Compliance with Annex 1: Sterile Fill Finish for Early Phase Clinical Supplies

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Many in the sterile manufacturing space are navigating the new requirements presented in the "The Rules Governing Medicinal Products in the European Union" 2023 revision of Annex 1 as it relates to their early phase drug products. Evaluation of some of the key requirements within Annex 1 (e.g., Contamination Control Strategy, Pre-Use Post Sterilization Integrity Testing, Isolator Technology, Unidirectional Airflow, Controls in the Critical Zone, Monitoring and Training) and how they relate to sterile fill finish processes are critical for compliance to Annex 1.

Contamination Control Strategy

The revised Annex 1 emphasizes the use of a Contamination Control Strategy (CCS) as a comprehensive approach that employs quality risk principles to control upstream and all contributing processes in the manufacture of the drug product to limit microbial, endotoxin, and pyrogen contamination. In many laboratories, CCS is used to monitor and control the bioburden load of the drug substance to low levels before a fill-finish is started. Removing the operator from access to the sterile, open product is a major tenant of the CCS and the most effective way to reduce contamination to the product. The ultimate CCS is built around isolator technology that completely removes the operator from the critical zone in accordance with this revised guidance. Annex 1, section 4.3 states that any alternative approaches to the use of Restricted Access Barrier Systems (RABS) or isolators should be justified. A comprehensive strategy also provides controls for viable and non-viable particulates, personnel training and gowning, control of parameters in the critical zone, and Aseptic Process Simulation (APS) or media fill.

Pre-Use Post Sterilization Integrity Testing

The revised Annex 1 reaffirms the European Union's position on Pre-Use Post Sterilization Integrity Testing (PUPSIT). This is an activity that has historically not been performed by US manufacturers due to perceived contamination risks. In this process, the sterile 0.2 micron filter is tested for its integrity (leaks, holes) using solutions and a testing apparatus before it is used to sterilize the drug product. Once it is shown to be integral, it can be used to sterile filter the bulk drug product. US providers are slowly implementing a PUPSIT strategy in compliance with Annex 1. Many companies employ a single-use, gamma sterilized, filter train that can be checked for filter integrity on a filter integrity tester (Sartocheck or similar). Once passing results are shown, the filter train can be used to filter the bulk drug product. After the sterile filtration is complete, the filter is tested again to demonstrate its integrity. It is also noted in Annex 1 that PUPSIT may not always be possible after filter sterilization due to process constraints (e.g., filtration of very small volumes of solution). In these cases, alternative approaches may be taken by providing a thorough risk assessment.

Isolator Technology and Unidirectional Airflow

One approach is to use robotic isolator technology (such as Cytiva's Microcell™) as the platform for sterile fill finish operations. The Microcell generates a Class A (ISO 5) environment in a closed isolator that is gloveless and completely removes the operator from the critical zone. Prior to filling into vials, pre-sterile vials and stoppers within caps are loaded into the Microcell. The chamber is decontaminated with a validated hydrogen peroxide cycle. This disinfection cycle can be developed and validated using an extensive series of hydrogen peroxide cycles with varying hydrogen peroxide volumes, temperatures and humidities that are designed to kill the 10^6 population of bacterial spores presented on biological indicator strips. The final process is verified through media fill studies.

Hydrogen peroxide levels within the chamber are measured in real time by integrated Drager tubes and digitally reported. Once the hydrogen peroxide has been cleared from the chamber, sterile bulk product is pumped into the Microcell where a pre-sterile, single-use fill needle is used to fill the vials. The Microcell is designed to provide uninterrupted, HEPA filtered, first air protection to open product vials. Airflow studies have shown the airflow within the Microcell to be unidirectional and horizontal as it sweeps over and away from the filled vials during the filling process. Because the Microcell is gloveless, the

unidirectional air cannot be interrupted by isolator gloves or operator interventions. Additionally, because it is gloveless, there is no risk to the product posed by leaking glove connectors or pin holes forming in the gloves.

Controls in the Critical Zone

Annex 1 directs that manufacturers take steps to move particle generating crimping procedures away from the filling area. The use of press-cap technology eliminates the generation of particulate matter historically associated with aluminum crimping procedures. Stoppers are pre-set within the press-caps, gamma sterilized, and used to seal the vials within the Class A chamber. In accordance with Annex 1, the Class A isolator environment is leak tested and continuously monitored for positive pressure, temperature, and humidity. The chamber is continuously monitored for particles ≥ 0.5 and ≥ 5.0 µm with a suitable airflow and alarm levels that warn the operators of excursions. Viable particles are measured within the single chamber using a tryptic soy broth (TSB) media sampling collector that is in close proximity to the vial filling. The TSB media is incubated immediately after the fill.

Single-use systems are employed to reduce the potential for contamination that is associated with permanent components (fill needles, fill lines, filter lines) that must be cleaned, sterilized, and re-installed before reuse. Additionally, single-use systems reduce the number of manipulations required thereby reducing the potential for operator errors. Many in the bio/pharma industry have implemented sterile, single-use filtration line sets configured with bulk drug product bags, and sterile, single-use, fill needle lines sets. Aseptic connections are made with intrinsic connectors that minimize any potential contamination from the surrounding environment.

Monitoring and Training

An Isolator system contained within a cleanroom that meets Class D background requirements is compliant for housing a closed isolator as required by Annex 1. As mapped by a company's CCS and detailed by SOPs, cleanrooms undergo regular periodic cleaning and monitoring for viable and non-viable particulate matter and viable surface contamination. All manufacturing personnel are trained on gowning, aseptic techniques, and aseptic process simulation (APS or media fill). The APS program must be designed to mimic the entire fill-finish process starting from TSB media dissolution, sterile filtration, and filling into vials. The APS should be challenged with interventions (few interventions are possible within a closed, robotic isolator), duration of fill, and number of vials filled. Filled vials are incubated for two weeks, at two different temperatures, and examined for any growth. APS should be conducted biannually, but any changes to the container closure, Microcell, or filtration set-up will trigger a new APS.