hot spots” of microbial growth. Alcohols, chlorine compounds, hydrogen peroxide, hydrogen peroxide/peracetic acid and phenols can be absorbed by rubber compounds, which leads to brittleness and decomposition over time.[5]

**Safety**
When creating a microbial control plan it is important that the biocidal objectives be aligned with concerns of personnel safety. Biocides are commonly eye and skin irritants, unpleasant to use, and highly toxic.

Below is a chart generalizing biocides toxicity levels to personnel based on industry standard MSDS at the highest concentrations of commercially available products.[5]

| | Alcohols ex. Isopropyl Alcohol (IPA) | Aldehydes ex. Glutaraldehyde | Chlorine and Chloride Compounds ex. Sodium hypochlorite | Hydrogen Peroxide | Hydrogen Peroxide / Peracetic Acid | Phenolics ex. p- t – amylphenol | Quaternary Ammonium Compounds ex. Benzalkonium chloride |
|---|---|---|---|---|---|---|---|
| Irritant | Medium | High | High | High | High | Medium | Low |
| Corrosive | Low | High | High | High | Medium | Medium | Low |

**Rotation**

Rotation of disinfectants has received significant attention by researchers, parenteral manufacturers and regulatory agencies. It is now generally accepted that microorganisms within cleanrooms do not develop resistance to disinfectants, as was believed[6 7] since the disinfectants are designed to provide 100% kill of these organisms. If there are no organisms left to mutate (as might occur, say, in the human body during antibiotic treatments), there is no resistance to be developed. Sutton[8] stated it very explicitly: "The need for rotation of disinfectants in a pharmaceutical cleanroom sanitization program is unsupportable from a scientific basis". In principle there should be no need to rotate disinfectants, as long as the environmental isolates have been properly determined, the disinfectants have demonstrated the necessary kill capability, and used properly.

Despite these findings, facilities still rotate disinfectants (i.e. switch back and forth from phenolics to quats on a pre-determined schedule). Reasoning for this may be historical ("we've always done it that way") or because the regulatory agencies seem to prefer to have disinfectants rotated[9].

Most facilities do choose to incorporate a sporicidal treatment into the disinfection protocols on a regular basis. Daily application of sporicidal agents is not generally favored because of their tendency to corrode equipment and because of the potential safety issues with chronic operator exposure. Weekly or monthly application of sporicides is usually considered adequate. [2]

**Application**

Clean-Rinse-Disinfect-Rinse

The efficacy of many disinfectants will be greatly diminished in the presence of organic matter, so it is critical to clean surfaces before applying a disinfectant. The physical action of cleaning and rinsing will remove all gross contamination as well as a large number of microorganisms. The addition of a surfactant (traditionally, a non-ionic surfactant or enzymatic cleaner) to the cleaning process will allow for wetting and removal of biofilms. Rinsing is typically performed using water for injection (WFI) or 70% IPA. Since IPA is quite volatile it can remove residues and dry the surfaces simultaneously. With lightly soiled surfaces, it is possible to reduce time and effort by using a biocide that can both clean and disinfect. The disinfectant of choice will then be applied, paying close attention to the contact times needed to kill the environmental isolates. It is important when applying disinfectant that the surfaces remain saturated for the required dwell time. Disinfectant residues should be removed using WFI or 70% IPA to prevent the buildup of disinfectants or cleaning chemicals.[5]

**How to Wipe and Mop**

With wipes there is a basic application techniques used for cleaning, disinfection, residue removal, and drying. Always begin cleaning from the area with the lowest contamination to the area of highest contamination. Fold a wipe into four distinct quadrants (Figure 1). Always present a clean wipe surface to remove the contamination with each unidirectional stroke. Each pass of the wipe should overlap the previous by about 10-25%. The wipe should be refolded after each pass to present a clean wipe surface. Use the lift and pull method seen in the Figure 2 to insure the effective removal of contaminants. Figures 3 and 4 show the actual pull and lift technique in use. Do not wipe in a circular pattern, as this will lead to the re-depositing of contaminants onto freshly cleaned surfaces.

Mopping has two application techniques that can be employed. The pull and lift method is effective for using with flat-paddle style mops, for cleaning walls, ceilings, and floors. Clean in unidirectional strokes that overlap the previous stroke by 10 to 25%. Again clean from the area with the lowest contamination to the area of highest contamination (i.e. on a wall you would clean from the top of the wall to the bottom – using only vertical strokes or horizontal strokes). For string mops use a modified S-motion to apply a solution.[5]

**Prove It**

Now that you have a way to disinfect, the next critical step is to prove it. It is important in USP <1072> and <797> that you show that your disinfection protocol is effective. Test the microbial control plan using your resident isolates and the ones identified by the Association of Analytical Communities (AOAC). Testing is required to demonstrate the disinfectants effectiveness in vitro and the actual performance under operating conditions. The standard tests employed are: in vitro lab testing, in vitro real use tests, and in use tests. Once the testing is complete you are ready to put your microbial control plan in action. [5]

**Summary**

The goal of rendering critical surfaces free of microorganisms is an on going challenge. Using biocides to eliminate these unique, insidious and dangerous microorganisms will depend on the execution of the protocols, validation of the processes, and the continued monitoring of environmental isolates.

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