VALIDATION

Hydrogen Peroxide Residue Effects Testing in Isolators

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Introduction

The intent of this paper is to briefly explain the testing that is needed to determine if hydrogen peroxide (H_2O_2) gas following an isolator decontamination cycle is retained in, on, or around product containers, rinse solutions, and Environmental Monitoring (EM) supplies at levels that will produce "false negative" sterility test results. USP <1208> suggests that B/F tests must be performed using actual test articles that have been exposed to all phases of decontamination[1]. This procedure is actually a mock sterility test using steps that are identical to those performed in a sterility testing suite to verify that residues on or around articles will not lead to a "false negative" product sterility result.

Testing for Reside Effects should utilize inoculated samples of simulated product (a sterile buffer solution in the actual product container filled to the standard fill volume) with selected organisms (≤100 CFU), EM samples, and recovery growth media[2]. The containers and all required sterility test supplies will then be exposed to the isolator's validated decontamination cycle (back-to-back cycles are commonly validated to account for potential system cycle aborts). After proper aeration and external exhaust of the Sterility Testing Isolator, a series of sterility tests and post-test EM samples will be performed by trained, certified laboratory personnel on the simulated product in accordance to the standard laboratory testing procedure or SOP. Unexposed positive and negative control samples will be tested in a horizontal laminar flow bench or a peroxide-free isolator in the Microbiology Laboratory[3].

Procedure

The procedure for performing the Residue Effects Study or "mock" sterility test begins with choosing six (6) USP Growth Promotion microorganisms to challenge the

[1] USP 30-NF 25, Sterility Testing – Validation of Isolator Systems <1208>, The United States Pharmacopeia, Rockville, MD (2007)
[2] USP 30-NF 25, Sterility Tests <71>, The United States Pharmacopeia, Rockville, MD (2007)
[3] USP 30-NF 25, Validation of Microbial Recovery from Pharmacopeial Articles <1227>, The United States Pharmacopeia, Rockville, MD (2007)

process. A common environmental isolate is typically added to the study. Testing will be performed using triplicate samples, although, decontamination of all samples can be performed during a single decontamination cycle. Load the isolator(s) and perform the normal (Production) cycle per SOP. After complete aeration of the isolator(s), begin the testing within the sterility test isolator. Upon completion of the test procedures and EM sampling within the isolator, collect all replicates of samples from the isolator and properly incubate under controlled conditions for a minimum of seven (7) days.

Acceptance Criteria Examples

- All of the exposed samples and the unexposed positive control samples are positive for growth within seven (7) days of incubation (exposed EM plate counts should be within a set limit of the unexposed plate counts)
- The unexposed negative controls are negative for growth
- Initial inoculum level for each test organism is ≤100 CFU
- Organism identifications are confirmed by colony morphology and/or subculture Gram stains

Conclusion

After successfully completing the Residual Effects Validation, the system owner will have documented evidence that the validated decontamination cycle will not lead to a false negative sterility or EM test result. The sterility test isolator system should not be considered to be in a "validated state: until these steps have been successfully completed.

Keywords: hydrogen peroxide, sterility testing, isolator, environmental monitoring, false negative