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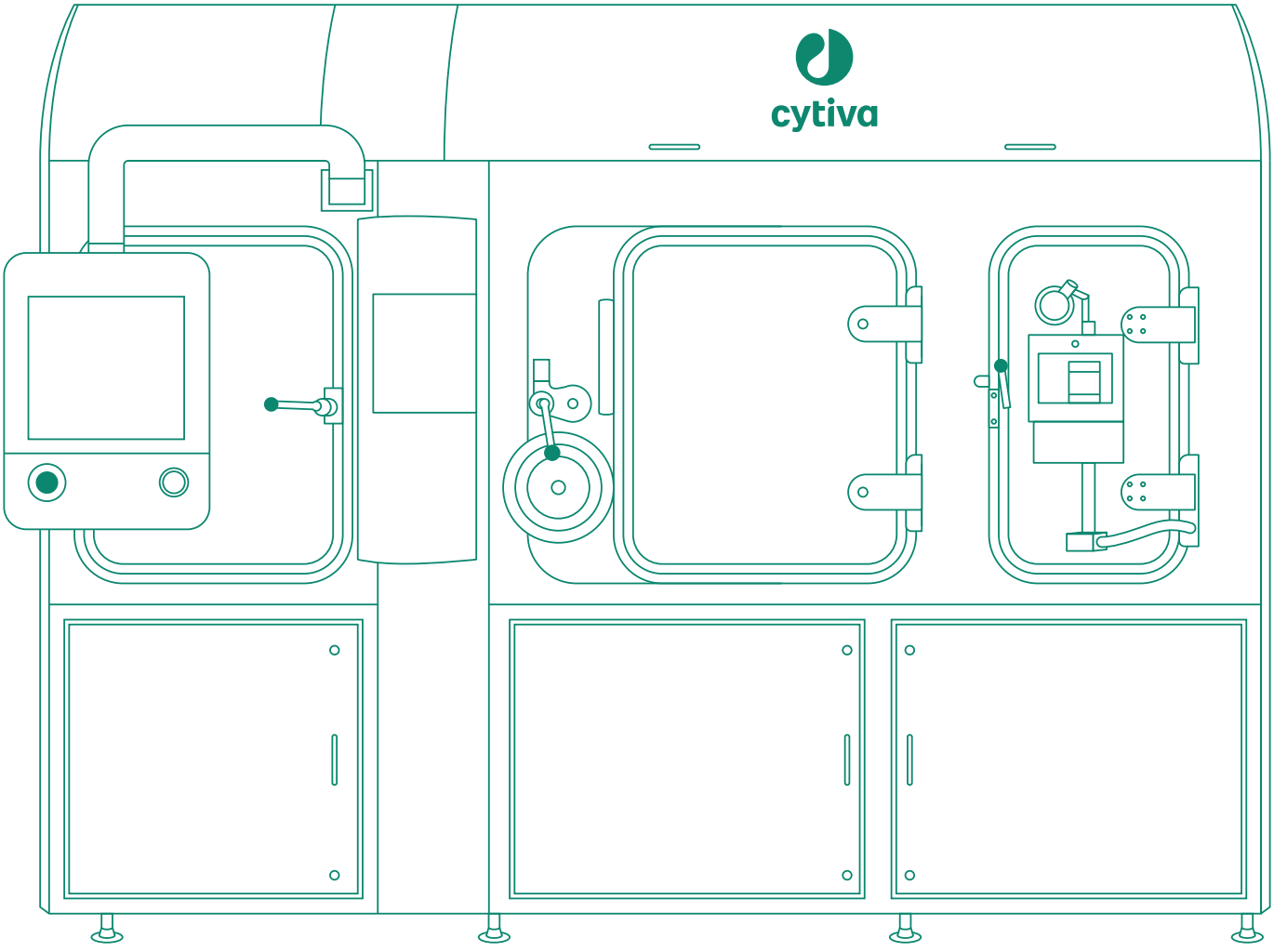
Aseptic process design and simulation under Annex 1 guidelines



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Introduction

Injectable drug manufacturers face growing challenges for aseptic filling processes; among these are the rise of cell and gene therapies and the risks associated with drug products that can't be terminally sterilized (1). New technologies — robotics, automation, modern isolator designs for aseptic filling, and others — are designed to address these challenges.

The 2022 revision of the European Union's (EU) Good Manufacturing Practice (GMP) Annex 1 is a direct response to these evolving conditions (2). Annex 1 provides detailed guidance for the manufacture of sterile products, covering facilities, equipment, systems, controls, and procedures, with a strong emphasis on quality risk management (QRM) and applying risk-based assessments (1).

Also, Annex 1 signals an industry-wide move toward barrier technologies, notably restricted access barrier systems (RABS) and automated isolators. These technologies have great potential to minimize microbial contamination risks associated with direct operator interventions in aseptic filling critical zones.

Aseptic process simulation (APS) provides a critical test to validate aseptic filling processes. APS should be a central component of end users' contamination control strategies (CCS) as drug manufacturers continue to retool the industry (2). This article will explore some of the key differences between automated isolators and RABS that pertain to the design and execution of APS studies. This includes a focus on Annex 1 guidelines, highlighting how the characteristics of these systems influence process validation and ultimately, sterility assurance.

Aseptic filling workspaces

Manufacturing sterile drug products in controlled environments requires specific technical and operational measures to separate the process segments and prevent product contamination. Technologies have evolved to minimize, or in some cases even eliminate, the largest potential source of contamination – human intervention. RABS and automated isolators are being used to meet Annex 1 requirements to decrease interventions and improve sterility assurance. These technologies are shown to maximize aseptic drug filling conditions and minimize microbial contamination associated with direct human interventions. But each of these systems provides a different level of protection to drug products and requires different considerations for APS design.

Restricted access barrier systems

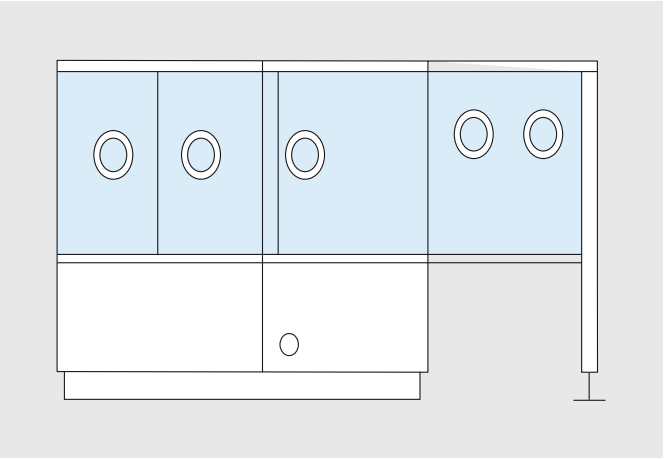

Fig 1. Restricted access barrier system (RABS).

RABS provide Grade A enclosed environments sealed off from the surrounding facilities to varying degrees. The inner surfaces are manually disinfected and decontaminated with a sporicidal agent. Automatic decontamination cycles aren't possible. Operators use gloves, half suits, rapid transfer ports, and other integrated devices to perform manipulations or transfer materials during aseptic filling. These operator activities occur within the critical zone where product and critical surfaces are exposed during filling. The external cleanroom environment for RABS must meet at least a Grade B level of cleanliness.

RABS offer a higher level of flexibility for aseptic filling operations than automated isolators but a lower level of sterility assurance. This is mainly due to the increased number of operator interventions allowed and required by RABS. These systems are available in two configurations: open RABS and closed RABS. The interior areas of open RABS are separated from the surrounding cleanroom with a rigid-wall enclosure and air overspill into the exterior environment. Closed RABS create completely sealed filling environments where air is recirculated through a self-contained airflow system.

Automated isolators

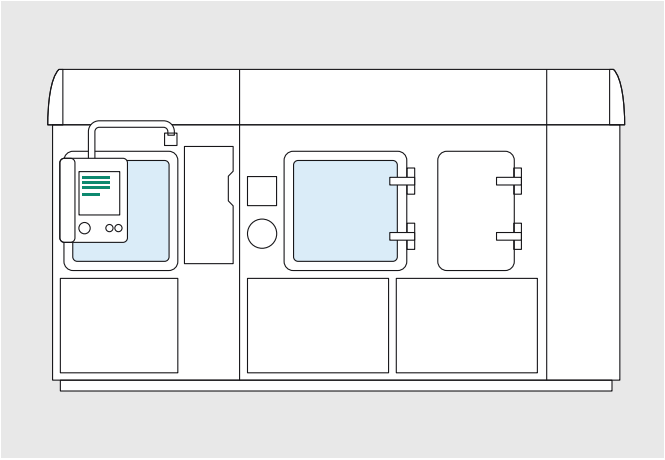

Fig 2. Cytiva's SA25 aseptic filling workcell, a robotic gloveless isolator.

In contrast, isolators are fully enclosed systems that minimize or eliminate operator intervention in the critical zone during aseptic filling processes. Manual, semi-automated, and automated (sometimes robotic) isolator formats can be used. The design strategy behind isolators aims to eliminate risk through design rather than merely mitigate it. The interior of isolators meets Class 100 (ISO 5) standards, and systems such as the SA25 aseptic filling workcell achieve total particle levels well within ISO 5/ Grade A air requirements (3).

Material enters and exits automated isolators through various mechanisms, including material transfer chambers, rapid transfer ports, or mouseholes. Once the isolator is closed, an automated decontamination cycle with a suitable agent — commonly vapor phase hydrogen peroxide (VPHP) — is performed to sanitize internal isolator surfaces and materials placed inside the isolator. Additionally, horizontal or vertical unidirectional airflow from the supply filter towards and over the filling zone is mandated by regulatory bodies to sweep particles away from critical operations. In contrast with RABS, automated isolators can be cleaned with wash-in-place cycles. This is due to the air leak tightness of these systems following ISO 10648-2 standards. As such, isolators can maintain positive pressure to prevent ingress of air from the background environment, thus reducing the risk of contamination.

Automated isolators maximize product isolation and greatly reduce the number of operator interventions. While aseptic filling processes in gloveless isolators are automated, some isolator designs have glove ports that allow operator intervention in the filling zone, when needed. Robotic gloveless isolators eliminate operator intervention during aseptic filling runs, removing the main source of contamination from the aseptic process (i.e., the human operator.).

Aseptic process simulations and Annex 1

Often referred to as 'media fills', APS are critical studies conducted on aseptic filling processes to evaluate their capability to consistently produce sterile products (4). The purposes of APS are to (1):

- Simulate the complete aseptic filling process, from setup through sealing of the container.
- Assess whether the facility and air handling systems are suitable for the intended use.
- Evaluate the process design, material handling, and operator interventions to ensure they're performed in a manner that prevents product contamination.
- Evaluate the proficiency of operators and assess whether gowning procedures are adequate.

During APS, drug products are replaced with a sterile microbiological growth media, and simulation runs are conducted as closely as possible to routine aseptic filling processes. Growth media must contact all surfaces and environments the product could potentially encounter. Microbial contaminants are more likely to grow and be detected in growth media than in actual drug products, which exposes potential flaws or weaknesses in the aseptic process.

APS must include all operator manipulations and interventions that occur during routine production, crucially those that occur near sterile zones where product is processed. These are broadly categorized in two ways. First, routine operator actions are integral to the aseptic filling process and occur prior to decontamination, such as aseptic assembly and manual environmental monitoring (EM). Second, operator interventions are nonroutine corrections or adjustments of the process, such as clearing jams or changing filling needles.

Both current good manufacturing practice (cGMP) regulations, such as those in 21 CFR parts 210 and 211, and Annex 1 require the validation of aseptic processing operations, including aseptic filling (2, 5). APS studies should be performed on a semi-annual basis for qualified processes.

APS is considered a crucial component of a facility's CCS outlining the controls in place to manage the risks of microbial, particulate, and pyrogen contamination throughout the manufacturing and aseptic filling process. While the CCS defines the critical control points and monitoring measures, APS is a tool to verify aseptic filling processes are correctly implemented and continuously perform as expected.

What differentiates Annex 1 from FDA guidance for APS

Overall, compared to the 2004 US Food and Drug Administration (FDA) guidance on sterile drug products, Annex 1 is much more prescriptive and detailed about aseptic drug processing (2, 5). Several regulatory hurdles differentiate Annex 1 from the FDA guidance. First, Annex 1 places particular emphasis on the importance of barrier systems to minimize contamination associated with operator interventions.

The regulatory landscape, particularly in the EU, has greatly increased its focus on APS (6). The 2022 revision of Annex 1 expanded the detail on APS from just five paragraphs in the earlier version to approximately four pages now (6). Annex 1 provides specific requirements for APS interventions and worst-case scenarios. It requires the inclusion of various aseptic manipulations, interventions and interactions, and worst-case conditions in the APS design. While interventions should be performed as they are intended and per approved instructions with trained and/or qualified operators, APS shouldn't be used to justify practices that pose unnecessary contamination risks (2).

Aseptic process simulation: RABS vs automated gloveless isolators

Table 1. Design considerations for restricted access barriers systems (RABS) and automated gloveless isolators that might impact aseptic process simulations

Feature	RABS	Automated gloveless isolators
Design	Semi-enclosed system with glove ports or manual access doors	Fully enclosed, sealed system with no glove ports
Decontamination	Manual process	Automated cycles
Gloves	Required for operation	Eliminated from design
Human interaction	Allows manual intervention via gloves or doors	Robotic operation; user interacts via HMI (no manual access)
Operator interventions	May occur within the filling zone	Eliminated during aseptic process
Sterility assurance	Moderate – depends on manual cleaning and operator discipline	Very high – automated decontamination (e.g., VPHP)
Cleanroom requirements	Typically requires Grade B background	Lower classification acceptable (Grade C/D)
Product protection	Good, but dependent on operator compliance	Excellent – minimal contamination risk

HMI is human-machine interface.

The choice of barrier technology used for aseptic drug filling bears directly on APS design (4). For example, direct interventions in RABS occur more often than with isolators. Conversely, robotic gloveless isolators eliminate human intervention in filling zones, limiting operator interactions to routine tasks prior to decontamination. As such, the interventions in automated gloveless isolators don't pose the same risk and eliminate the need to simulate operator gown changes, shift changes, or breaks (1). For automated gloveless isolators, the CCS is built around risk elimination through design. As a result, the validation emphasis shifts to demonstrating the integrity of the closed system, the effectiveness of automated decontamination cycles, and the use of presterilized components.

APS considerations for RABS

RABS operators use integrated gloves to perform manipulations or convey materials within the interior zones (i.e., for interventions), and so APS with RABS must simulate aseptic operator manipulations. Gloved systems require demonstration of good mechanical and chemical resistance, and routine physical integrity testing. Leaks in glove material can compromise batch integrity and are considered a critical failure mode (1).

Overall, RABS require much more involved APS to capture the multitude of interventions and interactions involved in their operation, and come with several considerations for designing APS (2):

- APS should simulate interventions performed through glove ports and any risks associated with glove integrity (4).
- APS simulation of worst-case scenarios is crucial (4).
- The disinfection process should be validated through APS (4).
- Barrier breaches (e.g., door openings) that could potentially introduce contamination should be simulated. Airflow pattern studies should be performed to demonstrate the absence of air ingress during interventions (4).
- Manual interventions should be delineated in an authorized list of allowed and qualified interventions that may occur during aseptic filling, including information about the frequency of occurrence of each intervention (4).

It's important to restate that open RABS aren't fully sealed and their design relies on positive airflow from the Grade A critical zone to the Grade B exterior environment. It's essential to maintain a proper airflow and pressure differential between adjacent areas with different classifications. Airflow patterns within cleanrooms and zones should be visualized to show no ingress from lower to higher grade areas (4).

APS considerations for isolators

APS for gloved isolators considers the same concerns for glove integrity that are encountered in RABS. However, for automated isolators, operator qualification via APS typically focuses on qualifying the automation system and process instead of on manual interventions within the aseptic zone (3). This is even more so the case for automated gloveless isolators.

Validation for isolators emphasizes the efficiency and robustness of design principles that contribute to minimizing operator intervention:

- Automated decontamination cycles, for example using VPHP.
- The integrity of the closed isolator system and its isolation from the exterior environment.

One additional design principle is worth emphasizing, specific to the SA25 aseptic filling workcell: the use of presterilized single-use flow paths, which eliminates the need for aseptic assembly. Also, the use of nested components eliminates indirect product contact surfaces and complex machinery, such as vibratory bowls, starwheels, etc. Both are typical sources of particle generation that can potentially increase the number of interventions needed in the process (e.g., as lines get jammed by particulates).

Continuous monitoring of environmental conditions within the isolator, such as air pressure and total particle load (e.g., using real-time biofluorescent particle counters to assess real-time viable and total particulate load), confirms the state of control. APS for these systems simulates the process, including material transfers into the isolator and automated operations, while ensuring the integrity of the sterile boundary is maintained (3).

Manual operator interactions are still relevant to automated gloveless isolators and should be appropriately challenged in APS, but they largely occur before decontamination. With an automated gloveless isolator like the SA25 aseptic filling workcell, manual operator interactions may include tooling changeover, scale calibrations, flowpath installation, purge bottle placement, and component unload and reload, to name a few. Once decontamination is complete, operator interactions with the system during critical processing occur exclusively through an HMI (3). Because aseptic filling processes are isolated inside the machine and carried out robotically, operator interventions are eliminated in the critical filling zone.

Other APS considerations

Testing multiple container closure formats

When an aseptic filling process involves multiple container-closure formats (e.g., different sizes of vials, syringes, or cartridges), it's essential to determine the appropriate approach for APS to adequately challenge the process across all configurations. There are three options:

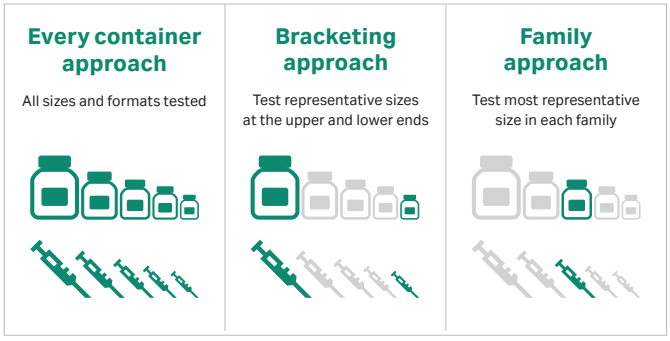

Fig 3. Comparison of APS approaches to testing multiple container-closure formats.

- **Every container format approach**. APS is performed using every size and type of container-closure format that will be processed on the line. This approach can be suitable when a limited number of distinct formats are used.
- **Bracketing approach**. This approach is typically applied with multiple sizes of containers of the same type. For example, APS might include the smallest and largest vial sizes within a family of vials. Annex 1 explicitly mentions that a bracketing approach may be considered where process equivalence can be scientifically justified. Worst-case conditions, such as the vial size with the largest opening or the slowest line speed, may be selected to challenge the process.
- **The family approach**. This approach groups containers and closures together into 'families' based on similarities in tooling, tub or nest design, decontamination processes, and the filling process. A representative combination from each identified family is selected and challenged during the APS. The rationale is that the differences between formats within a family are minimal, and there isn't a single worst-case configuration within the family. For instance, qualifying one size of nested vial (like 6R) can be representative of other sizes within that nested format (like 8R, 10R, and 15R) because they are packaged identically with similar orientations and dimensions, with height being the main differentiator.

Design standardization

Standardized designs in aseptic filling equipment allow for a standardized APS, which greatly reduces the complexity and time required to qualify a system. Custom-designed equipment often requires lengthy timelines for building, installation, and validation. However, standardized systems simplify the qualification process, easing the use of the same EM strategy across different installations.

APS outcomes and investigations

When interpreting the results of the APS, revised Annex 1 is clear: the target should be zero growth. Any contaminated unit identified during an APS run is considered a failed process simulation. This marks a significant shift, as older guidance mentioned a contamination rate of less than 0.1% with a 95% confidence limit as acceptable (2).

Any contaminated unit requires a thorough investigation to determine the most probable root cause(s) (4). This investigation should include species-level identification of the contaminant to help trace its source. Appropriate corrective and preventive actions (CAPA) must be determined and implemented. Production should not resume until after successful revalidation, which typically involves conducting a sufficient number of successful, consecutive repeat APS runs, normally a minimum of three. The investigation into a failed APS should also include a risk assessment of potentially impacted batches manufactured since the last successful APS.

Concluding thoughts

An increased focus in drug manufacturing on risk reduction by design is evident in the growing preference for RABS and isolator technologies over traditional manual approaches for aseptic filling. Both types of technology provide physical separation between operators and the critical aseptic processing zone. However, the minimization of operator interventions, increased sterility assurance, and the simplified approach to APS afforded by automated isolators suggests a considerable advantage over RABS.

While integration of isolators may need initial planning, the risk reduction benefits are substantial. Regulatory bodies like European Medicines Agency (EMA) and Health Canada have approved several products made using these closed, robotic isolator systems.

Ultimately, the emphasis placed by Annex 1 on QRM and on developing and maintaining a comprehensive CCS strongly suggests that technologies that eliminate risk through design are the future of aseptic filling. By removing the primary source of contamination and simplifying validation efforts through inherent risk elimination, isolators — automated gloveless isolators in particular — align well with the goals of modern sterile manufacturing: building quality and sterility assurance into the process design.

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