Annex 1, Sterility Assurance, and the Rising Bar for PUPSIT

The latest revision of EU GMP Annex 1 has transformed sterile manufacturing expectations from a primarily reactive, end-of-line mindset into a fully integrated approach to contamination control and sterility assurance. Among the changes drawing the most attention from sterile fill/finish teams is the clarified expectation for pre-use post-sterilisation integrity testing (PUPSIT) of sterilising grade filters.

PUPSIT is now widely viewed by EU regulators as a standard element of a compliant sterile filtration strategy rather than a discretionary add-on. For sponsors targeting EU approval, or planning eventual global expansion that includes Europe, it has become a practical "cost of admission" alongside isolator technology, robust environmental monitoring, and formal contamination control strategies.

What PUPSIT Actually Addresses

At its core, PUPSIT is a response to a simple but significant risk: a sterilising filter that appears to pass post-use integrity testing may in fact have been compromised prior to, or during, product filtration. Damage introduced during transport, handling, assembly, or sterilisation can be partially masked by product fouling of the membrane during processing. In these cases, a defective filter can meet post-use integrity limits because the product reduces flow through what is no longer a fully integral membrane.

PUPSIT mitigates that risk by verifying filter integrity after sterilisation but immediately prior to sterile filtration. In practice, operators wet the sterilising filter with a suitable medium – commonly water, buffer, or product, depending on filter design, validation data, and product characteristics – and connect a test instrument to conduct an automated integrity test. Parameters such as pressure, hold time, and acceptable test limits are established during filter and process validation. The outcome is a quantitative integrity readout with a pass/fail result before any product is exposed to the filter.

From a sterility assurance standpoint, this sequencing is critical. An integral filter at the time of actual use is a much stronger control than a filter only proven integral after product has already passed through.

Regulatory Expectations and When PUPSIT May Be Omitted

Annex 1 states that "the integrity of sterilised filter assembly should be verified by integrity testing before use (PUPSIT)," and in regulatory practice this "should" is treated as a default expectation. By contrast, the U.S. FDA has not made PUPSIT mandatory, which means global programmes must reconcile differing expectations when designing a single filtration strategy for multiple markets.

For products intended for the EU, omitting PUPSIT requires more than a procedural preference; it demands a robust, documented risk assessment that convinces regulators the likelihood of masked damage is acceptably low. Such an assessment typically examines:

- Filter type, manufacturer, and manufacturing method.
- Transportation, storage, and sterilisation methods.
- Packaging, handling, and inspection procedures.
- Product type, including potential for membrane fouling or masking defects.

Where very small batch volumes are at stake, the volume required for filter wetting and testing can consume a significant portion of the batch, making PUPSIT technically or economically challenging. In these situations, a sponsor may pursue a justified alternative strategy, but should expect extensive data requirements and close dialogue with the

Qualified Person (QP) and regulators. For many commercial programmes, the cumulative effort and uncertainty of "justifying out" PUPSIT outweighs the effort of designing it in from the outset.

Misconceptions and Practical Challenges

Despite its now central place in EU-focused sterile operations, PUPSIT still suffers from several practical misconceptions. One recurring concern is that in situ integrity testing increases the risk to the batch by introducing additional manipulations, connection points, and valves into an otherwise closed sterile system. Another is that PUPSIT will inevitably slow batch release and inflate costs relative to a post-use-only integrity testing approach.

The reality is more nuanced. The test method itself is not intrinsically complex; the complexity arises from executing it in situ on production equipment. Additional connection points, manual interventions, and the requirement to fully and consistently wet filters create operational failure modes that are not present in offline testing. In a large proportion of observed failures, the root cause lies in technique – insufficient wetting, air entrapment, inconsistent methods, or limited understanding of the underlying fluid dynamics – rather than in the filter or the test instrument. These are addressable issues, but they require design attention, operator training, and experience.

Facility and System Design: Building for Annex 1 and PUPSIT

One way to reduce the perceived burden of PUPSIT is to treat it as a core design requirement rather than as a retrofit obligation. When PUPSIT capability is integrated into the initial layout of a sterile fill/finish facility, the design team can optimise equipment configuration, utilities, and automation to support in situ testing without creating unnecessary contamination risks or operational complexity.

A purpose-built large-scale isolator-based fill/finish facility, for example, can be configured with:

- Isolator technology sized for commercial volumes and flexible vial formats, to minimise operator intervention at the critical zone.
- Clearly defined routing of utilities and piping, including the additional connections needed for integrity testing equipment.
- Walkable ceilings and external maintenance access to reduce interventions in classified areas over the facility lifecycle.
- Redundant clean utilities and equipment to support maintenance and unexpected downtime without compromising ongoing sterility assurance activities.

Such design choices support Annex 1's emphasis on proactive contamination control and sterility assurance, while providing the necessary infrastructure to deploy PUPSIT as a routine, low-disruption step in the process. They also allow fill lines – such as high-speed isolated vial fillers handling a broad size range – to be validated with PUPSIT in mind, rather than forcing later compromises to shoehorn additional testing into a fixed layout.

Modular Single-Use Assemblies and PUPSIT Execution

Beyond the facility and equipment level, the design of single-use assemblies has a significant impact on how reliably PUPSIT can be executed. A modular approach – using standardised sub-assemblies that can be configured for different products, filters, and process steps – offers clear advantages over a proliferation of bespoke, all-in-one part numbers.

In a modular architecture, tubing sizes, pre-filters, sterilising filters, flush bags, and integrity test connection points can be combined as needed to meet a specific process requirement without redesigning

the entire assembly. If a component proves problematic, it can be replaced without discarding the full build, and the same core components can support a large client base. For sponsors, this translates into:

- Increased flexibility when processes change or scale.
- Reduced lead times, as fewer unique part numbers need to be qualified and stocked.
- More predictable execution, since operators gain experience on a smaller set of reusable building blocks.

Critically, modular designs can be optimised for PUPSIT from the start. For example, they can include appropriately positioned test ports, pre-filtration options to mitigate air locking during wetting, and drain paths that facilitate efficient flushing of wetting media. The result is not only higher test success rates but also reduced operator burden during aseptic manipulations.

The Role of Operator Expertise and Training

No matter how advanced the facility and assembly design, PUPSIT ultimately depends on the people executing it. Inadequate wetting, inconsistent venting of entrapped air, or improper interpretation of integrity test results can all undermine the value of the control.

Organisations that routinely perform PUPSIT across a diverse portfolio of molecules and assemblies develop a body of institutional knowledge that can be difficult to replicate in lower-throughput environments. Experienced operators understand, for instance, when a filter is likely to be difficult to wet and when additional steps – such as using a pre-filter on the wetting agent to reduce air lock, providing sufficient back pressure on the system during wetting, or adjusting the flush volume – are warranted. That experience reduces false failures, helps distinguish between filter and technique issues, and preserves both batch integrity and schedule.

From a sponsor's perspective, evaluating a partner's PUPSIT capability therefore extends beyond the presence of test instruments and written procedures. It should include questions about the depth of operator training, the frequency and diversity of PUPSIT execution, how deviations are analysed, and how lessons learned feed back into assembly and process design.

Strategic Considerations for Global Programmes

Sponsors that begin their journey in the U.S.

often face a strategic question: implement PUPSIT from early development, or introduce it later when EU expansion comes into view. While deferring can appear attractive in the short term, it can create friction when scaling up or transferring processes across regions. Retrofitting PUPSIT into a mature process may require new assemblies, revised process validations, and additional regulatory dialogue.

Building PUPSIT into the development plan from the outset can offer several advantages for globally ambitious programmes:

- A single, harmonised filtration strategy that meets EU expectations and is acceptable in other markets.
- Reduced need for later revalidation and change control as regional scope expands.
- Earlier generation of data linking PUPSIT performance, filter robustness, and product quality outcomes.

In parallel, macro trends such as a renewed emphasis on domestic manufacturing in North America, evolving trade policies, and concerns about supply chain resilience are influencing where and how commercial capacity is deployed. Large, Annex 1-aligned, isolator-based facilities in the U.S. that are equipped for routine PUPSIT therefore occupy a growing role in many sponsors' long-term supply strategies.

PUPSIT as Part of a Broader Quality-by-Design Mindset

Viewed in isolation, PUPSIT can seem like one more discrete compliance box to tick. In the context of modern Annex 1 expectations, however, it is better understood as one component of a broader quality-by-design approach to sterile manufacturing. That approach ties together:

- Facility design that reduces inherent contamination risks and enables robust, isolator-based operations.
- Single-use assemblies and filtration strategies that anticipate PUPSIT and minimise operational complexity.
- Operator training and experience that translate written procedures into consistent practice.
- Data-driven risk assessments and validation work that align global regulatory expectations with the practical realities of manufacturing.

When these elements are developed cohesively, PUPSIT shifts from being perceived as a fragile, high-variability step to a predictable, low-risk part of routine batch execution. Rather than undermining confidence in the process, it reinforces sterility assurance by confirming that critical barriers are intact precisely when they are needed most.

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