Why and When You Should Perform Pre-Use/Post-Sterilization Integrity Testing (PUPSIT)

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Given that many biologic therapeutics rely on a sterilizing filtration step, it is crucial to be able to effectively evaluate the integrity of sterilizing filters. Historically, the requisite integrity testing was performed post-use. However, in the late 1990s, concerns arose in certain circles around the ability of post-use testing to detect microscopic flaws in the filters, which resulted in EU regulators including pre-use post-sterilization integrity testing (PUPSIT) as a requirement in the *EU Annex 1, Manufacture of Sterile Medicinal Products* in 1997.

PUPSIT EXECUTION

The integrity of the sterilizing filter can be determined using either a bubble point or diffusion/forward flow test. In order to effectively execute PUPSIT, sterility must be maintained on the downstream side of the installed filter during process operation and integrity testing, while avoiding uncontrolled bioburden on its upstream side. In practice, this requires the installation of additional components for a given sterile filtration setup, including sterilizing filters between the integrity tester and the filter being tested to avoid increased bioburden, as well as a sterile vent filter on the downstream side of the filter being tested to evacuate the test gas. In addition, due to the controlled nature of the room in which the filtration assembly is set up, there is a need for a sterile container, single use bag, or another sterile filter to evacuate the wetting liquid while maintaining a sterile barrier.

THE PUPSIT DEBATE

The benefits versus risks of PUPSIT have been the subject of debate for decades, with EU regulators generally in favor of the practice and US industry and regulators largely opposed to its inclusion as a regulatory requirement. The generally held opinion in the US is that PUPSIT introduces more risk for contamination into a process due to excessive handling of the sterilized filter and complexity of the set-up for PUPSIT. Conversely, as previously noted, PUPSIT is an EU regulatory requirement for sterile medicinal products that cannot be sterilized in their final container. The latest version of Annex 1, which went into force on 25 August 2023, states that "the integrity of the sterilized filter assembly should be verified by integrity testing before use (pre-use post sterilization integrity test or PUPSIT), to check for damage and loss of integrity caused by the filter preparation prior to use."[1] However the use of the word should generally denotes a recommendation rather than a requirement. Furthermore, the guidance also recognizes that "PUPSIT may not always be possible after sterilization due to process constraints"[1] and acknowledges that sponsors may be able to justify "an alternative approach provided that a thorough risk assessment has been performed."[1]

It is therefore important to understand the risks associated with performing PUPSIT versus not performing it. As previously noted, the risks of performing PUPSIT are related to the potential for contamination due to sub-optimal execution of PUPSIT. The

additional set up requirements and manipulations after the filter has been sterilized can be cumbersome and increase the risk of contamination. In addition, performing an integrity test after sterilization of the filtration assembly requires additional handling of the filter and filter assembly which could potentially cause damage to the filter. Integrity testing also requires either pumping air or liquid into the system (that is not normally part of the process) and sometimes at high pressure (at times close to the pressure limit of the filter). On the other hand, relying solely on post-use integrity testing increases the risk of using a filter with pre-existing flaws that are not identified post-use — i.e., "filter flaw masking". Specifically, it is possible that such pre-existing flaws in a sterilizing filter, which could theoretically be introduced during its manufacture, gamma irradiation, shipping and handling, or even during in-line steam sterilization could allow microbial contaminants to pass through during the sterile filtration process. Any blockage or clogging of the sterile filter during filtration would result in an elevated bubble point and allow the filter to pass the integrity test post-use. This theoretical "filter flaw masking" could potentially put patients at risk.

In order to perform a risk assessment to determine whether or not to perform PUPSIT, it is important to understand the risk level associated with filter flaw masking. In December 2017, a collaboration between the Parenteral Drug Association (PDA) and the BioPhorum Operations Group (BPOG) launched workstreams to study filter flaw masking. Results from one study, in which compromised filters were used to perform sterile filtration and a post-use filter integrity test was performed, demonstrated that filter flaw masking is unlikely during normal manufacturing conditions.[2] Data mining of bacterial challenge test (BCT) results of 0.2 µm and 0.45 µm filters for a variety of products corroborated these findings. The conclusion from both assessments is that filter flaw masking or significant bubble point inflation is rare, and the few fluids with elevated bubble point inflation ratios (above 1.0) tended to be those for which significant fouling (flow decay) occurs. This demonstrates why it is critical for manufacturers to perform a thorough evaluation of the risk of filter flaw-masking for their particular product formulation and filter combination.

EVALUATION OF RISK

Understanding the risks associated with performing PUPSIT versus those of foregoing PUPSIT, while also taking into account the EU guidance as well as the stage of development are key to an organization's decision regarding its use. Where justification needs to be provided for not performing PUPSIT, the recommended quality risk management approach as described in ICH Q9 should be followed in order to cover to assess the overall contamination control of the facility, including trending, to ensure that the facility stays in control. Categories for consideration in the facility risk assessment should include:

- Quality systems
- Equipment
- Facilities/utilities
- Incoming/starting materials
- Material transfer

In addition to a facility risk assessment, a process risk assessment that takes into consideration key parameters, such as those listed below, should be performed. Additional parameters that may be relevant for a particular product and/or sterile fill finish operation should be considered as well.

- The feed stream and product formulation
- The type of foulant in the product formulation if fouling does occur
- The filtration process conditions, e.g., flow rate and differential pressure
- Material of construction of the filter membrane, as some of the membrane materials are highly adsorptive and foul faster than others
- Pore sizes of the filter
- Number of filters in the process stream
- Complexity of PUPSIT set up and manipulation required to set up and operate process
- Vendor qualification of the filter and filter manufacturer including the gamma sterilization process
- Shipping vendor
- Batch volume
- Room classification where sterile filtration assembly occurs
- Results from BCT testing under process conditions

The output of the process risk assessment will determine if PUPSIT alleviates or elevates the risk of contamination.

MAKING A DECISION REGARDING PUPSIT

Upon completion of the facility and process risk assessments, a decision tree can be assembled to assess the cumulative risk and determine if there is a need to perform PUPSIT. In some cases, there may be sufficient justification to not perform PUPSIT. For example, PUPSIT may be precluded if the process stream consists of a low protein concentration with no known foulants that has never experienced flow decay and there are redundant filters in the sterile filling process. On the other hand, for a formulation that contains known foulants and has demonstrated flow decay during sterile filtration, PUPSIT is appropriate and will be a requirement.

Ultimately, the determination of whether or not to perform PUPSIT should not only take into consideration the facility and process risks assessments, but the regulatory implications as well, given the language in Annex 1.

References

1 European Commission, Guidelines, The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use, Brussels, 2022. Available from: https://health.ec.europa.eu/system/files/2022-08/20220825_gmp-an1_en_0.pdf

2 Morris, T., et al "PUPSIT and Annex 1 revision", PDA Letter, Aug 29, 2019. Available from: https://www.pda.org/pda-letter-portal/home/full-article/pupsit-and-the-annex-1-revision