EU GMP Annex 1: Manufacture of Sterile Medicinal Products

White Paper

Author: Dr Tim Sandle

Abstract

The new version of EU GMP Annex 1 was issued on 22nd August 2022[1], addressing the manufacture of sterile products. This is a legally binding part of EU GMP (as per Article 47 of Directive 2001/83/EC on the Community code relating to medicinal products for human use of the European Union). It also applies to the UK, as part of the Medicines Act of 2020.

The purpose of this white paper is to introduce Annex 1 and to describe the significant changes and the scientific and regulatory reasons behind these important updates. Where key sections of the Annex are referred to, the relevant clause is indicated (e.g., '8.12'). Because the Annex mixes up topics, this white paper has grouped topics thematically in an attempt to make the requirements of the Annex easier to follow.

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Introduction

Annex 1 has undergone no significant update for 15 years. A signal that a revision was underway appeared in 2017 through the issuing of a draft for public comment[2]. No drafts of Annex 1 should be referred to, only the official text applies. However, it is notable with the final version and the drafts that came before it that the sections of sterilisation methods have been expanded and areas like cleanroom classification and cleaning and disinfection have been shortened.

While there are several essential points to consider for the contamination control strategy, those that appear to be given the greatest weighting are:

- Maintaining the critical processing zone
- The aseptic assembly of filling equipment
- Aseptic connections (these should be sterilised by steam-in-place whenever feasible)
- Special focus on aseptic compounding and mixing
- The risks abound the replenishment of sterile product, containers and closures
- Concerns around the removal and cooling of items from heat sterilisers
- Staging and conveying of sterile primary packaging components
- Aseptic filling, sealing, transfer of open or partially stoppered vials, including intervention
- Loading and unloading of a lyophilizer

It is unsurprising that each of these relate to aseptic processing, the highest-risk area of pharmaceutical manufacturing.

The table below provides a short summary of the legal status of Annex 1 and the revision and implementation process.

What is Annex 1?	Annex 1 to EU GMP provides detailed information for the manufacture of sterile medicinal products; it supports the core chapters of EU GMP
What is the legal status of Annex 1?	It is the first annex of EudraLex "The Rules Governing Medicinal Products in the European Union" and it forms part of Volume 4 of the European guidelines
When was the new version of Annex 1 issued?	22nd August 2022
What is the new version replacing?	The new version replaces the 2007 version (and its 2008 amendment for vial capping)
How many drafts were issued?	Two drafts were released for public comment in 2017 and 2020
What is the date of implementation?	25th August 2023 (one year from the date of publication) for all parts except 8.123
What is the date of implementation of part 8.123?	25th August 2024 (two years from the date of publication)

Table 1. The legal basis of Annex 1

The scope of Annex 1 applies to all sterile medicinal products manufactured in the European Union and the UK, as well as those manufactured elsewhere and exported into the European Union. In addition to seeking the assurance of sterility (that is, being free from viable microorganisms), sterile medicinal products also need to be free of visible particulates and to be apyrogenic. In keeping with previous guidance, the default manufacturing route should be with terminal sterilisation, with aseptic processing selected only where a terminal sterilisation method would damage the product.

The scope of the Annex provisions extends to:

- Finished products
- Active substances
- Packaging materials
- Products provided in any size and combination
- Any manufacturing process
- Any manufacturing technologies
- Any manufacturing scale, where the objective is to provide a sterile product
- The design and control of facilities, equipment, systems and procedures

Core principles

The core principles of the 2022 version of Annex 1 can be broken down into ten essential points. These are summarised as:

1. The Annex covers manufacturing, packaging and distribution
    a. Manufacturing begins with the suitability of raw materials (the assessment of bioburden and endotoxin)
    b. Scientific principles for designing processes should apply
2. The Annex only sets out the minimum requirements, especially where limits are outlined in the text
3. It is not simply sufficient to qualify or validate facilities, equipment and processes – there needs to be systems for continuous verification. In addition, regular reviews must take place
4. People involved with manufacturing, packaging and distribution must have suitable qualifications and experience, be appropriately trained and demonstrate appropriate behaviours to protect sterile products
    a. The overall process must be overseen by people with process understanding, and those with engineering and microbiological knowledge
5. Interpretation and implementation of the Annex must be based on the principles of Quality Risk Management (QRM). This signals the necessity to follow ICH Q9 (Quality Risk Management)[3]. Importantly with QRM:
    a. It should be used proactively and be based on:
        i. Design
        ii. Good procedures
        iii. Effective monitoring
    b. It should connect and shape Quality by Design principles
    c. It can be used to address problems with procedures and processes
    d. It needs to be part of deviation management
    e. It can be used to implement alternatives to the Annex that are equivalent or better, but it cannot be used as the basis not to adhere to any provision within the Annex. The core principles are rationale, risk assessment and risk mitigation
    f. It must operate throughout the product lifecycle
    g. It must be documented
    h. Risk mitigations must be supported by a rationale
    i. The quality governance process must regularly review QRM
6. Annex 1 should be embedded into the Pharmaceutical Quality System as per ICH Q10 (Pharmaceutical Quality System) and in line with Chapter 1 of EU GMP
7. Parts of the Annex can be considered by manufacturers of non-sterile products to reduce the likelihood of microbial, particulate or pyrogenic contamination. Here, non-sterile manufacturers are required to cross-refer to applicable parts of the Annex and explain how they are adopted
8. There have been advances in sterile manufacturing technology, especially with RABS and isolators. There have also been advances with rapid microbiological methods, which the Annex acknowledges. There is a statement encouraging manufacturers to adopt technologies that increase product protection from microorganisms and microbial by-products like toxins
    a. This leads to an important principle of seeking to protect the product from people and from the environment
    b. Methods that achieve rapid detection are encouraged (such as rapid microbiological methods)
9. There is the requirement for a formal, holistic contamination control strategy (which is often abbreviated to ‘CCS’ in the new draft)
    a. Contamination control involves connecting interrelated controls and measures. It is a holistic concept
    b. The expectation now appears to be for a formal document which reflects the site-wide strategy for minimising contamination control with respect to sterile manufacturing[4]. The CCS enables the facility to fully-understand and review:
        i. Design
        ii. Procedural
        iii. Technical
        iv. Organisational controls
    c. Contamination control is a wide-ranging, interrelated and continuous concept[5]
    d. The CCS should focus on proactively preventing contamination, based on the principles of continuous improvement
    e. The CCS should be subject to regular review
10. Simply testing and monitoring does not ensure sterility. In other words, a detailed contamination control strategy based on quality risk management leading appropriately designed premises, equipment and processes and executed by qualified personnel following appropriately designed procedures contributes towards the assurance of sterility

Contamination Control Strategy

As indicated, the CCS occupies a central place in the implementation and continued operation of Annex 1. To re-emphasise, the CCS is made up of a series of discrete elements which needs to be considered as an interconnected whole.

Contamination under Annex 1 is not simply about microorganisms.

Defining contamination	
Microorganisms	Bacteria and fungi (viral risks are not specifically addressed in Annex 1)
Microbial by-products	Cellular material e.g., endotoxin
Non-endotoxin pyrogens	Other cell materials
Particles	Visible and non-visible (such as glass fragments)

Table 2. Types of contamination addressed in Annex 1

In terms of the scope of the CCS, it should contain different sections and consider assessment, controls and monitoring. Below is a suggested framework:

CCS subject area	**Assessed**			**Controlled**		**Monitored**
	Quality by design	**Process design**	**Technical and scientific assessment**	**Procedural controls and documentation**	**Organisational and personnel controls, including training**	**Monitoring, including real-time and rapid methods**
Plant						
Process						
Premises						
Equipment						
Personnel						
Utilities						
Raw materials						
In-process / intermediate manufacturing						
Product containers e.g., bags, vials						
Product closures e.g., stoppers						
Component suppliers						
Single-use systems						
Outsourced sterilisation						
Critical service providers						
Process risk management						
Process validation						
Sterilisation validation						
Planned preventative maintenance						
Unplanned maintenance						
Cleaning and disinfection						

Table 3: An anatomy of the contamination control strategy

In addition, the CCS must be supported by:

- Trending
- Detailed investigations using appropriate tools
- Root cause analysis and determination
- Corrective actions
- Preventative actions

The CCS should be used to drive continuous improvement throughout the facility. It is also important that all changes made through change control refer back to the CCS for an impact assessment, and that the CCS is updated as appropriate.

Premises

The first main section of the Annex (section 4) relates to premises, focusing on cleanrooms. These areas will ideally be visible for Quality Assurance personnel (4.17), either through viewing windows or through the use of CCTV.

Cleanroom design

The Annex outlines the cleanroom and clean zone grades – these are largely unchanged. In describing Grades A, B, C and D, the expected types of operations are outlined (in 4.4) and with the requirement that these are qualified according to EU GMP Annex 15. With Grade A, the necessity of demonstrating and qualifying unidirectional airflow across the entire Grade A area is emphasised. The use of air velocity measurements and airflow visualisation studies is the common way to demonstrate this. Such studies must correlate to air velocities.

All cleanrooms are required to be equipped with filtered air, designed to provide the required cleanliness class (as per 4.1). This is a change from the 2007 edition, where Grade D cleanrooms were not required to have HEPA filtered air. Attention needs to be paid to the fabric of the cleanroom, including the minimisation of ledges and avoiding sliding doors (4.6). Other verification requirements for cleanrooms are:

- Particle concentration in the air (which is the most important determinant of cleanroom classification). For cleanroom classification, the total of particles equal to or greater than 0.5 and 5 µm should be measured. This measurement needs to be performed both at rest and in simulated operations (4.27)
- Microbial assessments need to be made, both in the 'at rest' and 'in operation' states. These assessments normally start with a given number of environmental monitoring occasions (saturation monitoring) which becomes reduced following risk assessment (4.30,4.31)
  - While the 'colony forming unit' (CFU), which is used in tandem with cure-based methods, is referred to, alternative methods can be used if justified. Alternative methods may express data in a different form to the CFU, such as data obtained in relative light units from spectrophotometric particle counters
- Installed filter system leakage and integrity testing
- Airflow tests – volume and velocity
- Air pressure difference test
- Airflow direction test and visualisation
- Microbial airborne and surface contamination
- Temperature measurement test
- Relative humidity test
- Recovery test
- Containment leak test

These tests are conducted as per ISO 14644-1 (initial qualification), -2 (verification) and -3 (methodology). An important addition to Annex 1 is that the qualification (and re-qualification) exercise should ideally be performed during operations, such as media fills. For particle classification, while ISO14644 methods are referred to, the Annex advises that additional locations for particle measurements, based on risk assessment, should be adhered to (for both 'classification' and 'routine' monitoring). These additional samples should include the areas of greatest risk in terms of contamination transfer, such as point of fill in relation to aseptic filling. Particle classification needs to include the 'clean up' rate, in terms of the particles in the room returning to the required level after the classification has been exceeded within 20 minutes or a shorter time frame.

Installed filter system leakage and integrity testing:

i. Airflow tests – volume and velocity
ii. Air pressure difference test
iii. Airflow direction test and visualisation
iv. Microbial airborne and surface contamination
v. Temperature measurement test
vi. Relative humidity test

Appropriate controls need to be in place to maintain the cleanroom design parameters (as per 4.1). An important design verification tool is the airflow visualisation study (4.15), an exercise that needs to be digitally captured. The cases where airflow visualisation is required have been expanded to include:

- Track air between lower and higher-grade areas (here there should be no ingress)
- Track air movement within cleanrooms (air should not rise up from the floor and air should not move over operators or equipment)
- Track transfer from a higher grade to a lower grade area
- Airflow visualisation is required for all unidirectional airflow environments
- Airflow studies must simulate interventions (both through gloveports and any that are direct)

Airflow studies are required for 'at rest' and 'in operation conditions' (a state where the maximum number of people are present, in addition to equipment running and processing taking place) and areas of contamination transfer concern must be rectified. The output from airflow visualisation studies can be used to inform about selecting environmental monitoring locations.

Other aspects of cleanroom design are summarised in the table below (drawing on 4.1, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.14, and 4.16).

Cleanroom Grade	Requirements
A	• To be supplied with filtered air • To be maintained at a positive pressure to Grade B. Air pressure indicators must be in place, with a warning system • Localised unidirectional airflow across all areas. This is assessed by achieving velocity that conforms to a homogeneous air speed in a range of 0.36 – 0.54 m/s (unless an alternative justification can be made) • Barrier from RABS or isolator • All exposed surfaces must be smooth, impervious and unbroken • No recesses that are difficult to clean (including doors) • Ceilings must be sealed to stop contamination ingress • Materials must minimise generation of particles • Materials must be able to withstand detergents, disinfectants and sporicides • Sinks and drains are not permitted due to microbial risks
B	• To be supplied with filtered air • To be flushed effectively • To be maintained at a positive pressure to Grade C (minimum 10 Pascals). Air pressure indicators must be in place, with a warning system • Continuous monitoring of air pressure differences • All exposed surfaces must be smooth, impervious and unbroken • No recesses that are difficult to clean (including doors) • Ceilings must be sealed to stop contamination ingress • Materials must minimise generation of particles • Materials must be able to withstand detergents, disinfectants and sporicides • Sinks and drains are not permitted due to microbial risks • Airlocks must be at the same grade in the at rest condition (viable and particulate) • Separate entry and exit for personnel and materials is required
C	• To be supplied with filtered air • To be flushed effectively • To be maintained at a positive pressure to Grade D (minimum 10 Pascals). Air pressure indicators must be in place, with a warning system • All exposed surfaces must be smooth, impervious and unbroken • No recesses that are difficult to clean (including doors) • Ceilings must be sealed to stop contamination ingress • Materials must minimise generation of particles • Materials must be able to withstand detergents, disinfectants and sporicides • Any sinks or drains must be fitted with breaks; floor drains must be designed to prevent backflow. A process for regular cleaning, disinfection and maintenance must be in place, with the frequency justified in the CCS • Airlocks must be at the same grade in the at rest condition (viable and particulate) • Separate entry and exit for personnel and materials should be considered as part of the CCS
D	• To be supplied with filtered air • To be flushed effectively • To be maintained at a positive pressure to the surrounding environment (minimum 10 Pascals). Air pressure indicators must be in place, with a warning system • All exposed surfaces must be smooth, impervious and unbroken • No recesses that are difficult to clean (including doors) • Ceilings must be sealed to stop contamination ingress • Materials must minimise generation of particles • Materials must be able to withstand detergents, disinfectants and sporicides • Any sinks or drains must be fitted with breaks; floor drains must be designed to prevent backflow. A process for regular cleaning, disinfection and maintenance must be in place, with the frequency justified in the CCS • Airlocks must be at the same grade in the at rest condition (viable and particulate) • Separate entry and exit for personnel and materials should be considered as part of the CCS
First stage changing rooms	• To contain hand washing facilities

Table 4: Cleanroom requirements

With the criteria in the table on page 7, emphasis is given to air pressures. This is because low and negative air pressures to adjacent areas of a lower grade, presents a contamination transfer risk. The set points and any time delay between pressure readings needs to be justified in the CCS.

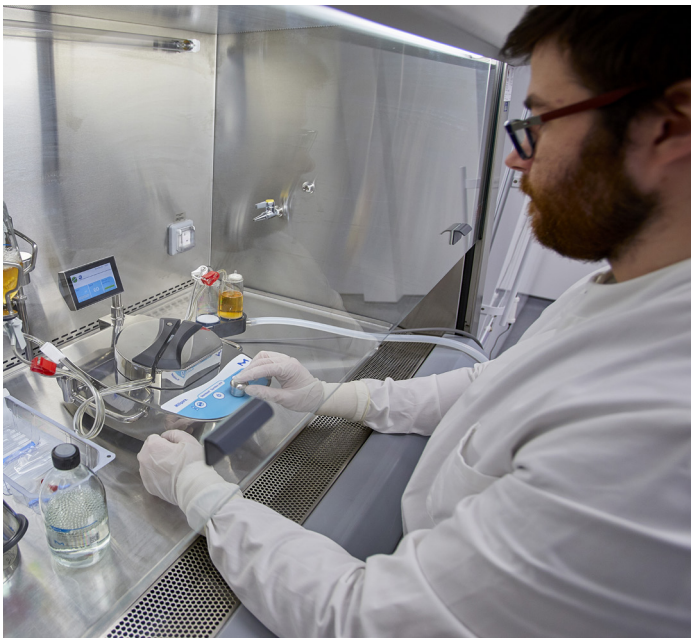
Cleanroom separation

As part of the facility design, cleanrooms for different uses should be separated (4.2) and there needs to be procedural controls in place to address the problems of contamination and of mix-up. Where toxic substances are handled, the air being expelled from the cleanroom will need to be decontaminated (4,14).

Entry of people and materials

Entry into cleanrooms should be via separate airlocks for personnel and equipment, as set out in 4.1. An effective control mechanism for airlocks is active flushing with filtered air (4.11), supported by cleaning, disinfection and barriers (such as wrapping). The local flushing of air extends to pass-through hatches. This would need to be at a rate that recognises the contamination risk presented by people and materials. For personnel, these airlocks are changing rooms. It therefore becomes important that items are not transferred in via changing rooms but instead go through a defined decontamination process. No two doors for an airlock or pass-through hatch can be opened simultaneously (as per 4.13); the Annex discusses having an interlock mechanism in place for Grade A and B areas, although it would seem logical to apply this principle to all cleanroom grades. All airlocks and pass-throughs must have audible warning systems and time delays (again based on risk assessment).

The Annex discusses the need to minimise transfer across cleanroom grades (in 4.10) and for this to be recognised as a major contamination risk area. This will be another area where QRM need to be used to develop this aspect of the CCS.



The biggest risk is with protecting Grade A, hence strict controls need to be in place and a logical design in place to support Grade A and Grade B transfer. To achieve this:

- Transfer must be one-way (4.10), such as by using a double-ended autoclave and in having separate entry and exit routes for personnel (the Annex does allow for a time-based system to be used if there is only one way in or out, although this carries greater contamination and mix-up risks)
- For Grade A and B, only approved materials can enter (4.12)
- Any item intended to go into Grade A must be protected in Grade B
- Transfer of all items must be risk assessed and monitored
- The hierarchy of control for items in terms of their relative contamination risk is (based on 4.11):

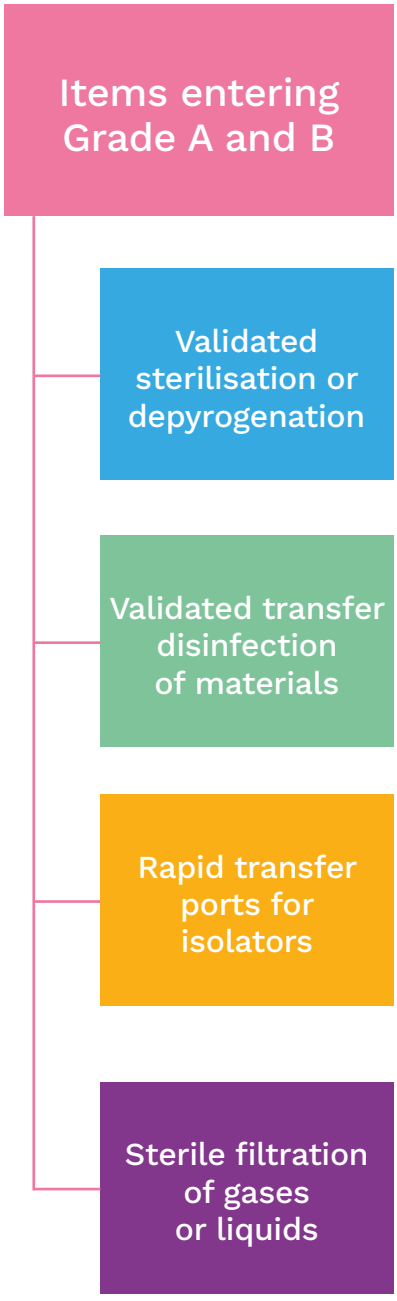


Figure 1: Hierarchy of control for Grade A and B transfer

Cleanroom monitoring

Cleanrooms need to be monitored, as per 4.1, in order to confirm that the design parameters and the required environmental conditions are being maintained.

Barrier technology

The Annex places considerable emphasis upon barrier technology for minimising contamination through separation principles and, in particular, separating the person from the product in order to maintain Grade A conditions (in 4.3). This is achieved by the use of Restricted Access Barrier Systems (RABS) or isolators, and any alteration from this requirement requires a scientific rationale (and presumably regulatory approval). Any aspect of the operation that results in personnel not using barrier and glove port technology (what is described as a 'direct intervention') must be avoided and presumably risk assessed.

In terms of the core requirements with barrier technology / separative devices (4.18-4.22):

Barrier technology type	Requirements
Open isolators e.g. provided by a depyrogenation tunnel or connected to an oversealer	• Maintain Grade A conditions • Ensure first air • Ensure unidirectional airflow, capable of sweeping away contamination • Located in a Grade C cleanroom (minimum) • Airflow visualisation must focus on open area interfaces • Be subject to leak / integrity testing • Gloves must be examined before and after each use • Gloves must be integrity tested at the start and end of each campaign or more often
Closed isolators	• Maintain Grade A conditions • Ensure first air • Air does not need to be unidirectional, but the risk of turbulent air must be understood • Located in a Grade D cleanroom (minimum) • Be subject to leak / integrity testing • Gloves must be examined before and after each use • Gloves must be integrity tested at the start and end of each campaign or more often
RABS	• Maintain Grade A conditions • Ensure first air • Ensure unidirectional airflow, capable of sweeping away contamination • Ensure positive pressure differential between the RABS and the cleanroom (with the setpoint based on risk assessment) • Located in a Grade B cleanroom • Airflow visualisation must relate to interventions, including open door interventions • Gloves must be sterile and decontaminated after each use • Gloves must be examined before each use • Gloves must be periodically integrity tested

Table 5: Essential requirements for barrier technology

Both RABS and isolators must be subject to bio-decontamination, and this must be preceded by cleaning in order to ensure residues which might interfere with the disinfection process are removed from surfaces (4.22). None of the agents used must impact on the subsequently processed product (this either means bringing residues down to an occupationally safe level and / or testing the product for the presence of cleaning and disinfection agents). For isolators, a sporicidal agent is required (gaseous or in vapour form) and the cycle must be reproducible and validated, to show no surviving microorganisms. The cleaning and disinfection of a RABS must periodically include a sporicide, with all surfaces contacted through the use of a validated process.

Other requirements need to be developed from a risk assessment and captured within the CCS. A risk assessment should include:

- Set-up and contamination transfer risks
- Bio-decontamination method and programme
- Operation and automation
- Glove / gauntlet manipulations and the impact on first air
- Impact of a loss of barrier integrity
- Impact of the loss of glove / gauntlet integrity (for isolators, gloves need to be tested at a minimum of the start and end of a campaign with the determined frequency captured in the CCS)
- Transfer mechanisms
- Impact of maintenance

'First air' refers to unidirectional filtered air that has not been interrupted prior to contacting exposed product and product contact surfaces. If the air is disrupted before it makes contact, the potential to add contamination to the air prior to reaching the critical zone arises.

It is good practice to have undertaken a thorough risk assessment of each isolator or RABS device using an established methodology like Failure Modes and Effects Analysis (FMEA). Requalification intervals remain unchanged, with Grade A / B at six-monthly and Grade C / D at annual (as minimum qualification periods). Following certain deviations or replacement of critical aspects of the cleanroom like HEPA filters, as part of remedial actions, will also trigger cleanroom requalification. The change control system should provide the optimal means for making requalification assessments (4.32).

Equipment

With equipment (5.1 to 5.3), the Annex requires that an inventory is maintained along with the initial qualification and process / instrumentation diagrams. To qualify equipment, the standard Annex 15 approach should be followed. This applies to (among other items):

- Sterilisers
- Water systems
- Air handling systems
- Particle counters

The qualification process should account for maintenance, especially how repairs and maintenance can be conducted whilst maintaining contamination control. This includes restricting activities, limiting personnel in the area, evaluating and monitoring the environmental impact, and determining suitable cleaning, disinfection and sterilisation through an impact assessment. A formal process of reinstatement is required and one that assesses sterility assurance. This also applies to unplanned maintenance activity.

With equipment sterilisation, both direct and indirect product contact parts need to be sterilised. These are defined as:

Direct product contact part	Equipment where the product passes through, such as filling needles or pumps
Indirect product contact part	Equipment parts that do not contact the product, but may come into contact with other sterilised surfaces, such as stopper bowls, guides and any sterilised components

Table 6: Direct and indirect product contact parts

In the event of leakage (e.g., of hydraulic fluids), critical equipment needs to have a warning alarm in place (as per 6.22).

Equipment used in cleanroom must be able to be disinfected. This includes electronic devices (7.9). The types of items deemed suitable need to be defined in the CCS.

Utilities

With utilities, the criticality of each system needs to be assessed as part of the CCS and covered by a risk assessment (6.1 to 6.6). Higher risk utilities, in descending order of criticality, include:

Figure 2: Relative criticality of utilities

The critical parameters and critical quality attributes must be trended and assessed, this means looking at cleanroom pressure differentials, steam quality, online water data and so on. As with equipment, diagrams, schematics and qualification data should be compiled for each utility. This is down to the level of pipe lengths and diameters, the types of valves, material design etc.

Water systems

The key requirements for water systems are focused on the avoidance of biofilm formation. These include:

Area for assessment	Annex 1 requirement (6.7–6.15)
Annex 1 objective	• To produce water free from microbial contamination and of the appropriate quality, as per European Pharmacopeia (microorganisms, endotoxin and particles) • Testing should be conducted and trended, using historically derived alert levels. Alert breaches must be assessed • Action level extrusions require investigation and product impact assessments • Sampling must be frequent and representative, including a sampling point each day
Design requirements	• Ensure correct slope of piping • Avoid deadlegs • Turbulent flow (at the validated rate)
Contamination control	• A scheduled decontamination process must be in place (e.g., sterilisation or disinfection) • Decontamination must follow maintenance works • Testing must follow maintenance works
Special risk factors	• Seasonal variation
Water for injections (WFI)	• Produced by distillation or equivalent (e.g., reverse osmosis and electrodeionisation) • Maintained in a sanitary state, e.g. >70°C • Filter controls in place for tanks (integrity testing and condensation avoidance) • Continuous TOC and conductivity monitoring must be in place

Table 7: Design and control requirements for water systems

Steam used for sterilisation

With steam, the requirements are:

Area for assessment	Annex 1 requirement (6.16–6.17)
Annex 1 objective	• To obtain steam of chemical purity and absence of endotoxins (microbial sampling is optional based on the CCS)
Feed water	• Feed water needs to be purified using a validated system
Steam quality	• The steam must be free of additives • Steam must meet European Pharmacopeia WFI requirements • Testing must be regular • Testing includes chemical purity, endotoxin, non-condensable gases, dryness value (dryness fraction) and superheat

Table 8: Design and control requirements for steam

Gases

The requirements for gases are:

Area for assessment	Annex 1 requirement (6.18–6.20)
Annex 1 objective	• Gases that come into direct product contact must be of the appropriate chemical, particulate and microbial quality
Design	• The design should prevent backflow
Gas quality (general)	• A water content must be specified • An oil content must be specified
Gas quality (aseptic processing)	• A water content must be specified • An oil content must be specified • Gases must be passed through a sterilising grade filter (0.22 µm) and filters subjected to integrity testing per batch • Transfer piping must be sterile • Gases must be subject to microbial monitoring at point of use on a regular basis

Table 9: Design and control requirements for gases

Cleaning and disinfection

The revision to Annex 1 affords more attention to cleaning and disinfection (4.33 and 4.36), and with this a greater emphasis upon cleaning (although the text is somewhat shortened compared with the drafts that were issued). The basic requirements are:

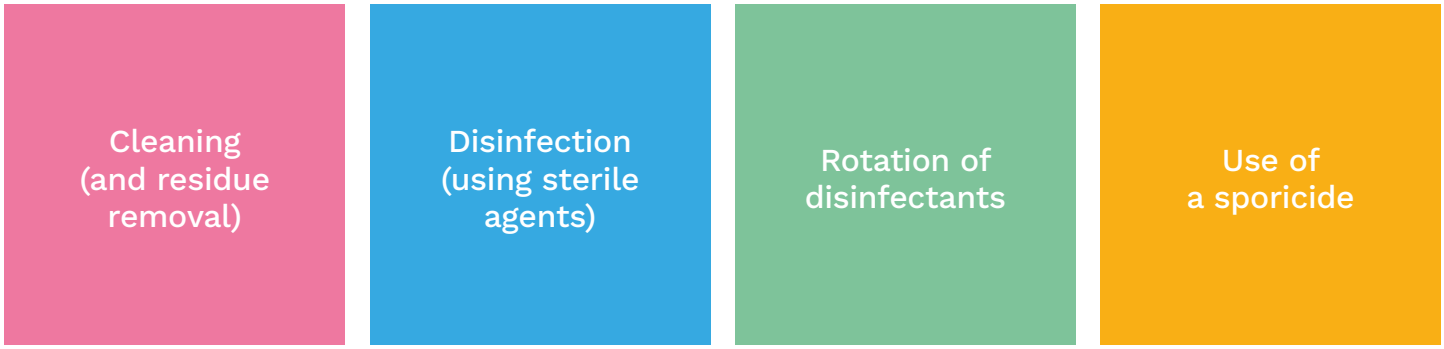


Figure 3: Elements of a cleaning and disinfection programme

Cleaning needs to take place before disinfection, as disinfectants have poor penetrative ability and therefore soils will need to be removed. However, cleaning must not leave residues, as these may interfere with disinfectants. Disinfectants must be rotated between agents of different modes of efficacy in order to increase the spectrum of kill. A spodicidal agent needs to be included within the disinfection regime, across all grades of cleanroom. These disinfectants should be sterile for Grade A and B areas and may need to be sterile for Grade C and D areas as per the CCS. In section 8.47, the disinfection procedure for transfer of items into Grade A and B areas is outlined together with the necessity of demonstrating that the disinfection process is effective in reducing any contamination on packaging to acceptable levels. This will increase the focus on surface efficacy studies for disinfection and the types of materials used for packaging. The overall transfer disinfection process needs to be validated (8.49) and be assessed through the environmental monitoring programme.

The cleaning and disinfection regime must be supported by the use of validated disinfectants and be assessed through the environmental monitoring programme, including profiling organisms that are potentially resistant. More space could be afforded in the Annex to methods of validating disinfectants and the process of performing surface studies and field trials. The Annex requires any disinfectant that is diluted to be assessed for its microbial content, including hold times and expiry times.

The use of fumigation, such as with hydrogen peroxide in the vapour phase, is listed as an alternative disinfection step. This process requires validation. While the Annex does not spell this out, validation should include defining worst-case room load plans and supporting this with biological and chemical indicators.

Cleaning validation of equipment is called out separately to cleanrooms (in 5.4). Successful cleaning of equipment is defined as being capable of removing residues (to the visual cleanliness level) and being able to minimise microbial and particulate contamination. Too often cleaning validation neglects the microbial, so it is refreshing to see this within the Annex.

Personnel

Considerable space is afforded within the Annex to personnel.

Figure 4: Controlling personnel related contamination risks

The section on personnel discusses clothing requirements. Here low particle shedding, sterile garments must be worn prior to entry to Grade B areas and visually inspected for their integrity. The CCS needs to define the gowning qualification and testing process (this is particularly important for relaundered gowns). For gowns worn by operators in Grade B areas, the maximum wear time of the gown needs to be qualified (for example, from a historical overview of exit suit plate data).

In Grade C and D areas, the requirement to wear gloves and facemasks will be operationally dependent and defined in the CCS.

The personnel controls in the Annex relate to pre-assessment, training, gowning, and general cleanroom behaviours. The text is not the easiest to follow, so the requirements have been more logically sorted and they are presented in the following table. The information in the table can be used to verify cleanroom training programmes and to develop core competencies for personnel.

Activity	Requirements	Annex 1
Basic hygiene		
Hygiene (for any grade of cleanroom)	• All personnel must practice personal hygiene each day • Any medical conditions of concern must be reported daily and assessed by a competent person • Personnel who have handled microbial cultures cannot enter cleanrooms unless they have undergone decontamination • Entry into a cleanroom must begin with handwashing • Outdoor clothing for Grade C and B areas must be removed and factory clothing worn, including socks	7.7, 7.8, 7.10, 7.14
Training		
General training (any grade of cleanroom)	• To cover – microbiology, hygiene, cleanrooms, contamination control, aseptic techniques, product protection / patient safety	7.3
Entering Grade C and D areas		
Entering cleanrooms (general, any grade)	• Wristwatches, make-up, jewellery, and personal phones are not permitted • Only minimum numbers are permitted (each cleanroom needs a maximum occupancy)	7.2, 7.9
General gowning (any cleanroom grade)	• Garments must be checked for cleanliness and integrity before entering the cleanroom • Garments must be packed so that no part of the garment lands on the floor • Staff must not allow any part of the garment to touch the floor when they get changed	7.11, 7.13
Entering Grade B areas		
Entering cleanrooms (general, any grade)	• Wristwatches, make-up, jewellery, and personal phones are not permitted • Only minimum numbers are permitted (each cleanroom needs a maximum occupancy)	7.2, 7.9
General gowning (any cleanroom grade)	• Garment packaging must be inspected for visible signs of damage prior to opening • Garments must be checked for cleanliness and integrity before entering the cleanroom • Garments must be packed so that no part of the garment lands on the floor • Staff must not allow any part of the garment to touch the floor when they get changed	7.11, 7.13
Gowning (aseptic areas, Grade B) – training	• Gowning training and assessment • Visual assessment • Microbiological monitoring, gloved fingers, forearms, chest, hood, facemask • Trainees must be shown airflow visualisation studies *(this training is a minimum of annual)* For the routine gowning practice, refer to Figure 5.	7.4, 7.18
Working in cleanrooms		
Working in cleanrooms	• All movements must be slow, controlled and methodical • In aseptic areas, aseptic technique must be continually practiced • Airflows in critical zones must not be disrupted • Practices need to avoid entraining Grade B air into Grade A areas	7.18
Unsupervised access	• Unsupervised access is only permitted for staff who have participated in an aseptic process simulation (APS) • Unqualified personnel must be supervised, and their activities recorded	7.5
Gloves	• Gloves must be regularly disinfected • Damaged gloves (and gowns) must be immediately replaced	7.16
Gauntlets (aseptic processing specific)	• RABS: Gauntlets visually examined before and after each use • Isolator: Gauntlets visually examined before and after each campaign and after any manipulation	4.21
Exiting cleanrooms (any cleanroom grade)	• Gowns must be checked for integrity on exit	7.11
Disqualification		
Disqualification (from Grade B)	• Personnel can be disqualified from working in aseptic areas based on trend data or a failed APS • Requalification requires pre-set criteria and participation in an APS	7.6

Table 10: Personnel competencies for working in the cleanroom

For working in an aseptic processing area, the gowning process needs to be documented and follow predefined steps. The diagram below has been constructed to make the requirements clearer:

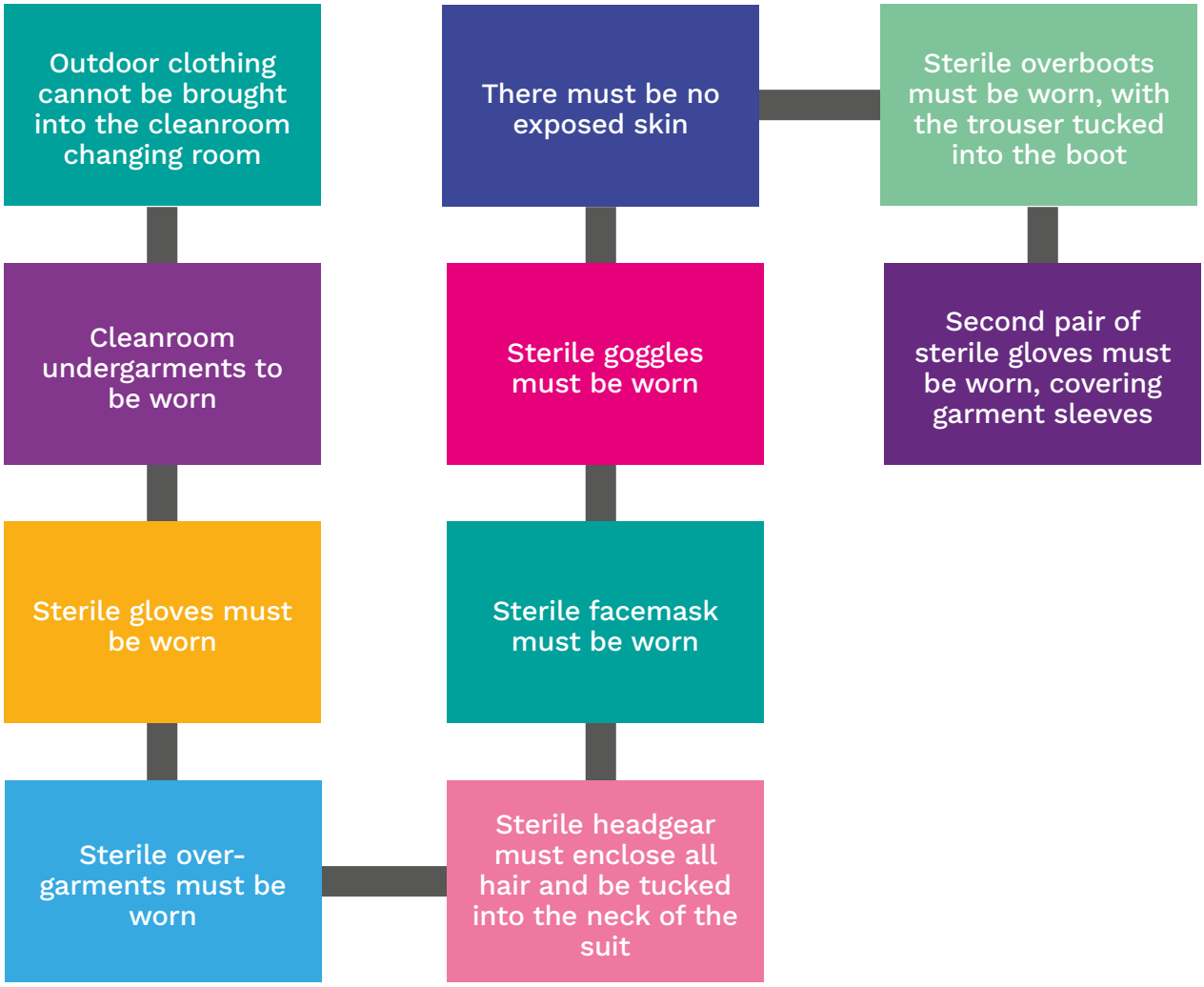


Figure 5: Steps for entering the cleanroom and gowning

Production technologies

The section in the Annex on production technologies for terminal sterilisation does not differ significantly from the previous version. With aseptic processing, the requirements are closely bound with the CCS and QRM. The CCS should identify risks and discuss their acceptability, including the necessity of justifying residual risks. This is part of the governing philosophy within the Annex where contamination risks need to be documented and understood. Contamination is defined as microbial, pyrogenic, and particulate (including fibres).

The Annex encourages the use of barrier technologies (RABS and isolators) and calls out robotics specifically (8.7 – 8.9). Such technologies are designed to minimise interventions and robotics can allow for glove ports to be done away with completely. Non-media fill qualified interventions require prior approval of Quality and all interventions need to be assessed as part of the batch release process (8.16), along with stoppages.

The Annex tightens up on the operations that need to be performed within Grade A, including set-up. For set-up, all sterile items must only be unwrapped in Grade A, and Grade A conditions (as assessed through viable and particle monitoring) must be maintained. As many items as possible should be pre-assembled. In addition, connections must also be performed under Grade A unless a qualified single-use system is used.

Sterilisation

A large section of the Annex is given to sterilisation technologies. In discussing sterilisation, the importance of scientific principles, annual validation (physical and biological), and demonstrating repeatability and reliability feature strongly. Of the different methods of sterilisation available, the Annex sees heat as the superior method.

The requirements for the qualification of biological indicators has been expanded, with controls required for transportation and storage. For the user, confirming population, purity and identity is a pre-use requirement.

Item identification and segregation (as between sterile and non-sterile items) is an important consideration, as is investigating failed sterilisation cycles. The records from sterilisation activities need to be reviewed by a suitably knowledgeable person and be assessed for batch release purposes.

Some general advice is provided for sterilisation, which can be summarised as:

Document structure / requirement	Source	Reference
Policy		
Heat sterilisation is the method of choice	EU GMP Annex 1	8.37
Validation		
Validation assessments need to include: ● Risk assessment to show suitability of the sterilisation process ● Material / product composition ● Storage conditions ● Maximum time between preparation (including any cleaning) and sterilisation	EU GMP Annex 1	8.36
Assessment by physical measurements and biological indicators	EU GMP Annex 1	8.36
The whole of the product, and surfaces of equipment and components, need to be subject to the required treatment (that is, sterilisation is a penetrative technology)	EU GMP Annex 1	8.36
Validation should define loading patterns	EU GMP Annex 1	8.38
Validation must include maximum and minimum loads	EU GMP Annex 1	8.38
Biological indicators should be stored in defined conditions and subject to purity, population and identity checks by the receiving laboratory. The CoA must be assessed for D-value and Z-value suitability	EU GMP Annex 1	8.43
Validation must assess the maximum hold time post-sterilisation	EU GMP Annex 1	8.48
Validation must consider the integrity of the sterile protective barrier system	EU GMP Annex 1	8.48
Transfer methods for getting items into Grade B and A areas must be validated	EU GMP Annex 1	8.49
Routine use		
Sterilisation indicators should be used but these cannot replace the assessed sterilisation parameters	EU GMP Annex 1	8.44
Sterilisation records must be available for each sterilisation run	EU GMP Annex 1	8.45
Items to be sterilised should be in appropriate packaging	EU GMP Annex 1	8.46
The maximum hold time after sterilisation must be assessed	EU GMP Annex 1	8.46
Requalification		
Worst case load patterns are subject to revalidation at least annually; other load patterns should be revalidated at a risk-based frequency	EU GMP Annex 1	8.38

Table 11: Important considerations for sterilisation processes

Other areas of aseptic processing are outlined in the Annex, including blow-fill-seal and form-fill-seal. With both processes, particulate control is mentioned (8.96 to 8.120).

The Annex has sections on different sterilisation technologies. Some of these would be undertaken directly by the pharmaceutical manufacturer and others will be outsourced. Concerns are expressed with ethylene oxide (8.37-8.78) and here it is stated “This method should only be used when no other method is practicable.” The reasons here are connected to safety (mutagens) and residues. A preferred method is radiation for heat sensitive products (8.71-8.72). Although the method of radiation is not stated, ultraviolet radiation (like UV-C) is deemed not to be an acceptable method of sterilisation. This leaves technologies like gamma, electron beam and X-rays as suitable irradiation processes.

With heat as the preferred method of sterilisation, the Annex sets out the requirements for moist heat and dry heat. Moist heat includes steam and superheated water (8.59-8.65). An importance is placed on packaging, package integrity, and the necessity of items being dry when removed from the autoclave (the avoidance of wet loads).

Validation requirements include:

- Load design and configuration
- Calculation of equilibration time
- Exposure time
- Correlation of pressure and temperature
- Minimum and maximum temperature range (including heat penetration studies)

Dry heat sterilisation is described in terms of tunnels and ovens designed to achieve depyrogenation (8.66-8.70).

The key requirements for this technology are:

- Air speed and distribution of heat to achieve uniformity, with air supplied through HEPA filters
- Airflows and pressure control
- Heat penetration into the units being treated
- Belt speed
- Loading patterns
- Minimum and maximum temperatures

Hence, in terms of the preferred methods of sterilisation, the Annex signals:

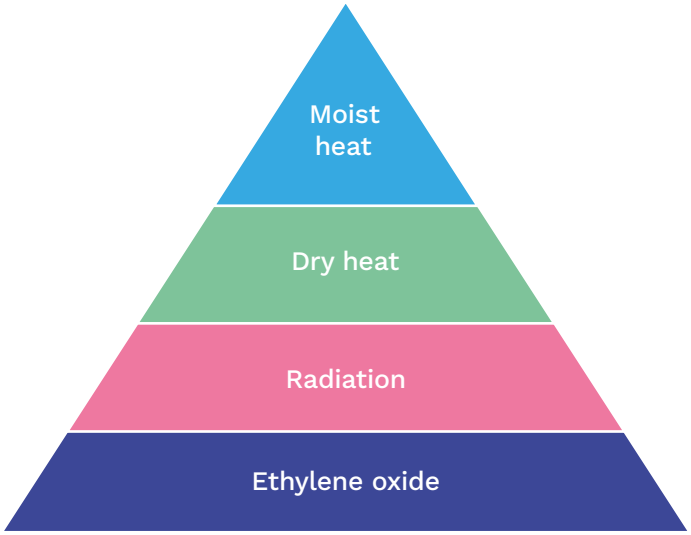


Figure 6: Hierarchy of sterilisation technologies as indicated in Annex 1

Notably, the use of hydrogen peroxide in vapour form does not feature as a sterilisation technology. The technology is considered too fragile in terms of its reproducibility and robustness. This explains the earlier mention of the sterilisation of direct and indirect product contact parts for RABS and isolators.

Sterile filtration

Sterilising filtration is discussed in greater detail within the revised Annex (8.79 to 8.95). A sterile filter remains defined as one with a porosity of 0.22 µm or smaller, where a controlled filtration process is designed to produce a sterile filtrate.

The important requirements for sterile filtration are:

- Use of bioburden reduction filters prior to the sterilising filter
- Carrying out sterile filtration as close to the point of fill as possible
- Filters must be subject to pre- and post-use integrity testing. This includes pre-use post sterilisation integrity test or PUPSIT (pre-use post sterilisation integrity testing)
- Filters must be evaluated through bacterial retention testing, ideally using actual product

In terms of filter selection and design:

- Filters should not introduce impurities into the product, such as fibres
- The filter should not present a risk in terms of adsorption, leachables or extractables
- The filter must demonstrate good product computability

Operationally, when using a filter:

- During filtration, conditions must be controlled such as time, pressure, temperature, and flow rate

For small volume products there is a risk framework that can be followed should PUPSIT not be technically possible.

Lyophilisation

With freeze-drying, the same attention paid to aseptic filling needs to be considered with the loading of freeze-dryers to validated load patterns (in that Grade A conditions must be maintained). It is also important that freeze-dryers are sterilised and that the hold time is assessed as part of the media fill. Sterilisation increases in importance, for each load loaded or unloaded, where an old-fashioned manual loading system is in place (where humans are interacting within Grade A environments). Here vials must be in sealed containers if no automated loading system is in place (8.121-8.126).

Single use systems

A new addition to the Annex concerns single-use, sterile, disposable technology (referenced in the Annex as 'Single Use Systems'), as per 8.131-8.139. Key concerns with single-use systems relate not only to the sterilisation methods, and maintaining sterility through system integrity, but also to the reactivity between the product and the material that the system is composed of and hence adsorption, leachables and extractables. These product-material interactions need to be considered post-sterilisation. The qualification of single-use technology is through inclusion in an aseptic process simulation.

Where single-use systems are not available, care must be taken with closed systems in relation to their sterility and with undertaking connections under Grade A conditions. This is as per 8.127-8.130.

Aseptic filling

With aseptic filling, there are a series of steps that require risk assessment and representing in media fills (these are discussed below). Each of these has a time-based element. These critical steps are:

- Hold times for equipment for cleaning, drying and sterilisation
- Hold times for sterilised equipment
- Hold times for decontamination environments including RABS and isolators
- Time between product sterilisation and the start of filling
- Hold time for sterilised products
- Run times for filling activities, divided into total aseptic processing time and filling time
- Maximum stoppage times during filling

Figure 7: Essential requirements for inclusion in media fills

These activities must be supervised and overseen by Quality personnel.

Finishing

For the finishing of sterile products, each unit must be held under Grade A air until completely sealed. The sealed unit should be checked for integrity and products held under vacuum need to be assessed to show that the vacuum is maintained. The assessment of the container closure is designed to show that ingress of microorganisms is not possible and this requires qualification using a pharmacopeial approved method. These methods are divided into deterministic and probabilistic, with deterministic methods being preferred. These assessments needs to account for the transportation of the product, especially the extremes of temperature and pressure to which the product units will be subjected to.

Considerable weight is given to visual inspection, assessment of defects and particular concerns with particulates. The Annex guides the facility to consider areas where particles can enter the product (such as from packaging or capping). Visual inspection should address:

- Operator eyesight e.g. corrected vision
- Background used for the inspection
- Levels of illumination
- Inspection time
- Frequency of breaks and operator fatigue
- Line speed
- Container sizes

Figure 8: Essential requirements for visual inspection

The above should have Quality oversight and be trended.

Environmental monitoring

Environmental monitoring is an important verification tool, once control has been established. As section 10 of the Annex alludes to, this should be overseen by persons with knowledge of microbiology and sterility assurance. The design of the monitoring programme must be intimately connected with the CCS. To be effective as a signal for the state of asepsis, all environmental monitoring results need to be considered together.

Environmental monitoring consists of:

- Viable monitoring
- Particle monitoring
- Temperature monitoring
- Humidity monitoring
- (And for aseptically filled products – aseptic process simulations)

Temperature and humidity are factors that not only affect operation, but also personnel comfort. Hence, there is a direct connection to contamination control.

The above provides an indicator of cleanroom suitability and non-compliances can be signalled through the use of appropriate alert and action limits (alert levels should be set based on historical data so that any deterioration in conditions can be detected. Whether action levels are set below the recommended values in the Annex requires consideration within the CCS). To draw meaningful assessments, the data points need to be trended and the data used as part of batch release.

Trending includes:

1. Total counts
2. Types of microorganisms, including those that signal increased resistance
3. A change in the pattern of alert or action levels (that is, they appear at a higher frequency)
4. Consecutive alert levels
5. Action level executions connected to specific activities

Such data is also essential for investigations to address non-compliances. Investigations into action level excursions require a root cause analysis to be conducted and a product impact assessment to be made. From this, corrective and preventative actions (CAPA) need to be set. With alert level investigations, the emphasis is upon corrective actions.

Viable monitoring

The viable monitoring programme needs to be risk based, with QRM used to establish:

- Locations for samples, especially in Grade A and Grade A / B interfaces. Airflow visualisation studies are useful for assessing where microbial transfer presents the highest potential risk. Locations will include:
  - Sterile equipment surfaces
  - Areas where containers are located
  - Areas where closures are located
  - Where product is exposed
  - Additional locations can be considered when conducting microbial excursion investigations or to evaluate the effectiveness of a corrective or preventative action (such as a revision to a disinfection regime)
- Types of samples and methods, including the orientation of monitoring equipment. Viable monitoring should use a combination of methods:
  - The Annex makes reference to settle plates, air samplers, finger plates, contact plates, swabs, and gown plates. In addition, rapid microbiological methods are encouraged, especially where these generate superior data. Rapid methods may express data differently to the classic viable monitoring methods, that is not necessarily the colony forming unit (CFU)
  - The methods selected should be qualified. This includes an understanding of the recovery efficiency of each of the methods
- Consideration of whether the act of sampling presents a risk of contamination intrusion (especially into Grade A)
- Sample sizes and volumes (assessing these is particularly important for active air samplers and particle counters)

- Time of sampling (e.g., surface sampling conducted at the end of the operation). Time also needs to account for:
  - Higher frequencies for new facilities
  - In operation
  - Specific activities like transfer disinfection (as mentioned in 8.49)
  - Following room disinfection
  - Prior to start-up
  - Following shutdowns
  - Inactive rooms
- Frequency of sampling. For aseptic processing, the monitoring needs to be continuous (beginning with set-up) in relation to particle counting and settle plates (as a minimum)
- Strategy for personnel monitoring in relation to aseptic processing. This will include gloves and potentially gowns following interventions and gloves and gowns on exit from Grade B areas
- Responsibilities for monitoring need to be described (including checks by Quality where the responsibility is delegated to production personnel)
- Impact of equipment operating within the cleanroom
- Incubation conditions, to capture both bacteria and fungi
- The need to assess for anaerobes as well as aerobic organisms
- Microbial identification strategy
  - All organisms isolated in Grade A and B need to be identified
  - For organisms in Grade C and D, this will include those exceeding action and alert, as well as others based on the CCS

Most of the above items appear in the Annex between sections 9.4 and 9.13; and 9.22 to 9.31.

In terms of viable monitoring limits, these are:

Grade	Air sample CFU / m^3	Settle plates (diam. 90mm) CFU / 4 hours[a]	Contact plates (diam. 55mm) CFU / plate[b]	Glove print, including 5 fingers on both hands CFU / glove
A	No growth[c]			
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

Table 12: Viable monitoring limits (taken from Annex 1)

The above table is Table 6 in Annex 1. The main change is with Grade A, which is no longer expressed as "<1 CFU". By using 'no growth' this further steers a path towards the adoption of rapid microbiological methods. The other main change is the removal of any reference to 'average' values. This avoids any temptation by the facility to seek to use averaging to bring data down to below an assigned action level (the so-called 'averaging into compliance').

The lower case (a) is an indicator that settle plate exposure per four hours must be qualified, to avoid plates undergoing excessive dehydration and loss of growth promoting properties. The lower case (b) indicates that contact plate limits also apply to gowns (a limit for cleanroom suit monitoring thus appears for the first time). The lower case (c) states that any microbial recovery in Grade A requires an investigation. Agar plates intended for Grade A must be wrapped until they enter Grade A or be protected by Grade A air (as set out in 8.10 for the Annex).

Particle counting

Like viable monitoring, particle counting needs to be part of a formal programme. Alert levels need to be set and action levels considered in relation to the recommended levels. With the recommended levels, there are two important changes. First, the ≥ 5 µm level at Grade A for 'at rest' and 'in operation' increases from 20 counts per cubic metre to 29. This brings the ≥ 5 µm limit in line with ISO 14644. In addition, for Grade D 'at rest', a recommended level has been added for the first time (9.15). The particle levels are shown in Table 13 below:

Grade	Maximum limits for total particle ≥ 0.5 µm/m^3		Maximum limits for total particle ≥ 5 µm/m^3	
	at rest	in operation	at rest	in operation
A	3,520	3,520	29	29
B	3,520	352,000	29	2,930
C	352,000	3,520,000	2,930	29,300
D	3,520,000	Not predetermined[a]	29,300	Not predetermined[a]

Table 13: Particle counting limits (taken from Annex 1)

The previous table is Table 5 in Annex 1. The lower case (a) indicates that each facility needs to determine an appropriate action level. With ≥ 5 µm at Grade A, the Annex cautions that low level counts, below the action level, may be a signal of an underlying contamination problem.

When monitoring batch processing, particle counting at Grade A needs to be continuous (most facilities would also include Grade B where RABS are used) with alert levels on an alarm system promptly reacted to (this may include additional microbiological monitoring as a reaction to particle count fluctuations). The only exception to continuous monitoring is where powders or other particle generating substances are handled – here alternative approaches can be defined in the CCS.

For particle counters, the Annex (in 5.9) indicates that a rationale is required for the particle counter tube diameter and bend radii, and that the overall tubing length cannot exceed 1 metre. Bend radii was traditionally greater than 15 centimetres, although the Annex seems to allow alternatives provided these can be justified. The number of bends must be minimised.

Further with particle counting, the Annex requires isokinetic probes to be used in Grade A (these function to straighten the airflow), for these to be as close as practicable to the activity, so that counting is representative, and for the probe to be orientated towards the airflow.

Aseptic processing simulations

Media fills, rebranded as 'aseptic processing simulations' (APS), are largely described in section 9 of Annex 1 within environmental monitoring (although some references to the exercise are made in section 8 as well). The APS is a key qualification exercise and probably deserving of a standalone section. For media fills, the acceptance criteria is now firmly set at zero. In other words, the growth of a single unit requires the suspension of the line and an investigation.

Essential to a successful APS is the design of the process. Here all steps involved in the aseptic operation should be captured. This includes manipulations and interventions, which needs to be based on product fills and replicated for their type and frequency. The types of manipulations and interventions included should only be those that would routinely be executed – the media fill should not seek to mimic or simulate bad practices.

In designing the media fill, a matrix approach can be used for the same container-closure configurations and the worst-case container sizes and line speeds selected.

Decisions to be made in the planning include:

- Simulating maximum hold times for filtered product
- Simulating the maximum time between filtration and freeze-drying (if applicable)
- Selection of a suitable culture medium and showing growth suitability using compendial strains and facility isolates
- Capturing idle process time that is representative of normal filling
- Manipulations and interventions should reflect normal filling operations
- Determining the volume filled per container, to ensure sufficient air space for microbial growth and to allow for all surfaces to be contacted
- Determining the run time and setting the duration to capture shift changes, fatigue (including maximum permitted time by an operator in the cleanroom), all interventions, and overall capacity
  - With run time, each working shift that would be involved in the product fill being simulated needs to be captured
- The batch size should be sufficiently large to represent normal operations (a recommended number of vials no longer features in the Annex, although the minima are described as 5,000 or 10,000 units)
- Simulating campaign filling

- Any inert gas that might lead to microbial inhibition needs to be substituted
- Demonstrating that microbial contamination can be detected upon post-incubation inspection e.g., use of clear glassware
- Inversion and agitation of each unit is required prior to incubation
- Incubation conditions should be based on optimally promoting microbial recovery

Additional considerations appear with freeze-drying. The Annex makes things clear that freeze-drying is an important part of media fill design, including transportation to the freeze-dryer, loading of the freeze-dryer, using a representative duration of the chamber dwell, unloading and sealing. The maximum time interval between freeze-dryer sterilisation and use needs to be captured in the media fill design. Care must be taken that the lyophilization process does not affect the viability or recovery of contaminants.

Another important area is vial reconciliation, especially in terms of accounting for all filled units and having appropriate justifications in place should not all filled units be incubated. Such decisions require detailed documentation.

In terms of how many APS need to be run:

Circumstances	Number of media fills or timing of media fills
New filling lines	Three media fills minimum
Modification to the HVAC system Change to equipment Changes to process Changes to the number of shifts and numbers of personnel Major facility shutdown	Three media fills minimum
Each aseptic process, each filling line and each shift	Twice per year
Last event before shutdown	One media fill per line
Each operator	Once per year
Prior to line decommissioning	One media fill
Prior to line relocation	One media fill
Upon line relocation	Three media fills minimum
Following a media fill failure	Three media fills minimum

Table 14: Requirements for aseptic process simulations (media fills)

With media fills, where a failure occurs, a detailed investigation, establishment of the root cause, and the setting of appropriate corrective and preventative actions is required.

Other areas of microbial testing

In addition to the direct monitoring of the environment, a bioburden control strategy is required. The Annex, slightly confusingly, bundles these under the final substantive section titled 'Quality Control'. Reference in section 10 is made to screening raw materials, components and ingredient products for microorganisms, particles and endotoxins as per the CCS. These concerns extend to intermediate manufacturing and assessing bioburden pre-final filtration (aseptic processing) or immediately prior to terminal sterilisation.

The sterility test has been a pharmacopeial requirement for 90 years. However, the significance of the test result when a pass is obtained is limited (notwithstanding attention paid to where the samples are taken from within the batch) and the Annex emphasises that a pass with the sterility test is not a guarantee of sterility assurance should other control measures break down. Hence, the overall sterility assurance programme needs to be carefully considered.

As with assessing isolator decontamination, the Annex now contains a warning that the method used to decontaminate the sterility test samples should be examined to show that any residues are non-inhibitory and do not undermine the validity of the test.

Good Distribution Practices

Annex 1 extends to the distribution of the product until it reaches the intended recipient, and therefore the Annex connects with Good Distribution Practices (GDP). This includes ensuring that processes associated with the finishing, storage and transport of sterile products should not compromise the sterile product.

The following need to be assessed:

- Container integrity
- Risks of contamination
- Avoidance of degradation

The reference to degradation is another sign that Annex 1 extends beyond the microbial by considering overall product stability, such as by ensuring that products are stored and maintained in accordance with the required storage conditions. In terms of container integrity, this needs to be assessed under 'worst case' conditions, testing out temperature and pressure (such as mimicking the conditions within the hold of an aircraft) for the maximum distribution time duration.

Batch release

For the release of sterile medicinal products, the Annex requires that those who undertake certification or release of sterile products (Qualified Persons) have access to manufacturing and quality information and trends. It is also important that such personnel have additional training covering the manufacture of sterile products and are cognisant as to the critical quality attributes that show sterility and contamination controls.

As part of batch release, all non-conformities (the Annex lists sterility test failures, environmental monitoring excursions or deviations from established procedures) need to be reviewed. These must have been investigated and be provided with an impact assessment for the process and in relation to product quality. It may be necessary to extend the scope of the investigation to other batches and to other product types.

Summary

This white paper has presented an overview of the key elements of Annex 1, highlighting the most important changes and offering interpretation of some of the points made. The paper has also sorted the flow of information into a more logical order, bringing together similar topics.

Key takeaways from the Annex and this white paper are:

- Quality risk management is essential for all areas of sterile manufacturing
- Separating people from products is the most important consideration, especially where other aspects are suitably controlled and validated
- Rapid and real-time technologies are encouraged, such as rapid microbiological methods
- There is an expectation for each facility to have in place a formal, holistic contamination control strategy, focused on minimising contamination control with respect to sterile manufacturing. Key decisions and rationales should be contained within this strategy

In concluding, it is important to recognise that the Annex only sets out the minimum requirements. Pharmaceutical manufacturers should continually focus on preventative strategies, including proactive risk assessments and controlled changes, supported by trending, investigations, corrective and preventive actions (CAPA), root cause determination and the use of robust investigational tools. Producing sterile medicinal products is all about continuous improvements.

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About the author

Dr Tim Sandle

Dr. Tim Sandle has over twenty-five years' experience of microbiological research and biopharmaceutical processing. He is a member of several editorial boards and he has written over six hundred book chapters, peer reviewed papers and technical articles relating to microbiology. Dr. Sandle works for a pharmaceutical manufacturer in the UK, and is a visiting tutor at both the University of Manchester and UCL.

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Web: **www.rssl.com**