Application Method in Disinfectant Efficacy Testing (DET): Friend or Foe?

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ABSTRACT

Disinfectant validation is a critical component of a Contamination Control Strategy (CCS) for the aseptic medicinal product manufacturing industry. Disinfectant validation is also a clear expectation of various global regulatory bodies.[1,2] However, there is not a clearly defined best practice or method to detail how aseptic manufacturers should meet this expectation. Laboratory studies are necessary to qualify a disinfectant (i.e., liquid chemical disinfectants, sporicides, and sanitizers) for use in an individual manufacturing facility's classified areas. End user disinfectant qualification is accomplished through Disinfectant Efficacy Testing (DET), which involves in vitro laboratory testing on representative surface coupons for a facility against standard (e.g., ATCC (American Type Culture Collection) strains) and representative microorganisms.

The purpose of these studies is to determine and demonstrate the effectiveness of different disinfectants against the range of microorganisms that could be encountered in a facility. This ensures that disinfectants routinely used in a contamination control program can chemically inactivate the potential microbiological contaminants in a facility. It is imperative that the agents selected to design a contamination control program can effectively inactivate the full range of microorganisms, including challenging fungal and bacterial spores to protect the product, and ultimately patients, from microbial contamination. There are many different potential choices that can be made when designing a disinfectant efficacy study that require a deep understanding of the potential implications. These choices can have a serious impact on the reliability of the data that is generated, which thereby can affect the subsequent design of a contamination control program.

This poster will cover best practices in performing laboratory disinfectant efficacy testing (DET), focusing on why mechanical action and application method (e.g. wiping, mopping, spraying, etc.) should not be included in disinfectant efficacy studies, such as the inaccurate efficacy conclusions associated with including mechanical action and application method in laboratory disinfectant efficacy testing, and the scientifically sound, defendable option to evaluation application method and mechanical action in disinfectant validation--in situ field studies.

DET Regulatory Landscape

DET has been a clear regulatory expectation for decades. The FDA aseptic processing guide states, "The suitability, efficacy, and limitations of disinfecting agents and procedures should be assessed."[2] USP <1072> Disinfectants and Antiseptics states, "To demonstrate the efficacy of a disinfectant within a pharmaceutical manufacturing environment, it may be deemed necessary to conduct the following tests...This is considered necessary because critical process steps like disinfection of aseptic processing areas, as required by GMP regulations, need to be validated, and the EPA registration requirements do not address how disinfectants are used in the pharmaceutical, biotechnological, and medical device industries."[3] The 2023 revision of Eudralex Annex 1 also clearly demonstrates the expectation for aseptic manufacturers to perform DET stating, "The disinfection process should be validated."[1]

The regulatory guidance and guidelines around DET do not specify or dictate how studies should be performed. Additionally, there can be confusion regarding the interpretation of the regulatory guidance. This has led to DET being performed in a manner that is not scientifically sound, which can lead to inaccurate conclusions about various disinfectants' efficacy profiles and their potential misuse within a contamination control program.

The language of Annex 1 surrounding disinfectant validation has led some in industry to perform DET in a manner that can result in misleading data. Specifically, the following wording, "Validation studies should demonstrate the suitability and effectiveness of disinfectants in the specific manner in which they are used" (emphasis added), illustrates that it is a regulatory expectation that application method is evaluated as a part of disinfectant validation. However, there are multiple options to evaluate application method within a complete disinfectant validation, with one option standing out as being the clear best practice, as it is scientifically defendable. A complete disinfectant validation is comprised of:

- In vitro laboratory studies performed on surface coupons to qualify an appropriate wet contact time (i.e., disinfectant efficacy testing)
- In situ field studies (i.e., phase III studies described in EN 14885[7]) to demonstrate the effectiveness of the qualified disinfectants selected for a contamination control program in the specific manner in which they are used in the facility
- Ongoing Environmental Monitoring (EM) tracking and trending, to evaluate if the contamination control program is maintaining a state of control

Why Application Method Should not be Included in Laboratory Coupon Studies

Table 1: *Bacillus cereus* Data Demonstrating How Mechanical Action in DET Can Lead to Inaccurate Efficacy Conclusions

Organism	Method	Surface	Disinfectant /Contact Time	Log Reduction (≥2.0)
B. cereus spores	MicroFiber Mop-Mechanical Action	Stainless Steel	Phenol A /10 Minutes	2.2 (Pass)
B. cereus spores	No Mechanical Action	Stainless Steel	Phenol A /10 Minutes	-0.1 (FAIL)
B. cereus spores	MicroFiber Mop-Mechanical Action	Wall	Phenol A /10 Minutes	2.2 (Pass)
B. cereus spores	No Mechanical Action	Wall	Phenol A /10 Minutes	0.0 (FAIL)

Table 1 contains data for the same microorganism against the same disinfectants on the same surface materials with the same wet contact time, comparing the chemical inactivation of the disinfectant to the effect of mechanical action and physical removal of viable cells. Joseph Lister began using phenolics in 1865 as an antiseptic for surgery. Consequently, phenolics have been thoroughly studied, and it is well known that phenolic agents are not expected to exhibit efficacy against bacterial spores. The data in Table 1 with mechanical action and physical removal of viable spores leads to a log10 reduction that suggests that the phenolic is an effective sporicidal agent. However, when physical removal of spores (through mechanical action) is not included in the test and the chemical inactivation of the biocide is the primary study variable being evaluated, no reduction in bacterial spores was observed, aligning with over 150 years of understanding of phenols. When inaccurate efficacy conclusions are reached due to mechanical action being included in DET coupon studies, disinfectants can be used inappropriately, which can significantly increase contamination risk and potentially lead to serious adverse events for patients.

Additionally, when incorporating an application method into surface coupon studies, it is not possible to accurately represent the specific manner in which a disinfectant is used in a facility. Surface coupon laboratory studies involve small coupons (e.g., 2 cm, 5 cm), that make it impossible to evaluate the actual application method in a classified area. There is not an ability to effectively represent pull and lift motion of a wipe or mop and there will be differential pressure when applied to a small surface coupon compared to a large wall, floor, or isolator work surface, for example. Use of a spray application in a laboratory coupon study often involves fully saturating a surface coupon. However, in an actual facility, surfaces are not fully saturated using a spray application; different levels of interfacial tension between a surface and biocide will inevitably lead to beading of some disinfectants on the surface, rather than achieving confluent complete coverage of a large surface in a classified area. Another consideration is the potential impact of the subjectivity of individual laboratory operator technique in regard to applied force of mechanical action. In surface coupon testing a small volume, highly dense inoculum is applied to the surface coupon. Small differences in pressure of mechanical action by a wipe, for example, can be the difference between a passing log reduction and a failing log reduction. If a coupon is inoculated with 5×10^5 colony forming units (CFU)/0.05 mL, with a detection limit of <10 CFU and log reduction acceptance criterion of 3 log, differential removal of as little as 0.0005 mL of inoculum between two operators can shift a result from passing to failing and vice versa. Some methods that are intended for use by disinfectant manufacturers to register disinfectants for sale in specific geographic regions standardize this pressure through the use of a very specific dimension and weight granite block. This is necessary to ensure that products meet the performance bar for sale as a disinfectant, but this standard is not intended for disinfectant end users and does not represent the specific manner in which a disinfectant is used in a classified area.

This all demonstrates that the best practice for applying a disinfectant to a surface coupon in a laboratory study is by pipetting an aliquot of the disinfectant onto the inoculated area of the surface coupon, ensuring that the disinfectant remains only on the surface of the coupon and not immersing the entire coupon in disinfectant, following the methods in prescriptive surface disinfectant coupon testing standards, such as EN 13697 and ASTM E2197.[4,5] This allows for an evaluation of the chemical activity of a disinfectant irrespective of potential physical removal of viable cells or other artifacts associated with including an application method in laboratory studies, which leads to inaccurate efficacy conclusions.

Figure 1: Challenge of Representing Actual Application Method in Coupon Studies

Figure 2: Displaying Commonly Used 2 cm Coupon

In Situ Disinfectant Field Studies

In situ field studies demonstrate the suitability and effectiveness of the disinfectants in a contamination control program "in the specific manner in which they are used". Laboratory studies are neither suitable nor actually able to evaluate disinfectants "in the specific manner in which they are used". In situ field studies involve performing EM before and after a cleaning and disinfection event, typically surrounding situations that warrant a triple clean. In situ field studies involve evaluating the ability of the qualified disinfectants (sporicides are considered to be a special class of disinfectant) to effectively reduce worst case levels of microorganisms that are found in an actual facility and to effectively return the area to a state of control, based upon the area's classification. It is never recommended to intentionally and artificially introduce microorganisms (e.g., inoculate actual cleanroom surfaces with a microorganism suspension) as a part of an in situ field study. Opportunities to generate in situ field study data include EM Performance Qualification (EMPQ), planned shutdowns, construction and maintenance events, and natural disasters. EM samples are taken prior to cleaning/disinfection to determine baseline levels of microorganisms. EM samples can then be taken stepwise to measure the reduction of each application (e.g., after disinfectant application 1, after disinfectant application 2, and after sporicide application) or only taken before cleaning and disinfection and after the sporicide application. In situ field studies demonstrate the effectiveness of the qualified contamination control program in the actual facility, according to the facility's cleaning and disinfection procedures, by the actual personnel, in the specific manner in which the disinfectants are used.

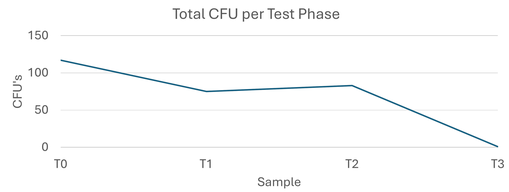

Figure 3: Example of In Situ Field Study Data[6]

Conclusion

An effective contamination control program is essential to maintaining the quality and safety of a manufacturing process and product. DET is the first step in establishing the contamination control program and a critical component of a CCS. It has been clearly demonstrated here that including application method and mechanical action in a laboratory DET coupon study can lead to inaccurate efficacy conclusions and is not representative of the specific manner in which a disinfectant is used in a classified area. Ultimately, the inclusion of application method in DET is not scientifically sound and does not achieve compliance with Annex 1. Evaluating the chemical activity of a disinfectant, irrespective of application method, allows for making effective decisions about designing and implementing a contamination control program. After qualifying a wet contact time for a disinfectant through laboratory studies, in situ field studies allow for demonstrating effectiveness of disinfectants in the specific manner in which they are used. Combined, these best practices allow for an actionable, defendable disinfectant validation based upon good science.

References

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