# Table of Contents

## Auditor Information

| Annex 1 Questions & Answers | |
|---|---|
| **Authors** | |
| Dr Ingrid Walther (Chair Annex 1 Task Force, co-author of the CCS Guide)<br>Dr Friedrich Häfele (member of the Annex 1 TF)<br>Robert Schwarz (member and co-author of the CCS Guide) | |
| **Version** | |
| Version 1.0<br>Version 1.1 | March 2024<br>April 2024 |
| **Technical Review** | |
| **Legal Representative** | |
| ECA Foundation<br>c/o VHP Auditing Firm and Legal Trustee<br>Attn Mr J. Ruland<br>Hebelstr. 7<br>68161 Mannheim<br>Germany | |

## Disclaimer

We received numerous questions during our Annex 1 events, at comment meetings and directly from our members. The answers were given by members of the Annex 1 Task Force, the speakers at the Annex 1 events and a representative of the supervisory authorities to the best of their knowledge, technical expertise, and experience. Dr. Ingrid Walther, Dr. Friedrich Haefele, Robert Schwarz are named here as representatives. However, in some cases there is still a lack of practical experience and the respective local authorities have room for discretion and interpretation. Factors such as the product to be manufactured, the given premises, production processes and much more also play a role in the implementation of Annex 1. For this reason, this Q&A document is intended to provide suggestions and assistance, but cannot represent a general guidance for the processes in individual companies.

As far as possible, we have assigned the questions to individual subject areas in Annex 1 and listed them under a corresponding heading.

# 1 Scope Annex 1

## 1.1 Is there a formal process for identifying to which scope the Annex 1 needs to be applied to API manufacturing? How does it have to be applied during the API manufacturing and how to make those decisions systematic?

It depends if the API is claimed sterile, it falls under the requirements of Annex 1.
For APIs (e.g., claimed "low bioburden" / controlled endotoxin level), Annex 1 provides guidance for state-of-the-art manufacturing whenever contamination control is required. It targets at the control of microbial, endotoxin, particulate contamination.

As outlined in the Scope of Annex 1 "…*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.*"

The application of the requirements and recommendations of Annex 1 can also be helpful for the manufacture of non-sterile products such as ointments etc.

## 1.2 Will also authorities of non-EU countries adopt Annex 1 (e.g. US-FDA)?

According to EMA representative, Annex 1 is expected to become applicable in all ICH member states. This is also supported by the fact that the new PIC/S document published recently on sterile medicinal products is identical to Annex 1. WHO was also involved in the revision and follows Annex 1 in its documents on sterile production.

## 1.3 Difference between aseptic and terminally sterilised product: Annex 1 is not very clear what is applicable for what product group. Should we identify the GAPs the same for both but risk assess it differently since it is aseptic or a terminally sterilized product? Or is there just not as much difference anymore between the two (aseptic and terminally sterilized process)?

Unfortunately, it is true that there are requirements in Annex 1, for which the attribution to either aseptic manufacturing or terminal sterilization technique is not explicitly stated. Thus, the only answer that can be provided is: QRM has to be applied to define the adequate solution for the individual process.

## 1.4 Is there a relation between Part IV - GMP requirements for Advanced Therapy Medicinal Products and the Annex 1 requirements?

Annex 1 applies for ATMPs only if explicit reference is made in EU-GMP Part IV, which is the case in the context of sterilization. Otherwise, from a formal point of view, Annex 1 is not applicable for ATMPs. However, it is advisable to consider Annex 1 requirements as state-of-the-art in general sterile manufacturing.

# 2 Utilities

## 2.1 Is the examination of all parameters in return loop of WFI needed? Thinking about the non-homogenously distribution of (micro)biology in a water loop while chemical and physical parameters are homogeneous distributed. In other words, is online monitoring at the return loop sufficient or is offline monitoring additionally needed?

Online monitoring in general determines TOC / conductivity. TOC can be an indicator for a microbiological contamination. CFU needs to be determined offline. Of course, parameters such as temperature or flow rate can also be measured. Sampling locations and frequency should be determined based on risk assessments.

Modern alternative methods for online measurement of all viable counts are now available, but the results cannot yet be correlated with the cfu results of the offline methods, i.e. comparability cannot be proven.

Therefore Sec. 6.13 in conjunction with sec. 6.15 clarifies, that daily sampling (off-line) is required at worst-case location and this is to be performed in addition to continuous TOC/conductivity in-line monitoring.

6.13 …. *Sample plans should be based on the qualification data, should consider the potential worst-case sampling locations and should ensure that at least one representative sample is included every day of the water that is used for manufacturing processes.*

6.15 *WFI systems should include continuous monitoring systems such as Total Organic Carbon (TOC) and conductivity, as these may give a better indication of overall system performance than discrete sampling. Sensor locations should be based on risk.*

### 2.2 WFI produced using R/O and distributed using cold loop: Is this acceptable if the site is using electro-deionisation (EDI), ultra/micro-filtration, ozonation and UV treatment and periodic thermal sanitization?

WFI should be produced by distillation or by a purification process that is equivalent to distillation. This may include reverse osmosis coupled with other appropriate techniques such as electrodeionization (EDI), ultrafiltration or nanofiltration. Ozonation, UV treatment and periodic thermal sanitization could be part of a company's biofilm-management. This management is general required but no technical solutions are given. Annex 1 does not define technical solutions or requirements on RO water systems. For technical support on this topic there is a EMA Q&A paper (EMA/INS/GMP/443117/2017).

### 2.3 Chapter 6 Steam: Does this also apply for autoclaves? (not used for terminally sterilised product but within aseptic manufacturing with sterilised equipment)

Annex 1 says in No. 6.17 "*Steam used as a direct sterilising agent should be of suitable quality and should not contain additives at a level that could cause contamination of product or equipment. For a generator supplying* ***pure steam used for the direct sterilisation of materials or product-contact surfaces (e.g. porous hard-good autoclave loads)****, steam condensate should meet the current monograph for WFI of the relevant Pharmacopeia (microbial testing is not mandatory for steam condensate)*."

### 2.4 What frequency is microbiological testing of gases used in processes expected to be? Currently we have a risk assessment, which states that due to filters used in process, this is not required. Is it now an absolute requirement to do the testing?

Testing should be done at least annually, but the depends of usage of gases. There is no frequency defined in Annex 1, however, "no testing" is not an option, as sec. 6.19 clearly requires periodic monitoring at the point of use. Monitoring frequency is to be defined, but depends on the manufacturer's experience, frequency of use, existing monitoring data and – as a general requirement – should be based on QRM principles.

## 3 Single Use Systems

### 3.1 How do you distinguish between SUS (Single Use Systems) and closed systems? (As the terms are often used identical)

There is no one-to-one correlation between SUS and "closed system":

a. Closed systems do not necessarily have to be SUS, e.g. also isolators, bioreactors, transfer vessels can be closed systems
   and
b. SUSs does not necessarily have to be a closed systems.

The definition of single use systems and closed systems are given in the glossary of Annex 1.

### 3.2 What is meant by large volume containers in integrity testing chapter? Does it also include SUS used in manufacturing?

CCIT requirements related large volume containers (>100mL) refer to the volume of the primary packaging. For details, refer to Section 8.22. However, this section does not describe requirements for SUS.

### 3.3 Many suppliers for SUS apply only ISO 9001:2015 standards. We cannot force them to apply Annex 1 expectations during manufacturing SUS, is this correct?

Annex 1 is not applicable for suppliers of SUS. It is the responsibility of the pharmaceutical manufacturing company to select qualified supplier according to their needs taking EU GMP guideline Chapter 7 into consideration.

However, there are ISO standards for the manufacture under biocontamination control, e.g. the former ISO 14698: Cleanrooms and associated controlled environments — Biocontamination control, now replaced by EN 17141 Cleanrooms and associated controlled environments — Biocontamination control.

## 4 Barrier Systems

### 4.1 Question about RABS/Isolators: open=RABS and closed = Isolator (vials in/out not robotics): asking because of background of Isolator: is Zone D sufficient for isolators (not robotics)?

For closed isolators, grade D is sufficient, for open isolators, grade C is required. RABS require grade B background. Risk from background environment is mainly caused by material being introduced into the isolator/RABS.

### 4.2 What would be a suitable way to perform an isolator glove integrity test for both, nitrile gloves and latex gloves? We face problems with a self-sealing effect of latex gloves, any tips?

Usually for isolator intervention, neither latex nor nitrile gloves used in production isolators. Generally, for isolator gloves two tests in combination are industry's standard: Pressure drop test and visual check for integrity. Typical materials for isolator gloves are EPDM (steam sterilizable) or CSM. Latex and nitrile are used for single use gloves. e.g. when gowning. However, these are then visually checked and often two pairs are worn on top of each other.

### 4.3 Is there any requirement for room classification of a QC Microbiological laboratory? Do Isolators used for (sterility) testing need a grade D background?

There is explicit no requirement for the room grade in Annex 1. Ph.Eur. 5.1.9 "Guidelines for using the test for sterility" says: "*Aseptic conditions for performance of the test can be achieved using, for example, a class A laminar-air-flow cabinet located within a class B clean room, or an isolator*", without specifying the background.

Even if the following guideline is not legally binding within Europe and is not mentioned or referenced in EudraLex Vol. 4, it could still provide helpful points of reference: ISOLATORS USED FOR ASEPTIC PROCESSING AND STERILITY TESTING' of sept 2007 (https://picscheme.org/docview/3441 ). It states at 2.3: " Controlled environments for aseptic operations are currently mainly provided by conventional clean rooms, of Grade B, containing workstations, of Grade A complying with the PIC/S and EC guide to GMP. A smaller number of controlled environments are provided by clean rooms, of Grade D or better containing equipment called isolators providing a Grade A environment. When isolators are used for sterility testing

there is no formal requirement for them to be placed in a Grade D environment. The environment should be controlled e.g. allow access only to trained staff, but not necessarily classified."

However, for the isolators applied for sterility testing, it is common practice to locate those in grade D environment.

### 4.4 Regarding Barrier systems: Is it required to perform a smoke study to prove egress, pressure cascade in a pass-through panel from a C to D grade in a low bioburden facility?

a. Usually, smoke studies (airflow visualisation) are performed, where unidirectional airflow shall be achieved or a backflush prevention (mouseholes at an open isolators) needs to be shown. Between rooms of different cleanroom grades, in general differential pressure is measured instead of performing a smoke study
b. Annex 1, e.g. sec. 4.15, 7.18, 8.109 define, in which cases airflow visualization studies have to be performed. Furthermore, in case of changes to rooms and equipment, which might impact the airflow situation, visualization studies would also be required in context with requalification.

### 4.5 Use of VHP is not considered as sterilization method for gloves, However, by autoclaving then VHP, the gloves become sticky and shorten the usage span of glove. Is autoclaving really a good practice?

a. For isolators gloves are not exchanged frequently. For RABS It is rather recommended to change glove material (EPDM = steam sterilizable) or supplier to still use steam sterilisation in an autoclave on them rather than changing the process of bioburden reduction to an inferior process (disinfection/decontamination vs sterilization).

### 4.6 How to deal with significant incident (e.g. breakage and blockage of line) within isolator during critical operations?

Usually this could only be solved by opening of the isolator which leads to ending the batch and campaign due to its non-integrity. Breakage of single vials, cartridges etc. may need no intervention. Time needs to be recorded and correlated to the vial/cartridge count. That section of the filled containers is specifically inspected for glass particles and if necessary discarded. This should be assessed upfront in the CCS. This is industry practice of e.g. insulin manufacturing, where a filling campaign may last week(s). If there is a complete blockage of the line, the isolator needs to be opened and batch is over.

## 5 CCIT

### 5.1 Regarding CCIT (§ 8.20 - 8.29). How to perform CCIT at prefilled syringes?

a. Usually, it is performed offline. Based on Annex 1 risk-based sampling during routine production. Method based on process, determination risk-based
b. The requirements are:
   - Validated method
   - Frequency based on knowledge & experience
   - Scientifically justified sampling plan
   - Justification for sample size (e.g. supplier management, packaging components, process knowledge)

### 5.2 What is mean with 100% CCIT Control?

a. 100% CCIT is required only for small volume parenterals (< 100ml) closed by fusion.
b. Large volume parenterals closed by fusion see Annex 1, sec. 8.22: "*Where final containers are closed by fusion, e.g. Blow-Fill-Seal (BFS), Form-Fill-Seal (FFS), Small and Large Volume Parenteral (SVP &*

*LVP) bags, glass or plastic ampoules, the critical parameters and variables that affect seal integrity should be evaluated, determined, effectively controlled and monitored during operations. Glass ampoules, BFS units and small volume containers (≤100 ml) closed by fusion should be subject to 100% integrity testing using validated methods. For large volume containers (>100 ml) closed by fusion, reduced sampling may be acceptable where scientifically justified and based on data demonstrating the consistency of the existing process, and a high level of process control. It should be noted that visual inspection is not considered as an acceptable integrity test method.*"

c. Products closed by other than fusion technique see Annex 1, sec. 8.23 or question 1

## 5.3 Is it a requirement to have your process challenged end by end, so from the point of sterile filtration until closure of the container? Or can you split up the process, so for example formulation and filling separately challenged??

a. Related to aseptic process simulation, it should only be the exception to subdivide the process into separate unite operations. If so, then you need to have a documented justification (annex 1, sec. 9.33 v. "*Separate simulations of individual unit operations (e.g. processes involving drying, blending, milling and subdivision of a sterile powder) should be avoided. Any use of individual simulations should be supported by a documented justification and ensure that the sum total of the individual simulations continues to fully cover the whole process*")

b. For manufacture you should validate these activities "end by end", which are performed automatic without interruption by product separation.

## 5.4 Is the issue with CCIT in Annex 1 also relevant for the pharmacy production or are here other regulations, for example ApoBetrO relevant?

a. This cannot be answered uniformly at European level, as national legislation plays a role here. Germany, for example: If the registered pharmacy or hospital pharmacy also manufactures for third parties, it requires a manufacturing authorization and falls under the GMP guidelines and Annex1. If this is not the case, the German ApoBetrO applies. Interpretations of additional national law (e.g. ApoBetrO) needs to be agreed with the national competent authority.