**Azzur Labs, LLC**

# Important Aspects to Consider When Performing a Microbial Cleaning Validation According to USP <1072>

Microbial cleaning validation. These three words can elicit a number of questions. Where to begin? Which tests need to be performed? What materials are needed to conduct the testing? What else needs to be considered? The answers to these questions will be discussed as they relate to the United States Pharmacopeia (USP) chapter <1072> Disinfectants and Antiseptics.

Where to begin? Cleaning is a process and there may be more than one way to validate the process, although the available testing guidance must be considered. Ultimately, the test of any validation process is whether the collected data show that the system performs as expected and the results consistently meet the acceptance criteria.[1]

Determining a disinfectant's ability to reduce the microbiological load of common pathogens, as well as environmental isolates, provides the user with the scientific data to support or deny established cleaning practices or to determine the contact time and concentration that is appropriate for use, prior to the cleaning procedures being implemented.

USP <1072> discusses the EPA requirement that companies who register a public health antimicrobial pesticide product must ensure the safety and effectiveness of their product. It is the manufacturer's responsibility to conduct the studies to prove that their products are safe and effective. AOAC International and the United States Pharmacopeia provide the official test methods for these studies.

Although the manufacturer's are required to prove that their products are safe and effective, it is the user's responsibility to provide evidence that the disinfectant is effective at reducing the microbiological load of environmental isolates on the hard surfaces found in their facility. Inappropriate use, ineffective cleaning and ineffective disinfectants can individually or in combination affect the quality of the product. It is important to ensure that the microbiological quality of the product is not compromised by the inability of the cleaning agent to perform as necessary.

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Which tests need to be performed? Before jumping into the actual testing, it is critical that the purpose of the cleaning validation and the data that is to be captured are clearly defined. First, the lethal and inhibitory concentrations as determined by the manufacturer must be confirmed. Second, there must be sufficient evidence that the disinfectant is effective that reducing a large microbiological load on the surfaces found in the facility. Finally, comparisons of the organisms present before and after cleaning with a new disinfectant must be conducted. To accomplish this, the three required tests used for determining the efficacy of the disinfectant as found in USP <1072> are the use dilution test, the surface challenge test and a comparison of the frequency and number of organisms recovered before and after the use of a new disinfectant.[2]

The use dilution test, which is performed by a laboratory, is used to determine the effectiveness of the disinfectant at specific concentrations and exposure times by inoculating the specific concentrations of disinfectant with the determined organisms. Each dilution is plated and the plates are counted. From this data, the lethal concentration and inhibitory concentration can be determined for each organism in the study.

The surface challenge test, which is performed by a laboratory, is representative of the actual use of the disinfectant in the facility. This type of test consists of inoculating surface coupons, made of the identical surface materials from the facility, with a specified population of an organism. The drying time of the inoculums should be considered, in order to mimic actual facility conditions. Then a predetermined volume of the prepared concentration of disinfectant is added to the surface coupon and is allowed to have the specified contact time. This may just be a contact time or this may be a wet contact time. If a wet contact time is required, additional disinfectant can be added to ensure the coupon remains wet for the entire contact time. There is also the option of cleaning the surface or just allowing for disinfectant contact on the surface. Opting to just do the contact time allows for a worst case scenario, ensuring that the required log reduction is achieved without the act of cleaning. A predetermined surface area is swabbed and the swab is transferred to a solution that has been proven to neutralize the disinfectant. From this point the swab is processed as required to free the organisms from the swab itself and the neutralizing solution is serially diluted to achieve countable plates. Multiple dilutions must be plated to determine the remaining population. Surface rinse samples can be used instead of swabs for the recovery.

The comparison of the frequency and number of organisms recovered before and after the use of a new disinfectant would be performed within the actual facility, as part of an environmental monitoring program. If a new organism is routinely being recovered, it needs to be determined whether or not this type of organisms was covered in the cleaning validation. If not, there is not sufficient proof that the disinfectant is effective against that type of organism and testing to include this new organism should be performed. Additionally, if a new disinfectant is introduced into the cleaning regimen, validation will be necessary.

Prior to performing any of the actual tests, a neutralization efficacy test must be performed. Neutralization efficacy is an essential part of any cleaning validation. It is critical to neutralize the residual disinfectant in order to allow for any surviving organisms to be able to grow. Without confirming that the chosen neutralizer has the ability to effectively neutralize any residual disinfectant, the testing performed cannot be considered valid.

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USP <1227> Validation of Microbial Recovery from Pharmacopeial Articles offers specific neutralizer options for the biocide classes. Additionally the method for a neutralization efficacy test is addressed. Exhibiting that <100cfu (colony forming unit) of each organism can be recovered through the use of a neutralizer in the presence of the disinfectant is imperative. Controls must also accompany this testing, as the original population of the culture must be confirmed to be <100cfu and that the neutralizer itself which has been inoculated with <100cfu can also show sufficient recovery of the inoculums. It is recommended that three replicates be performed for each organism, with the recovery of the organism in the presence of the disinfectant being within at least 70% of the recovered inoculums control. If 70% cannot be met, a different neutralizer may need to be considered.[3]

A variety of different controls are required. For the surface challenge test, negative controls should be done for each surface coupon tested and should be representative of the test that was performed. As an example, when performing a surface challenge test using a large floor tile, a predetermined surface area on a section of the tile that has not been inoculated would be swabbed and processed in the same manner as the test samples to ensure that the cleaning of the panel prior to the test being conducted significantly reduced the existing bioburden.

## References:


1. FDA Guide to Inspections Validation of Cleaning Processes (7/93). http://www.fda.gov/ICECI/Inspections/InspectionGuides/ucm074922.htm

2. United States Pharmacopeia (USP) (2014) Chapter <1072> Disinfectants and Antiseptics. Pharmacopeia Forum 37: 781-785.

3. United States Pharmacopeia (USP) (2014) Chapter <1227> Validation of Microbial Recovery from Pharmacopeial Articles. Pharmacopeia Forum 37: 1163-1166.


## About the Author:

Abby Roth has ten years of experience in laboratory testing and analysis, with emphasis on pharmaceutical microbiological testing. She began her career at Azzur Labs as a microbiologist and then spent a few months as Quality Director. She now is the Laboratory Manager, with responsibilities including method and equipment implementation, setting up client contracts, and ensuring the laboratory operates as expected. In addition to a strong background in pharmaceutical microbiology, Abby also has extensive knowledge in USP<797>, microbial cleaning validations, and water testing. She has assisted many clients in the setup of their environmental monitoring programs and has played an integral role in training clients to perform environmental monitoring, using proper aseptic technique. Abby is a Certified Quality Improvement Associate and Certified Manager of Quality/Organizational Excellence through ASQ.


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