ASK THE EXPERT

Strengthen Annex 1 Compliance with Expert-Driven Validation Services

with Markus Junior Maring and Marine Cannuel

Extractables and leachables (E&L) studies are critical in ensuring the safety and integrity of single-use systems in biomanufacturing processes. *Extractables studies* involve applying worst-case test conditions to identify all potential compounds that could be released from materials, such as plastic bags. *Leachables testing* mimics actual process conditions, including contact time, temperature, and the nature of the solution interacting with the single-use system. Such testing can be conducted under real process conditions or simulated in laboratory settings using scale-down bags. For a February 2026 Ask the Expert webinar, Markus Junior Maring (product manager, filtration consumables) and Marine Cannuel (manager of validation services) of Sartorius spoke on strengthening European Union (EU) Annex 1 compliance with fill–finish validation services, including considerations for E&L studies.

The Presentation

Container–closure systems require inspection for visible particles. Such inspection can be performed manually or with equipment; mandatory training is required for personnel involved in the process. One challenge arose when a Sartorius customer needed to detect foreign material in intravenous bags before and after filling. Unlike glass vials, the opacity and transparency of bags posed distinctive difficulties. To address that, Sartorius provided training kits containing bags with specific-sized particles, which enabled the customer to perform visual inspections both before and after filling to detect visible particles and evaluate their behavior.

Regulatory developments such as Annex 1 emphasize contamination-control strategies and validation strategies to ensure process control and product quality. Contamination-control strategies focus on process design and quality risk management to select appropriate materials and establish controls. Validation is often a collaborative effort between service providers and process owners, with the ultimate responsibility lying with the process owner. Selecting materials (e.g., sterile filters, bags, tubing, and assemblies) requires evaluating risks such as chemical compatibility, integrity, sterilization methods, stability, and binding. Those factors influence the validation strategy and tests needed to mitigate risks.

Because single-use systems primarily comprise polymers and plastics, E&L testing must be thorough. Chemical-compatibility testing assesses how process formulations interact with single-use systems and potential leachables entering the process stream. Integrity testing ensures that sterile filters and assemblies meet predetermined values, and that applied sterilization methods do not compromise their operation. Stability considerations include pressure, flow, and thermal exposure, which can degrade polymers under extreme conditions. Binding studies evaluate how single-use materials affect process flow, complementing chemical-compatibility assessments. Those evaluations all should feed into a validation strategy, providing data to demonstrate low risk.

For fill–finish processes, validations focus on single-use assemblies and sterile filters. Chemical-compatibility evaluation, integrity testing, shipment simulations, and microbiological testing — such as bacterial challenge tests — are conducted to ensure safety. E&L testing is performed on final containers (including container–closure systems) to validate process steps and detect particles. Visual inspection kits can support validation efforts, and storage studies also can be conducted.

Pre-use post-sterilization integrity testing (PUPSIT) evaluates the impact of sterilization on filter performance, considering factors such as sterilization methods, wetting fluids, integrity test methods, potential for flaw masking, and process pressures. Collaborative strategies with service providers help simulate worst-case

conditions and validate the process. A bacterial-challenge test involves loading filters with inoculated test fluid, applying pressure, and analyzing contamination sources. That systematic approach demonstrates the absence of risk by confirming zero colony-forming units (CFUs).

A process risk analysis evaluates single-use systems under different exposure temperatures, contact times, and solution types. Risk levels are categorized as low, medium, or high, guiding validation efforts. Low-risk systems can rely on extractables data from manufacturers, whereas medium-risk systems require extractable assessments extrapolated to process conditions. High-risk systems necessitate laboratory-based leachables analysis. Such an approach optimizes validation efforts, focusing resources on high-risk systems.

E&L studies, contamination-control strategies, and validation processes are integral to ensuring the safety and quality of single-use systems in manufacturing. By evaluating risks, selecting appropriate materials, and conducting thorough testing, manufacturers can mitigate potential hazards and meet regulatory requirements. Collaboration between service providers and process owners can enhance validation strategies, further ensuring patient protection and product integrity.

Questions and Answers

Is it mandatory to perform leachables tests for all single-use systems within a manufacturing process? No, it is not mandatory. However, any leachables risk must be analyzed thoroughly to show that it will be well covered by appropriate mitigation strategies. That is why we recommend our customers to conduct leachables testing only when risk is high.

Our organization performs filter validation but has yet to implement PUPSIT. To obviate need for additional validation when PUPSIT is implemented, can we estimate worst-case conditions during filter validation? From a strategic quality perspective, we strongly suggest discussing implementation of a PUPSIT simulation within the bacterial-challenge test to avoid potential scrutiny at a later date when you do implement PUPSIT fully. Right now, it is not mandatory to include PUPSIT within a bacterial-challenge test. However, which path to take depends on the amount of risk that you have — e.g., the nature of your formulation, process parameters, flow rates, process pressures, contact time, and batch volumes. Although the strategy differs by use case, we recommend at least evaluating whether such testing is needed long term. If it is, then discuss that with the project managers who support filter validation.

What is the purpose of an extractables assessment? The idea of an extractable assessment is to use already existing extractables data from the system manufacturer and extrapolate those data to process conditions. The main factor that we use to perform this exercise is a system's surface area:volume ratio, because that parameter is typically very high for bags and tubes (≈6 cm^2/mL). During process conditions, that ratio might be, for instance, ≈1 cm^2/mL. We would use those two values to extrapolate results. Sartorius offers the ExSim tool, dedicated to performing that exercise for Sartorius products.

Will PUPSIT become mandatory in the near future? Within Europe, PUPSIT is becoming or has become mandatory to evaluate. We know that many manufacturers using stainless-steel filter housings are struggling with PUPSIT implementation. Some even state that it is increasing contamination risks within their processes. Having traveled globally, it is interesting to see that regulators in some countries are taking Annex 1 as their foundation for their guidance. We have seen that, for instance, in India and Latin America.

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