Global Pharmaceutical Manufacturing Leadership Forum (GPMLF)

Annex 1 White Paper

16 December 2025

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Introduction

The pharmaceutical industry is encountering an increase in regulatory scrutiny related to sterile manufacturing, this is in part due to the update to Eudralex and PIC/S issuance of the updated Annex 1 for the manufacture of sterile medicinal products partly due to events such as the Pseudomonas aeruginosa contamination of eye drops of 2023. Inspection observations highlight gaps in alignment with the current state of manufacturing science, particularly in aseptic processing and contamination prevention. These gaps often stem from legacy equipment and facility designs, inadequate or misapplied risk assessments, and incomplete execution or documentation of process validation and monitoring that does not align with expectations described in Eudralex and PIC/S GMP Annex 1 (2022) or the Code of Federal regulations, such as 21 CFR 210 and 211 as interpreted in the USFDA Guidance for Industry – sterile drug products produced by aseptic processing (September 2004). Despite the identified challenges, industry remains committed to supplying patients with high-quality medicines that meet evolving standards and best practices, even as expectations evolve with the progression of technology and scientific understanding.

To safeguard patient supply while strengthening compliance, a practical, risk-based path is needed — one that recognizes changing expectations and provides actionable principles to mitigate risk during transitions to new equipment, facilities, and controls. This white paper responds to that need by consolidating contemporary expectations across contamination control strategy, aseptic process validation, equipment and facility design, and personnel practices. These propose a harmonized approach across industry which will ensure consistency during the development of aseptic processes and regulatory oversight. It explains how risk management supports sterility assurance and supply continuity, emphasizing that monitoring and aseptic process simulations should guide evaluations of control measures and inform future strategies, rather than justify inadequate designs or practices.

The white paper is intended to bridge near-term operational realities with longer-term investments in robust sterile and contamination-controlled operations. It outlines how manufacturers can prioritize control and monitoring of critical activities while adopting facility and equipment strategies aligned with current expectations. It also reinforces the essential role of qualified personnel, proper gowning, and disciplined behaviors in maintaining controlled environments and preventing contamination.

Scope

This whitepaper provides principles and practical expectations for manufacturing operations within the scope of GMP Annex 1, focusing on four areas: environmental monitoring and control; aseptic process validation; equipment and facility design; and personnel practices. Each subject area was drafted as an individual chapter to the whitepaper by multicompany and multidisciplinary teams with oversight from the GPMLF committee to ensure consistency of messaging. Informal feedback was also obtained from regulatory agencies to ensure that the principles expounded are in line with regulatory expectations and do not have significant gaps or contradictions. This does not imply regulatory approval or endorsement.

Recognizing the need for alignment with current regulatory interpretations promotes risk-mitigating approaches that enable continuity of supply and approval of innovative treatments while the design, execution, and qualification of equipment and facility upgrades proceed. This whitepaper does not replace applicable laws or regulations; rather, it offers industry-oriented guidance to support compliance and sterility assurance during periods of change. It should be noted that the paper is written with the current state of available technology in mind and is not intended to prevent innovative practices being adopted. Companies should consider new and emerging technologies such as gloveless isolators, rapid microbiology methods and automated cleaning and disinfection systems, and the use of Artificial intelligence as next steps for the industry to move forward and further enhance current Contamination Control Strategies.

Group 1: Strategic Approach for Maximizing Use of CCS in GMP Facilities

Aim:

The 2022 revision of EU / PIC/S GMP Annex 1[1] introduces the concept of a Contamination Control Strategy to define critical control points and assess the effectiveness of controls and monitoring measures used to manage contamination risks.

This document provides strategic guidance to enable effective use of the Contamination Control Strategy (CCS) in quality governance for GMP-compliant manufacturing, with the overall aim of ensuring high quality product and protecting the patient. Strategies outlined are also applicable to facilitate consistent and effective communication of the organization's holistic approach to contamination prevention (e.g. during internal communication and regulatory inspections) and to illustrate quality, compliance and operational benefits associated with capital expenditure and other strategic business decisions relating to contamination control in a facility.

The scope of this document includes sterile, low bioburden and other areas of GMP activity[2] (e.g. sterility testing) where contamination control is a requirement. When developing a CCS for products not intended to be sterile, the manufacturing site should understand those parts of Annex 1 and CCS that are applicable to their product and process. The CCS owner must be able to articulate the risk-based justification for excluding aspects of Annex 1 content that are not applicable.

Background:

Several publications are available that provide guidance on the technical content and structure of a contamination control strategy. Examples include:

- Parenteral Drug Association (PDA)[3]
- BioPhorum (CCS guidance for low bioburden drug substance)[4]
- European Compliance Academy ECA[5]

However, a technically comprehensive CCS will only achieve the regulatory expectation if implemented effectively and integrated into a manufacturer's pharmaceutical quality system (PQS). This guidance aims to show how CCS can be (i) embedded in routine operations and (ii) inform quality and strategic decision-making by facilitating robust communication of how contamination hazards are identified, mitigated, and reviewed throughout the organization.

This guidance will outline how the CCS can be integrated to maximize impact in manufacturing operations, quality system oversight and strategic decision-making, using illustrative examples of how this can be achieved in practice.

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[1] EU GMP Annex 1; https://health.ec.europa.eu/document/download/e05af55b-38e9-42bf-8495-194bbf0b9262_en?filename=20220825_gmp-an1_en_0.pdf.
[2] This may also include non-sterile products, where certain Annex 1 principles may be used to support the manufacture of products not intended to be sterile.
[3] PDA Technical Report 90; Contamination Control Strategy Development in Pharmaceutical Manufacturing; PDA - Technical Report Portal.
[4] Contamination control strategies (CCS) in low bioburden biologic drug substance manufacturing: insights from BioPhorum - BioPhorum.
[5] ECA CCS Reference; CCS Task Force issues new Guideline - ECA Foundation.

Implementing a CCS in multi-site organizations:

Implementing a CCS across a multi-site organization can enable efficiency and ensure consistency across an organization by working to global procedures and standards, while recognizing local specifics.

A global CCS SOP and template is recommended to describe rationale, design and implementation of global systems that contribute to contamination control and/or monitoring. This provides a tool for education across the organization's network to understand the rationales for facility design, equipment selection, process design, maintenance, environmental monitoring, training, qualification, and material sourcing, etc. and how these contribute to contamination control individually and collectively. It also serves as a standard against which compliance may be verified (e.g. during self-inspection or audit).

Global approach can enable 60-70% of the CCS content be provided without duplication of effort at each site, while allowing local fine-tuning dependent on products, manufacturing processes, equipment, and facility layout specifics. The final document comprising a common summary that is augmented by site description/interpretation, forms the final document to integrate into the site's pharmaceutical quality system. It is important not to force-fit a global description of contamination control measures into site CCS documents without the ability to explain local modifications. This risks compliance failures when global descriptions mis-align with local practice.

Integrating the CCS into the Pharmaceutical Quality System (PQS):

CCS integration into the PQS ensures continued review of contamination risks and mitigations through routine QMS activities such as investigations, deviations, changes, and periodic review of data. (e.g., environmental, personnel, and utilities monitoring This ensures that changes in risk, improvements in process knowledge and revisions to control measures or monitoring strategies can be considered on an ongoing basis. The CCS is utilized daily, as a strategic tool to ensure a state of control.

The CCS may inform quality impact decisions during investigations (e.g. assessing opportunities to minimise introduction or proliferation of contamination in a facility, containment, and detection opportunities), or the PQS investigation may lead to actions to improve the CCS itself. Where relevant, the CCS changes may form part of GMP update training.

In addition to this event-driven review, there should be a periodic review of risks, mitigations and monitoring activities described in the CCS as part of the routine Quality Risk Management review cycle or in association with a site master plan review or annual product quality reviews. This may form part of Management Review or other quality system oversight, and it should be visible to senior leadership.

Use of the CCS as a training tool:

The CCS may serve as a training and educational tool across the organization. The interaction of contamination control and monitoring activities, including the assessment of effectiveness and residual risk described in the CCS, enables personnel to understand the impact of their work on critical quality attributes at other stages of manufacture or control. This understanding highlights their individual contribution to protecting the patient and positively impacts Quality Culture.

For example:

- Enabling personnel responsible for aseptic processing to understand the importance of procedural compliance in protecting the patient from product contamination due to:
    - The limited capability to remove viable contamination introduced during processing, particularly at final steps of finished product manufacturing (e.g. fill-finish).
    - The statistical weakness of end-stage sterility testing, where a contaminated batch may pass a sterility test.

- Enabling support functions, e.g. purchasing teams, to understand the impact of supplier selection decisions on material inputs or process controls. This includes appropriate balancing of cost while ensuring robust with supplier quality management, which can affect product or process contamination control and monitoring.

Use of CCS in Inspection and Audit:

The CCS enables communication of risks and mitigations to internal and external stakeholders as a single, holistic review of risks and control measures. The CCS process owner should be able to explain the general CCS approach and bringing in other SMEs as needed. Suitable summary materials (diagrams or other tools that provide an overview of relationship between control measures) may be useful for this purpose.

The CCS is recommended for review as early as possible in any external assessment audit or inspection. This enables the organization to clearly explain the identification of risk, together with cumulative impact of the control strategy in good facility and process design, minimizing potential for contamination ingress hazards from materials, environment and personnel, and monitoring environment and process for failure. Demonstration of the CCS as a strategic tool, utilized daily, within PQMS highlights a proactive culture to regulators.

Using the CCS as a tool in strategic business decision making:

The CCS typically does not include information relating to productivity, avoidance of waste or resource utilization. However, improved quality assurance leads to many long term cost benefits. Effective implementation of a harmonized CCS drives down Cost of Goods, reduces waste from duplicative drafting and maintenance of individual CCS documents and enables organization-wide planning for Continuous Improvement and capital expenditure.

The CCS may be referenced when making strategic investment decisions by providing a clear explanation of facilities and equipment, process controls, contamination risk mitigation and monitoring approaches. These impact operational expenditure, cost of goods and productivity. CCS content may put future investment decisions into context by illustrating the cost or complexity of current controls vs the business, quality, supply, and compliance benefits that may be achieved through investment in facility, equipment, and technology. Alternatively, when investment is prioritized towards other areas of the business, the CCS can be an effective tool to outline any additional risk mitigation measures required to maintain regulatory compliance and product quality. In essence, a forward looking CCS provides context of the business risks that can be avoided by implementing more reliable facilities, processes and systems.

Using the CCS summary of contamination risks and mitigations, an organization can quantify resources attributed to a particular process design strategy, and potential savings following investment (e.g. replacing a legacy ‘open’ aseptic process by investing in isolator technology/closed system may provide opportunity to reduce clean area classification, personnel gowning and qualification, environmental monitoring, process and environmental monitoring, allow for greater utilization of manufacturing areas, and other risk mitigation measures). OPEX savings from this process improvement may be an important aspect of the business case, and aid in the organization’s financial planning.

Group 2: Equipment and Facilities

Introduction:

This section of the white paper discusses the challenges involved regarding compliance within sterile medicinal product facilities and ensuring that Industry, suppliers and the regulators have a common understanding of the requirements that underpin compliant facilities and equipment. The white paper discusses a number of key areas including: Restricted Access Barrier Systems (RABS) versus Isolators, Indirect contact part sterilization (RABS and Isolators), PUPSIT and closed systems.

Although not directly covered by this paper, the team also encourages the adoption of suitable innovative technologies. These technologies include well designed robotics systems for aseptic filling (to further remove human interventions) and also the use of rapid and alternative monitoring systems to ensure that companies have rapid feedback on how their aseptic systems and controls are functioning, potentially enabling nearer to real time reactive measures to be taken.

RABS versus Isolators:

It is widely accepted that aseptic filling facilities should include a separative barrier between personnel and the critical zones. Barrier technologies provide protection of pen exposed sterilized packaging components (e.g., syringes, vials, IV bags, stoppers) and the filling process from the operators (which are considered one of the highest risks of microbial, and particulate contamination to aseptically prepared medicinal products). Currently, the main options for achieving this goal are either Isolators or RABS. Facilities of older design where there is no or little separation are no longer considered appropriate.

The recently updated EU and PIC/S Annex 1 for sterile medicinal products directs the manufacturer to use either RABS or Isolators. The USA FDA Guidance for Sterile Drug Products Produced by Aseptic Processing (September 2004) only refers to Isolators and discusses the need for barriers to achieve segregation of the aseptic processing line. FDA inspections of sterile medicinal filling facilities demonstrate that there is an acceptance of well designed and controlled RABS and isolator technology. At recent conferences a number of regulators have expressed a preference for Isolators over RABS, although currently open and Closed RABS are accepted as well as Isolators.

- Although the majority of new facilities and filling lines appear to be designed as Isolators, there is still a number of RABS lines that form a large part of the manufacturing capacity across the world and therefore a restriction on the use of RABS could limit new product applications.

- Although Isolators may appear to be the better options, in actuality, they may not be suitable for all processes and themselves may provide a challenge for some products and processes.

Some of the concerns over the use of RABS is based on the fact that there does not appear to be a harmonized approach to the design of RABS with some not being optimized to protect the patient. Generally, there are open and closed RABS which appears to be only two designs, however when looking at the operations of these systems, there is a wide variety of interpretations, especially regarding open RABS. These variations include (but are not limited to):

- Open door set up, but the setup is not optimized to minimize the durations of the door opening.

- Aseptic assembly of the filling system are not optimized e.g. the product pumps, filling needles and product manifold are sterilized separately and then assembled aseptically, sometimes through glove ports, sometimes with the RABS doors open. Rather than pre assembly prior to.

- Once set up of the filling system is complete then some companies do not allow any further open door interventions, whilst others will allow a wide range of open door interventions (potentially negating the benefit of the RABS).

It is therefore proposed that rather than pushing the industry into isolators due to the concerns relating to the inconsistencies in operation of RABS, the paper provides guidance as to what Industry considers the suitable operation of a RABS to be. There should also be guidance on the operations of RABS and definitions, and these can be found in the PDA Points To Consider (PTC) published June 2025.

It should be noted that the control measures for RABS or isolators should be clearly defined in the Contamination Control Strategy (CCS). The CCS is discussed in detail other sections of the white paper and will not be discussed further in this section.

Key requirements for existing RABS facilities:

- Equipment, including indirect contact parts, should be frequently sterilized (typically this is per batch or occasionally per campaign (if short) using validated methods such as moist heat sterilization.

- The sterilized equipment should be wrapped in such a way that it can be transported from the sterilizer to the filling line whilst minimizing risk of recontamination e.g. by a minimum of double wrapping.

- Open door interventions during set up should be minimized by optimizing the set up process to ensure that, as much as possible, can be performed through gloveports.

- Aseptic set up of the equipment should be optimized to remove the need for unprotected aseptic connections by either by pre assembly prior to sterilization or using intrinsic aseptic connections.

- Open door interventions on completion of the set up process should be prohibited.

For new installations of RABS, all of the above should be included in the consideration but in addition wider consideration should be given to the meaning of the S (System) in RABS. This should include items such as:

- CIP and SIP of reusable contact parts such as the filling system

- Automated sanitization of the RABS using systems such as VHP

Indirect contact part sterilization:

Regulators have had a concern regarding the set up and sterilization of indirect contact part in isolators for a number of years. Indirect contact parts are defined in clause 5.5 of Annex 1 for the manufacture of sterile medicinal products as:

“Indirect product contact parts are equipment parts that do not contact the product, but may come into contact with other sterilised surfaces, the sterility of which is critical to the overall product sterility (e.g. sterilised items such as stopper bowls and guides, and sterilised components).”

Regulators are concerned because these parts come into contact with primary container-closure system components and directly contact the sterile product (e.g. stoppers), and therefore they should themselves be sterile. However, the challenge has been what constitutes a true sterilizing agent. A number of companies have interpreted this as meaning that Vapour Hydrogen Peroxide (VHP) can be used as a sterilizing agent as it is capable of producing a six log reduction of highly resistant *Geobacillus stearothermophilus* spores. However, this view of VHP is not universally held due to issues seen in the application of this technology where it has failed to achieve sterilization due to unforeseen challenges. These challenges have included unidentified hotspots, changes in temperature/humidity and difficult to penetrate areas. This concern was first expressed in the MHRA Blog “VHP Fragility” April 2018 and has continued to be an area of debate to the current day. It is still argued that the conditions and differences in an isolator, such as different materials of construction, mean that the VHP process cannot be consistently controlled to ensure that there is no risk to the patient. Given these concerns this paper does not propose using VHP as a sterilization process, only as a robust decontamination.

The purpose of this section of the white paper is to propose the approach that should be taken to the sterilisation of indirect contact parts for isolators.

Key considerations for indirect contact part sterilization and assembly intended to be used in isolators:

Equipment design:

- As far as possible the equipment should be designed to allow ease of removal from the isolator (thus preventing damage) and transfer to and from the sterilisation equipment (typically an autoclave).
- It should allow loading into the sterilization equipment. Older designs of filling lines have had equipment, such as stopper descrambler bowls that have been too large and too heavy to move into the equipment.
- Allow a logical flow of equipment assembly and set up e.g. top down and inside of the isolator to the outside.
- Allows for the setup of the equipment using sterile tools.
- Allows for appropriate wrapping that supports the logical sequence of set up.
- Allows for the cleaning of the equipment and removal residues such as silicon from stoppers as well residual cleaning agent (e.g., including water).
- Allows for ergonomic handling of the parts both for health and safety reasons but also to aid in handling so as not to re-contaminate or compromise sterile surfaces.

Sterilisation:

- The equipment will be wrapped in such a way that it protects the equipment from recontamination during transport through the Isolator surrounding area (typically grade C).
- Wrapping of the equipment in such a way that different layers can be removed at the interface of the grade C surrounding the isolator but then allows the equipment to remain covered whilst the isolator doors remain open so that transfer of sterilized wrapped parts does not represent risk of introducing contamination into the isolator (sterilized parts as contamination vector). Furthermore, not only multilayer wrapping but also properly designed transfer and set up procedures play a role here.
- Loading into the sterilizer in a methodical manner that allows the set up in a logical manner.
- Wrapping that allows ease of removal, so as not to breach first air principles.

Primary packaging components addition:

This area of activity should include a number of considerations:

- If not pre-loaded in isolator prior to decontamination, (e.g., for very small batches), then introduction to the isolator of components such as stoppers should be either through transfer isolators or the use of Rapid Transfer Ports (RTP).

- Where RTP ports are used the method of opening the beta ports need to be designed so as not to breach first air that travels over the stoppers or indirect contact part systems (controlled beta port open should be considered).

- Vials should be transferred directly into the isolator using a dry heat sterilization/depyrogenation tunnel or use similar systems and considerations to those listed above for RTP systems.

- If using nested components, then these may be transferred into the isolator using 2 main methodologies, either using sound material contamination methods (i.e., VHP, electron beam) sanitization of in the tube or transfer chamber(s) and a No Touch Transfer (NTP) approach.

- NTP relies on the removal of multiple layers of sterilized wraps that are removed at the interface to each grade, and helps to avoid direct manual contact with sterilized supplies.

- The removal of each layer of Wrap either for E beam or NTP should be at a minimum be performed through glove ports but if possible using well designed robotic systems.

Set up process:

- The setup process will be performed with personnel enhanced gowning (e.g. sterile clothing, whilst the isolator doors remain open). Although this should be considered a contamination reduction step and a key part of the Contamination Control Strategy, it should not be considered an aseptic process with the surrounding background room remaining classified as grade C.

- The process could be performed using at least two operators designated as “primary/receiving” and “transferring/support” operator. This involves the second “non clean” operator removing the first outer sterilized wrap at the interface of the isolator and handing the equipment to the first (clean) operators without touching the next sterile layer. The “tube of tooth paste” method would be commonly used. The clean operators would then transfer the equipment into the isolator. Also, it should be noted that the operators will also use good aseptic behavior during the set up even though the process is not an aseptic process.

- The isolator must maintain a positive airflow during the door open process to ensure airflows from the isolator into the surrounding grade C area minimize the risk of biological burden into the isolator that would create a greater challenge to the VHP decontamination process.

- The company must have air visualization e.g. smoke studies of the setup process demonstrating appropriate airflows are maintained to minimize the risk of contamination during this process.

- The final wrapping will be removed with the Isolator doors closed and immediately prior to running the qualified decontamination cycle.

Note 1: The above proposals are intended to minimize risk of microbial contamination during the set-up process but should not be considered a truly aseptic process.

Note 2: It is generally agreed that isolators provide an increased level of protection from the operator once the process is running, but that there is a significant cost associated with implementation of Isolators rather than RABS. Caution should be taken not to apply requirements that are fully reflective of an aseptic process that would potentially inhibit industry from investing in Isolator technology.

Pre Use Post Sterilisation Integrity testing (PUPSIT):

Although not a new requirement, the updated Annex 1 published 2022 is far clearer that the previously published version from 2009 in the requirement to perform PUPSIT on the primary sterilizing filter during aseptic filling. (This clear expression of the expectation had led to divergent approaches amongst Industry and regulators). The industry is discussing how best to implement PUPSIT (where not already in place) and this section will detail expectations and options that will help harmonize the design, and therefore the inspection process. The Annex 1 does allow some area for risk assessment for a company to define when it does not feel that the use of PUPSIT is in the best interest of the patient (rather than just operational challenges, this risk assessment will include elements such as the filtration system design (e.g. pre filters etc.) and the likelihood of the product masking a damaged filter, but this risk assessment will be very specific to each product and will not be further discussed in this paper.

The implementation of PUPSIT should be taken as a minimum requirement. However, on the assumption that the company has performed the assessment and will perform PUPSIT the following are considerations for successful implementation:

Filtration design:

- The manufacture of the medicinal product will use either a single sterilizing filter or a dual sterilizing filter system; this will be based on product and process design and filter validation. In general, dual filters are recommended.

- Where a dual filter system is used then the manufacturer will define what and how the filters are designated, examples include:

    - Filter one (upstream) sterilizing filter, filter two (downstream) redundant

    - Filter one, redundant, filter two, final sterilizing filter

    - Filter one and two sterilizing filters

- Based on the definitions chosen, this will define the strategy used for PUPSIT and what action will be taken in the event of an integrity test failure.

- If both filters are designated as sterilizing filters the implication is that they are both integral to the final sterilization of the product. In this case both filters must have PUPSIT performed, and any failure implicates the batch.

- One filter sterilizing, one redundant then it is only required to perform PUPSIT on the filter designated as the sterilizing filter. In the event that this filter fails, then post integrity (pre use integrity data is required for both filers) can be performed on the filter designated as redundant. If this filter fails, the product would then be in question.

Filter location:

The are arguments for the location of the filters e.g., inside or outside the filling cabinet or isolators. The following considerations should be clearly considered during the design and subsequent description of the filtration process in the Contamination Control strategy.

- Inside the filling cabinet or isolator:

    - The filtration equipment has to be transferred into the critical zone and therefore adds potential risk to the critical zone.

    - PUPSIT has the potential risk of contributing microbial contamination due to activities such as venting of the non-sterile liquid during the wetting process.

- The design of the system should include appropriate vent filters (which themselves generally require integrity testing) and closed systems that allow the collection of unfiltered waste without compromising the critical zone.

- Filtration systems are often complex systems that can cause issues by breaching first air in the isolator and impacting airflows.

- Outside of the cabinet:

  - This removes the risk to the critical zone but does introduce the risk that if non integral then air that is not of a grade A quality could be introduced into the system after the final filtration of the product. However, this is mitigated by placement of this part of the equipment train in a specified area that is protected by extensive HEPA-filtration.

  - Annex 1 clause 8.80 states that:

    "Suitable bioburden reduction prefilters and/or sterilising grade filters may be used at multiple points during the manufacturing process to ensure a low and controlled bioburden of the liquid prior to the final sterilising filter. Due to the potential additional risks of a sterile filtration process, as compared with other sterilisation processes, an additional filtration through a sterile sterilising grade filter, as close to the point of fill as possible, should be considered as part of an overall CCS."

    It is not defined what "as close to the point of fill as possible" means and consideration should be given to ensure that the number of aseptic connections after the sterilizing filter should be avoided (via SIP) or minimized, and only performed in Grade A and also using intrinsic sterile connectors where possible.

The preference would be to locate the filtration system outside the filling cabinet or in a separate section but as close to the point of fill as possible to minimize the potential impacts discussed previously.

Closed Systems:

The updated Annex 1 has a new section relating to closed systems discussing both re-usable and disposable systems. The addition is indicative of a general industry move towards closed systems. The use of closed systems has a number of benefits including creating a secure barrier preventing contamination from external contamination. Closed systems take the form of fixed systems such as those used in API manufacture and older biological drug substance manufacture and single use systems such as commonly seen in vaccine manufacture. The annex gives a lot of guidance regarding the use of closed systems but is silent on key items such as the background to be used when using these systems. This section give guidance on key considerations for the selection of backgrounds. Much of the decision on the background environment will be based on the ability to detect a breach in the integrity of a systems, assuming that a robust system is used e.g. pressure decay or helium leakage rather than solely relying on manual visual inspection.

<u>Key Considerations</u>:

- Where it is not possible to detect a leak by post use integrity testing:

  - The background should be a higher grade e.g. Grade A, as the system itself could not be guaranteed as closed and therefore must be considered at risk of contamination from the environment.

- Where it is possible to assure integrity by the use of a validated integrity test and no manipulations are performed in the area, then the system could potentially can be used in a lower grade area below Grade A classification, e.g. grade D (see Annex 1 Table 4 which gives examples from Grade

A through D bearing in mind the appropriate risk). But any failure in integrity would potentially lead to rejection of the product.

Mitigation Strategies:

There is a concern that despite the annex having been effective for equipment and facilities since August 2023, some companies are not yet able to fully meet the expectations If this is the case it is necessary for each company to assess the specific risk(s) and Gap(s) identified and then assess the patient need versus the GAP and potential patient risk. This assessment will be company, facility, equipment and product specific so will not be addressed in this paper but general key considerations should include:

- Risk to patient
- Existing mitigation
- Identification and implementation of new control measures to reduce the risk
- Limitations of detection systems
- Record of the decision of risk acceptance (or otherwise)
- Longer term commitments to address the Gaps:
    - To include the longer term CAPA(s) to be formalized in the Quality system.
    - Senior management acceptance of the current state and commitment to the CAPA (especially where the CAPA may require large Capital Expenditure).
    - A periodic review of the current process to ensure that there are no changes in the level of risk identified and therefore the decision regarding risk acceptance.

Note 1: It should be noted that, due to the fact that the Annex was published in 2022 with an already extended implementation date of August 2023, the regulatory tolerance for acceptance of gaps will be reducing with passing time. However, it is also noted that the regulator is also adverse to the causation of product shortages. For this reason, it would be advisable for each company to have their regulatory outreach strategy defined, this may include a proactive approach to the regulator to ensure that they are aware of the gaps and accepting of the company's proposed strategy. Such strategies would need to be supported by qualification/validation and subsequently proven as durable and reproducible over time once implemented commercially.

Note 2: It should be noted that mitigation of risk should not be based solely on the output of monitoring systems such as Aseptic Process Simulations, Environmental monitoring and sterility testing. Mitigation is likely to be formed of a mix of operational measures (e.g. enhanced procedures and training) and Technical measures (e.g. design such as additional use of barrier technology and automation).

Group 3: Personnel Training and Procedures

It is a well-established truth that aseptic operators must be qualified to a level that allows a facility to perform its operations in a way that minimizes contamination risk to products and meets regulatory requirements for producing sterile products. To this end, companies operating in this space invest heavily in the recruitment and training of skilled and talented personnel to carry out aseptic operation functions. The rationale for such recruitment and training is clear: aseptic behavior is crucial in clean rooms to maintain product sterility and prevent product contamination. A key contamination risk in such an environment is human error and therefore the combination of experience, training and task execution as the criteria for operator qualification is vital to ensure product sterility and the assurance of product quality.

The criteria to ensure appropriate levels of qualification are well defined, highlighting the importance of ensuring robust training programs are in place with ongoing effectiveness monitoring and periodic requalification strategies. They include:

- ***Designing Equipment and Processes to Minimize Contamination Risk:*** Personnel errors are a significant source of contamination in aseptic processing. Good design sets operators up for success. In addition, proper training and qualification ensure that operators understand and implement behaviors and techniques to prevent microbial contamination and to ensure product sterility and that these techniques and behaviors are systematically implemented.

- ***Regulatory Requirements:*** Regulatory bodies acknowledge the importance of personnel training and qualification in aseptic processing. For example, the FDA's *Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing* outlines the need for knowledge and skill-based training on aseptic technique, cleanroom behavior, microbiology, gowning, personal hygiene, and job-specific training.

- ***Aseptic Process Simulation & Smoke Studies:*** A key aspect of qualification is ensuring operators can properly simulate manufacturing operations and that this can be confirmed prior to manufacturing via APS and Smoke Studies activities.

- ***Gowning Qualification:*** A key aspect of qualification is ensuring operators can properly don and wear appropriate gowning and other protective equipment to provide a barrier between themselves and sterile materials.

- ***Continuous Training:*** Initial qualification is followed by ongoing training programs to maintain and enhance operator skills and knowledge, covering theoretical and practical aspects of GMP, microbiology, and hygiene.

- ***Monitoring:*** Personnel monitoring, including routine checks of gloves and gowns, is crucial for detecting contamination and identifying adverse trends.

That said, operator effectiveness is not solely determined by training; it also depends on softer criteria such as mindset and behaviors. This includes the ability to make sound decisions, assess risks accurately, and exercise good judgment during production activities. Operators must actively recognize potential contamination risks and take appropriate actions to minimize them. To do so requires that they understand the “why”.

Despite these intensive levels of training, qualification, and monitoring, personnel issues continue to be prevalent regulatory observations. Key observational themes include:

- ***Inadequate Training***: Observations highlight the lack of proper training and qualifications for personnel, leading to non-compliance with procedures.

- ***Improper Gowning and Gowning Technique:*** Many instances of personnel wearing inappropriate gowning for aseptic processing, compromising sterile conditions.

- ***Inadequate Supervision:*** Vigilant supervision of daily operations is critical to provide feedback to operators and prevent contamination hazards.

- ***Poor Monitoring Practices:*** Inadequate environmental and personnel monitoring techniques, impacting the reliability of microbial data collection.

- ***Failure to Follow Procedures:*** *Observations* of personnel not adhering to established procedures, resulting in contamination risks and other issues.

As properly qualified operators are essential for various aseptic processing tasks, including Setting up and Operating Equipment, completing Documentation, Cleanroom Maintenance and Media Fill Procedures, non-compliance with established qualification criteria can have a significant impact. For example, contaminated product lot rejections, recalls, and the legal repercussions of non-compliance may all be outcomes of a non-robust training and qualification program.

So, what are some key strategies that can be employed by companies to ensure proper task execution?

- ***Quality Management System (QMS):*** Robust QMS Implementation that aligns with both operational needs and compliance requirements, including frequent internal audits by qualified quality assurance staff to proactively identify and address deviations before FDA inspections. In addition, the implementation and management of a robust document control system (ideally automated) to ensure proper version control, revision history, and approval processes for procedures and other relevant documents. Finally, a robust Corrective and Preventive Action (CAPA) program to investigate the root causes of deviations and implement effective measures to prevent recurrence.

- ***Reduce Human Error:*** Ensure SOPs are clear, detailed, and regularly updated to reflect current practices and regulations. Where possible, introduce simple, task-focused job aids and work instructions and visual aids to support SOPs. In addition to improving conformance to current standards, human error is also improved by identifying areas for continual improvement of facilities, equipment, and processes.

- ***Quality Culture:*** Ensure that the behaviors of everyone in the organization prioritizes the provision of safe, effective, and high quality pharmaceutical products to patients.

- ***Senior Management Presence:*** A strong and visible presence of senior leadership on the production floor is essential to sustaining a culture of operational excellence and quality. Leaders should regularly conduct Gemba walks to observe adherence to standards, engage directly with operators, and build trust-based relationships that encourage open dialogue about challenges and risks. This proactive engagement fosters a psychologically safe environment where operators feel empowered to speak up, enabling timely interventions and continuous improvement. Leadership's role extends beyond observation—it includes setting clear expectations, reinforcing accountability, and guiding teams back to standard when drift occurs. It's not just about operators; supervisors and managers play a critical role in shaping behaviors and ensuring consistency. Similarly, quality professionals must be embedded in the Gemba, not as auditors but as partners—ready to support, coach, and resolve issues in real time. "Quality on the floor" means being physically present to understand the reality of operations, simplify processes, and co-create effective control strategies. Even the best training and technology can erode over time if not reinforced by leadership and quality engagement—preventing the "slippery slope" where standards quietly shift and risks escalate.

- ***Aseptic Coaches:*** Designating qualified experts as aseptic coaches can provide another resource to operators to provide support on the shop floor, where these coaches can augment existing training programs and provide real-time feedback on aseptic behaviors and practices.

- ***Leverage Technology:*** Utilization of novel methodologies such as Virtual Reality, AI tools can play a critical role in the training and qualification of personnel. Even then, the most robust operator training programs can lose their effectiveness over time if not actively sustained. Initial qualification may ensure technical competence, but without periodic reinforcement, the deeper understanding of *why* certain behaviors and standards matter, especially in contamination control, can erode. The best programs ensure operators undergo intensive aseptic training that includes both classroom instruction and hands-on practice, covering topics like microbiology, cleanroom behaviors, and environmental monitoring. However, sustaining this level of awareness requires more than a one-time effort. Programs must include **recurring training and requalification** that revisit not just the *how*, but the *why*—why glove integrity matters, why first air must be protected, and why vigilance is essential in every movement. This emphasizes the need for periodic requalification and a culture that challenges the notion of "we've always done it this way". Without this mindset shift, even well-trained operators may default to habits that compromise product quality and patient safety. Examples of training methods to support understanding of the linkage between equipment and facilities with personnel could include a combination of:

    - Hands-On Simulation

    - Video-Based Instruction

    - Virtual Reality (VR) Modules

    - Classroom Instruction

    - Mentorship Programs

- ***Equipment Design:*** The complexity of drug product filling equipment such as isolators, Restricted Access Barrier Systems (RABS), and closed systems demands that operators understand not only how to use the equipment but also how their actions impact product sterility and regulatory compliance. Operators and supervision should be trained in the setup, operation, and maintenance of equipment including filling lines, glove ports, and transfer systems. Understanding sterilization cycles (e.g., SIP, VHP), aseptic assembly, and intervention protocols is essential. Operators and supervisors should understand cleanroom classifications (Grade A-D) and how environmental controls support sterility assurance therefore training must include procedures for working in classified areas without compromising airflow or surface integrity. Human factors consideration during equipment design should be executed to ensure feasibility of operators to utilize equipment as designed, Given Annex 1 emerging guidance, training should embed certain specific concepts such as isolator glove utilization given they are sanitized and not sterilized, as well as techniques for operators to avoid impacting first air at all times. To achieve this training, equipment training programs should consider:

    - ***Risk-Based Scenarios:*** Training should include simulations of contamination events, equipment failures, emergency interventions to build decision-making skills such as mock interventions (e.g., smoke studies) to demonstrate impact on first air.

    - ***Cross-Functional Collaboration:*** Design training that includes input from Quality Assurance, engineering, maintenance to ensure holistic understanding such as joint workshops where operators practice equipment set up with QA oversight and engineering support.

Finally, operations management (executives as well as shopfloor supervisors) should visibly champion and reward all of the above, in tandem with quality assurance managers.

In conclusion, there is no one thing which can be done to address the ongoing concerns highlighted by regulators with regard to the behaviors and expertise of the human in the aseptic environment. As manufacturing technology develops, so must the tools and techniques implemented to ensure that operators are appropriately trained and qualified, contamination controls are maximized, and patient-related risks, due to product quality and/or supply interruption, are minimized.

Group 4: Applicability of EU Annex 1 to Low Bioburden Biologic Drug Substance Manufacturing

Maintaining adequate contamination control in pharmaceutical manufacturing is critical for product quality and patient safety. This document explains how EudraLex, Volume 4, Annex 1 and Annex 2 guidelines intersect and complement each other in managing contamination control for low bioburden processes, emphasizing a risk-based approach tailored to specific manufacturing steps.

EudraLex, Volume 4, "***Annex 2***, ***Manufacture of Biological active substances and Medicinal product for Human Use"*** provides general guidance and considerations for low bioburden manufacturing related to Personnel, Premises and Equipment, Animal derived materials, Documentation, Production, Starting and Raw Materials, Seed lot and Cell bank system, Operating principles and Quality control. Maintaining overall low bioburden levels is a cornerstone of product quality and patient safety. ***"Manufacturing and storage facilities, processes and environmental classifications should be designed to prevent the extraneous contamination of products. Prevention of contamination is more appropriate than detection and removal, although contamination is likely to become evident during processes such as fermentation and cell culture. Where processes are not closed and there is therefore exposure of the product to the immediate room environment (e.g. during additions of supplements, media, buffers, gases), control measures should be put in place, including engineering and environmental controls on the basis of QRM principles. These QRM principles should take into account the principles and guidance from the appropriate sections of Annex 1 to EudraLex, Volume 4, when selecting environmental classification cascades and associated controls"*****.**

While EudraLex, Volume 4, ***Annex 1***, "***Manufacture of Sterile Medicinal Products"*** is associated with sterile manufacturing of medicinal products, the updated guidance (August 22, 2022) offers valuable considerations for non-sterile, low bioburden processes. The scope of the guidance indicates that the principles may be applied to other non-sterile processes where microbial controls are considered important.

"The intent of the Annex is to provide guidance for the manufacture of sterile products. However, some of the principles and guidance, such as contamination control strategy, design of premises, cleanroom classification, qualification, validation, monitoring and personnel gowning, may be used to support the manufacture of other products that are not intended to be sterile such as certain liquids, creams, ointments and low bioburden biological intermediates, but where the control and reduction of microbial, particulate and endotoxin/pyrogen contamination is considered important. Where a manufacturer elects to apply guidance herein to non-sterile products, the manufacturer should clearly document which principles have been applied and acknowledge that compliance with those principles should be demonstrated."

Annex 2 refers to Annex 1 specifically when choosing environmental classification cascades and associated controls, emphasizing that Annex 1 is cited because it remains the only current EU GMP source of guidance available on this subject. The use of Quality Risk Management (QRM) principles is also encouraged, to consider not to mandate full compliance with Annex 1 for low bioburden manufacturing, but to use its guidance as a valuable reference for informed decision-making. ***"Although the title of Annex 1 refers to the manufacture of sterile medicinal products it is not the intention to force the manufacture of sterile product at a stage when a low bioburden is appropriate and authorized. Its use is because it is the only EU GMP source of guidance on all of the classified manufacturing areas including the lower grades D and C."***

The importance of strategies related to contamination control is evident across both Annexes where the level of control can often increase in detail from early to later steps in the manufacture of biological active substances to drug products. A risk-based Contamination Control Strategy (CCS) or program is recommended to be in place for each manufacturing facility in order to define all critical control points and assess the effectiveness of all the controls (design, procedural, technical and organizational) and monitoring measures employed to manage risks to product quality and safety.

The development of the CCS requires assessment by those with detailed technical and process knowledge. Properly executed contamination control strategy assessment of low bioburden manufacturing facility considering applicable Annex 1 and QRM principles should help to deliver and justify proper level of controls required for given manufacturing steps. A governance structure to provide oversight of the CCS, the effectiveness of the contamination controls, the lifecycle management, and to escalate and remediate control issues is necessary to ensure the required outcomes are achieved and maintained.

Elements to be considered within a CCS for application to low bioburden manufacturing should include (but are not limited to):

- Design of both the facility and processes including the associated documentation
- Premises and equipment
- Personnel and training
- Utilities
- Raw material controls – including in-process controls
- Product containers and closures
- Vendor approval – such as key component suppliers, sterilization of components and single use systems (SUS), and critical service suppliers
- Management of outsourced activities and availability/transfer of critical information between parties (e.g., contract sterilization services)
- Process risk management
- Process validation
- Validation of sterilization processes, as well as processes intended to reduce or minimize bioburden
- Preventative maintenance – maintaining equipment, utilities, and premises (planned and unplanned maintenance) to a standard that will ensure there is no additional risk of contamination
- Cleaning and disinfection
- Monitoring systems – including an assessment of feasibility of the introduction of scientifically sound, alternative methods that optimize the detection of environmental contamination (e.g., Rapid Microbial Methods (RMMs).
- Prevention mechanisms – trend analysis, detailed investigation, root cause determination, corrective and preventive actions (CAPA) and the need for comprehensive investigational tools.
- Continuous improvement based on information derived from the above.

The CCS should also be periodically reviewed and updated to ensure that the contamination controls consistently deliver acceptable results. CCS should drive continual improvement of the manufacturing and control methods.

Note: Certain sections of Annex 1 are tailored exclusively for sterile manufacturing, for example, Section 8 (including Blow Fill Seal technology), Aseptic Process Simulation, PUPSIT of the final sterilizing filter and

Sterility Testing. These requirements do not need to be applied or evaluated in processes focused on low bioburden manufacturing.

Regulatory landscape – Inspectors from some health agencies have been using aspects of Annex 1 as best practices for the manufacturing of low bioburden or non-sterile drug substances. The 2022 update to Annex 1 places a strong focus on contamination control and continuous improvement. Even when products are not manufactured under sterile conditions, manufacturers are still expected to proactively identify and manage microbial or contamination risks. Annex 1 serves as a practical resource for developing robust contamination control strategies; it can essentially act as a blueprint for designing cleaner, safer processes.

- **Real-world example:** A company producing monoclonal antibodies will use microbial controls to maintain the integrity of their cell culture process but may not need to maintain strict aseptic conditions during the various stages of their process, where microbial levels are typically low, to avoid downstream contamination. The company in this case chooses to use closed bioreactors and automated sampling to minimize human contact and reduce risk. In low bioburden manufacturing, one of the key concerns is the ability of microorganisms to grow in the cell culture process.

Risk-based facility planning, one size doesn't fit all – Instead of strictly applying Grade A/B cleanrooms everywhere, EU Annex 1 and EU Annex 2 encourages a risk-based approach. That means designing environments to appropriately minimize likelihood of contamination and identifying effective applicable controls at each step.

- **Real-world example:** A fermentation suite might operate in a Grade D environment because the process is essentially closed, and microbial risks are low. But downstream purification, where the product is more vulnerable, might require Grade C or even Grade B conditions. In other cases where the process may not be fully closed, the control of higher grade conditions may be more applicable.

Build controls into the process – Closed systems reduce exposure and can allow for lower-grade environments in manufacturing and allow filling in a lower-grade environment, without the closed system in place, further consideration of the filling environment in required to control the environment.

- **Real-world example:** A drug substance filling operation for a low bioburden product uses a sterile closed filling operation to ensure a sterile boundary and may be moved into an (ISO 8) Grade C environment. This setup not only meets Annex 1's recommendation but also improves operational efficiency, reduced risk and environmental monitoring activities. Furthermore, this improves conforming product and reduces the risk of reject batches and potential market shortages.

Framing the CCS – The CCS is the playbook for minimizing the risk of contamination. It should cover everything from facility design to cleaning procedures, environmental and gowning monitoring, and how people, material and waste move through the facility into the production spaces. The playbook can help identify higher risks requiring greater controls, and lower risks with appropriate controls. This approach can help in maximizing quality, reducing risks, and controlling costs.

- **Real-world example:** A biologics manufacturer documents their CCS with detailed risk assessments, cleaning protocols, and microbial monitoring plans. They also track personnel movement and use color-coded zones to reinforce hygiene practices. Materials are transferred over lines of demarcation and go through contact times – all to reinforce contamination controls. Reducing unnecessary gowning and maintenance requirements in non-critical areas helped to reduce waste and meet sustainability goals at the facility without adding unnecessary risks.

Setting bioburden limits – the EMA's *Guideline on the sterilization of the medicinal product active substance, excipient and primary container*, developed in parallel to Annex 1 suggests a bioburden limit of 10 CFU/100 mL prior to final filtration can be a practical target. When appropriate, higher limits can be justified if the process includes effective microbial reduction steps with process specification requirements supported by data. Equally important is the characterization of your bioburden that is present in the product.

What is normal, what is an outlier, excessive, and everything in between, knowing establishing what is "typical" (through sound quality risk management and data analysis) helps identify any shifts and are typically associated with potential root causes. This can help in the rapid identification of sources and remediation, including organisms of concern (e.g., molds, gram negative and spore forming organisms). It is important to also establish microbial control strategies associated with upstream manufacturing processes. Upstream microbial control through risk assessment and process detection methods alone are not acceptable to rely on during upstream manufacturing.

- **Real-world example:** A company running downstream purification occasionally sees bioburden levels above 10 CFU/100 mL. The company has strong procedures in place for monitoring and identification of microorganisms above the bioburden limit. A further example, considering bioreactor set up, results in bioburden contamination during bioreactor assembly which can lead to batch contamination. With strong risk management, deviation, and trending program, the company implements corrective actions to control the bioburden limit, especially organisms that could impact the quality of the product and process.

Filtration – Filtration is key to controlling bioburden, but it is not just about the correct use of a 0.2 µm filter to remove microbial contamination (Ref Annex 1 clause 8.81). Demonstration, through validation, that the filter is effective under real process conditions is required. Pre-filters and bioburden filters also play an important role.

- **Real-world example:** The manufacturer validates filters using high-viscosity protein solutions. A QRM review confirmed that pre- and post-use integrity testing is required and already implemented. Proper product characterization supports filter validation and identifies potential masking effects.

Embracing technology – Modern tools can make microbial monitoring faster and more reliable. Traditional methods rely on incubation and manual inspection and only provide data after many days later. More companies are leveraging technology not only to reduce risk but also to come close to "real-time" results.

- **Real-world example:** A company uses a validated ATP bioluminescence to check surface cleanliness in minutes instead of days. Another company integrates automated air samplers with their building management system for real-time alerts, while minimizing unnecessary testing costs of traditional microbiological methods.

Culture, training and accountability – Contamination control is not limited to equipment and process controls, people are critical and building a culture of hygiene and accountability is essential.

- **Real-world example:** Operators at a biologics plant undergo quarterly training that includes gowning simulations and contamination case studies. Environmental monitoring results are shared openly to reinforce good habits. Ideas to reduce risks are openly discussed and integrated into facility procedures when beneficial.

Final Thoughts:

Low bioburden manufacturing is a balancing act between science, regulation, and practicality. By applying Annex 1 and QRM principles thoughtfully and learning from real-world examples, manufacturers can build robust, compliant processes that protect both product and patient. There is no singular control measure that gives microbial quality assurance and ensuring adequate contamination controls; the manufacturing process relies on building layers, forming a robust and integrated process with patient safety being at the focal point.

Key Takeaways:

- **Design smart, not sterile:** Design and qualification of the facility and equipment should come first, including automation, integration, and isolator/closed systems where possible. Then leverage other

QRM principles to manage residual risk through procedures and finally leverage detection mechanisms as confirmation that design controls are functioning correctly.

- **Build a strong CCS:** Document integrated risk and control strategies and revisit it regularly.
- **Validate your filters and justify your limits:** Data is your best defense.
- **Invest in technology and training:** They can pay off in reliability and speed.
- **Foster a contamination prevention culture: It starts with multi-functional leadership and grows with accountability.**
- **Consider sharing best practices and solutions by publication or at conferences.**

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