STERIS

Understanding the Impacts and Science Behind Disinfectant Residue

Dan Klein, Technical Services Director
STERIS

ABSTRACT

EU GMP Annex I, specifically states that "Cleaning programs should effectively remove disinfectant residues". Although residue in controlled environments, and disinfectant residue in particular, has been around since the advent of cleanroom disinfection, there is currently an increased sensitivity towards understanding and mitigating cleanroom residues.

This poster will focus on the science and microbiology of disinfectant residues including potential impact on rotational sporicides, the likelihood of trapping microorganisms, and best practices for addressing residue in the cleanroom. The active ingredients and surfactants that make up disinfectant residue of a formulated disinfectant are key components of a complex formulation. By understanding the nature of disinfectant residue and their impacts, continuous improvements can be made to optimize an overall cleaning and disinfection program.

Disinfectant Residue in Life Sciences Companies

Confusion about residue persists, and the science behind residue is often misunderstood.

Disinfectant residue is not new and is composed of key components of the disinfectant formulation, including the surfactants necessary for a 1-step cleaner/disinfectant and the active ingredient molecules that allow for the antimicrobial efficacy of the disinfectant.

Residue Components	Surfactants	Active Ingredients
Purpose	Ability to clean a surface	Killing of microorganisms

Figure 1: Components of Disinfectant Residue

Disinfectant residues should be studied with good scientific data in order to determine their impact on a rotational disinfection program. Little data exists to fully understand the impact, if any, of disinfectant residue on liquid sporicides used in Life Sciences companies.

This poster addresses several common myths that have been raised during discussions on this topic including the impact on liquid sporicides, the time it takes to build up, and the potential ability of disinfectant residue to trap or harbor microorganisms.

Monitoring of Disinfectant Residue

The most practical and widely accepted standard for disinfectant residue detection remains visually clean. Use of analytical detection to determine the presence of disinfectant residue can be misleading or provide conflicting data.

Disinfectant residue does not dry evenly on a surface, so the use of a sampling method over a large surface can give misleading results based on the micro-contour of the surface and application technique.

Analytical methods tend to be non-specific. Conductivity, for example, will measure more than just the surfactants or active ingredients found in a formulation. Creating analytical values for disinfectant residue levels collected from inconsistent measurements can create a standard that must be met in the future.

Disinfectant Residue Myths

Does disinfectant residue trap microorganisms?

In these studies, coupons were treated with the test disinfectant, either 1X or 10X, and allowed to dry after each application. The dried residue was then inoculated with either 10 µL or 100 µL of a *Pseudomonas aeruginosa* solution and $\log_{10}$ reductions calculated per baseline.

Although it is difficult to design a study that conclusively shows that disinfectant residue is not capable of providing a protective or occluding condition for microbial contamination, this study demonstrates that the active ingredients, both quat and phenolic, found in the disinfectant residues studied here maintain their antimicrobial activity when resolubilized. It is difficult to imagine that microorganisms would be protected in this hostile environment.

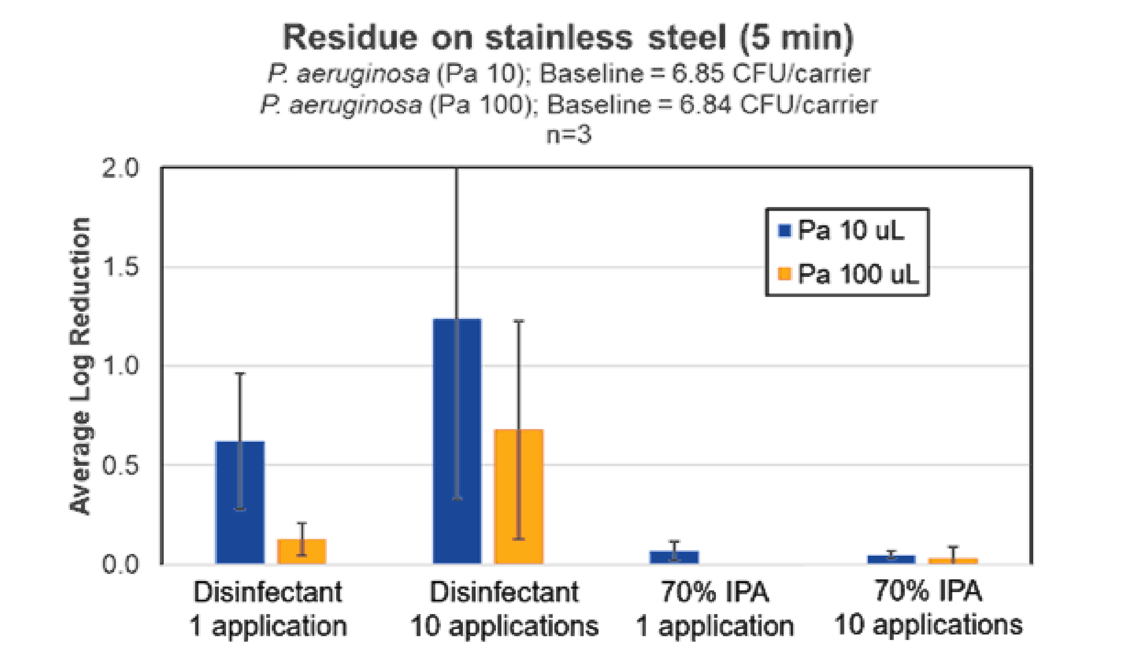

Figure 2: Efficacy of Residue from Low pH Phenolic Disinfectant

This study was expanded to include a low pH phenolic disinfectant, a quaternary ammonium compound disinfectant tested with *Staphylococcus aureus* in addition to *P. aeruginosa* with even greater efficacy exhibited.

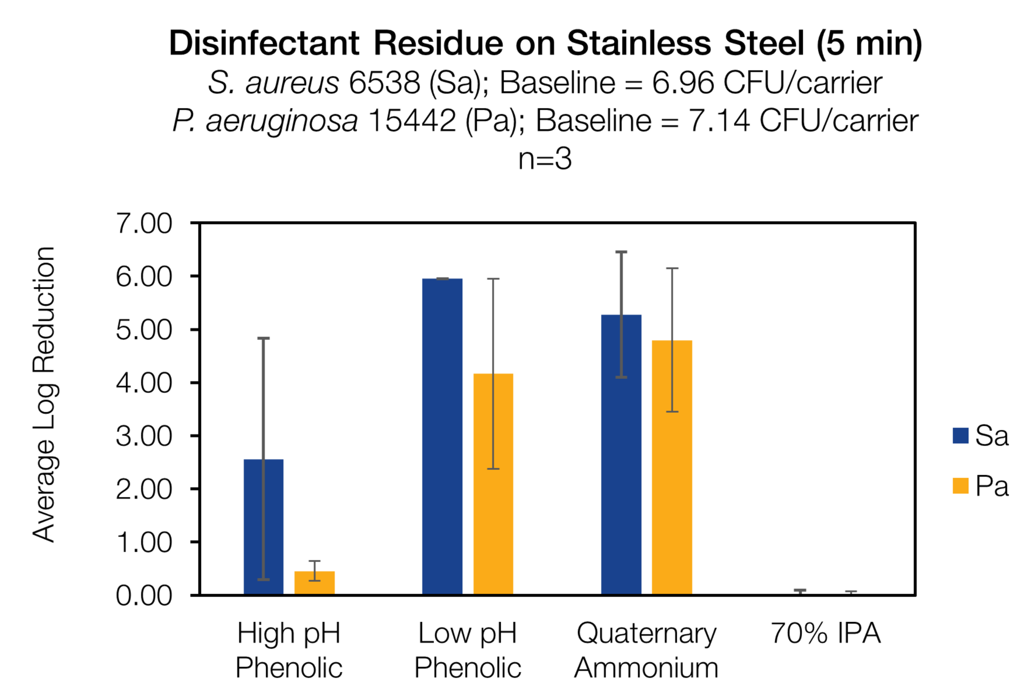

Figure 3: Efficacy of Residue from Multiple Disinfectants

Does disinfectant residue inhibit sporicides?

In this study, we looked at the impact of dried disinfectant residue on the efficacy of a Peracetic acid/hydrogen peroxide based sporicide. A worst-case 1:20 dilution of each disinfectant was prepared, and 2.36 g of the solution was placed into a weigh dish and dried in a biosafety cabinet. 5 g of sporicide was then added to the weigh dish. The mixture was then inoculated with a spore suspension of *Bacillus subtilis* at 3×10^8 colony forming units (CFU)/mL and left for the appropriate contact time; $\log_{10}$ reduction values were calculated and compared to a sporicide control with no residue added.

The results show no negative impact of the disinfectant on the sporicide efficacy, but instead suggest a slight synergistic impact from residual surfactant boosting the efficacy.

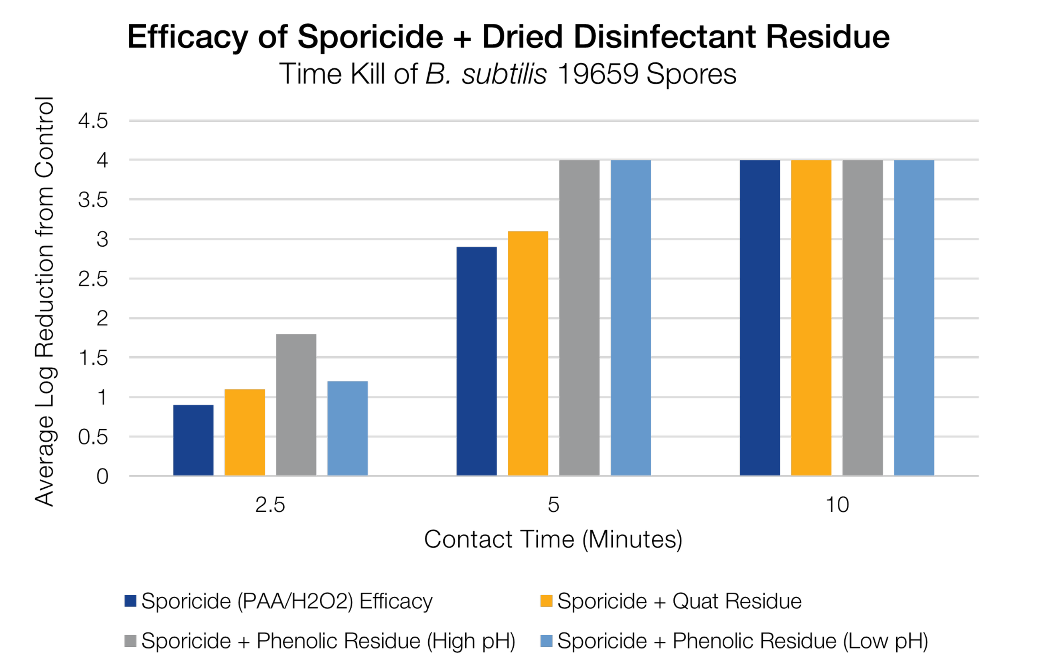

Figure 4: Impact of Disinfectant Residue on a Liquid Sporicide

Does disinfectant residue build up to extreme levels?

In this study, disinfectant was applied 15 times by wiping to 316L stainless steel coupons and allowed to dry completely after each application. Analysis was performed at 1, 5, 9, and 15 applications using total organic carbon (TOC) analysis.

Using this wiping application, it appears as if residue levels stabilize as the wiping effectively removes some of the residue previously deposited on the surface, as would be expected with disinfectant wipes with surfactants based on their cleaning properties.

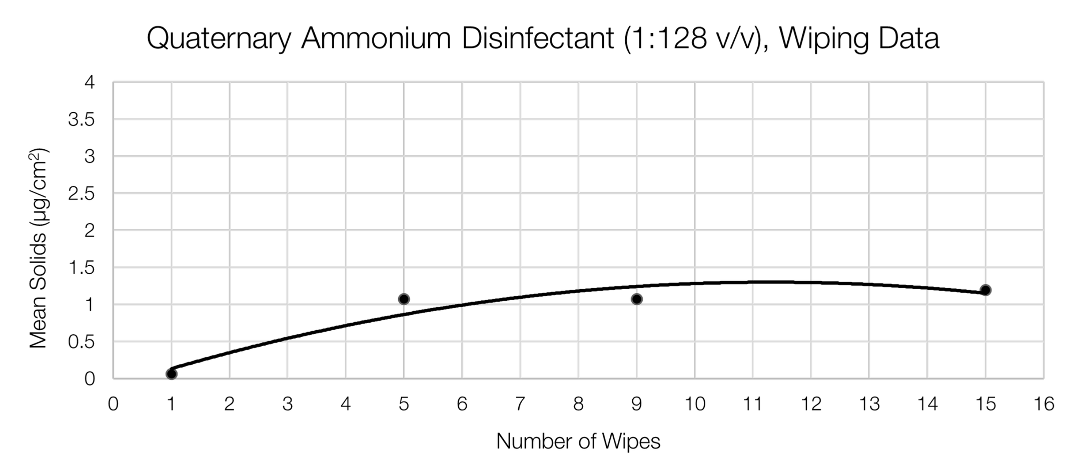

Figure 5: Build up of Disinfectant Residue After Multiple Wipes

Rinsing

A rinsing step should be incorporated into a disinfection program to prevent visible residue buildup and ensure that the facility remains ready for inspection. The frequency of rinsing to remove disinfectant residue should not be overly burdensome and can easily be incorporated into a rotational disinfection program on a monthly or other periodic basis.

The best way to remove any residues from the floors or walls is by using a mop wetted with water for injection with mechanical action. For smaller surfaces, wiping with an alcohol saturated wipe will be highly effective.

Using proper rinsing or wiping techniques is as important as using proper disinfection techniques. A bucket system for rinsing should be used in the same manner as a bucket system for disinfectant application.

A. Rinsing step using water for a three-bucket system:

The first bucket contains water (PW or WFI) (Water Rinsing Solution). The second bucket contains water (PW or WFI) (Water Cleaning Solution). The third bucket is empty (Waste).

i. Immerse the mop into the bucket "Water Rinsing Solution" and wring-out the mop slightly with the wringer. The mop should be wetted to wet the surface and remove detergent residue from the surface.
ii. The rinsing with the wet mop must be performed in overlapping strips in the form of a stroke from the back to the front (toward the door).
   a. Press on the mop to remove the residue on a defined area. The area is defined based on the capacity of the mop to wet the surface while removing detergent residue (e.g., 10-meter square).
   b. Note: If the surface is highly wet (pooling or did not dry within 1-2 minutes), do not allow the surface to dry, but use a dry mop to remove the excess solution from the surface.
iii. For further use of the mop, immerse it into the bucket "Water Cleaning Solution" and wring-out the mop slightly with the wringer in the third bucket and continue as described above, starting at step ii.
iv. Visually evaluate the floor to determine if a second water rinse is necessary.
v. When the rinsing is completed, dispose buckets' solution and clean the bucket and mops (if not single use).

Figure 6: Best Practices for Residue Removal with Mopping

Figure 7: Best Practices for Residue Removal with Wiping

The use of unidirectional, overlapping strokes for wiping is critical for both disinfectant application and residue removal.

Conclusion

Residue in a cleanroom or controlled environment can seem complicated based on a lack of understanding, aggressive marketing claims, and imperfect regulatory guidance. It is critical to fully understand key details about disinfectant residues including sources, characterization, detection, myths, and mitigation approaches that utilize the best scientific approaches and best practices in the Life Sciences industry. Our studies have indicated that excessive focus on addressing disinfectant residues at the expense of efficacy is not desirable.

References

1. European Commission. (2022). EudraLex - Volume 4 - Good Manufacturing Practice (GMP) guidelines, Annex 1.
2. STERIS White Paper, "Science Beyond the Surface".
3. "Use of Conductivity as a Tool for On-site Residue Management and Definition of Smart Cleanroom Regimes". Krause, et. al. EJPPS. Jan, 2023.
4. STERIS Laboratory Report #4354, "Interaction of Vesphene™ III se Phenolic Disinfectant Residue and Spor-Klenz™ Ready-To-Use Cold Sterilant".
5. STERIS Laboratory Report #4350, "Interaction of LpH™ III se Phenolic Disinfectant Residue and Spor-Klenz™ Ready-To-Use Cold Sterilant".
6. STERIS Laboratory Report #4346, "Interaction of Vesta-Syde™ SQ Quaternary Ammonium Disinfectant and Spor-Klenz™ Ready-To-Use Cold Sterilant".
7. STERIS Laboratory Report #4345, "Interaction of Vesta-Syde™ SQ 64 Ready-To-Use Quaternary Ammonium Disinfectant Residue and Spor-Klenz™ Ready-To-Use Cold Sterilant".
8. STERIS Laboratory Report #4344, "Determination of Vesta-Syde™ SQ Quaternary Ammonium Disinfectant on Surfaces due to Routine Application".