# Review of Annex 1 2022: Environmental Monitoring Changes

## Introduction

In August 2022, a new revision of the EU GMP Annex 1 regulatory standard for sterile drug products was released, replacing the most recent draft from 2020 and the existing revision from 2008. The deadline for operational use of the new standards is August 25, 2023: a year after the release. These requirements regulate the manufacturing of sterile drugs made in and imported to the EU. Pharmaceutical manufacturing is performed in controlled environments to reduce contamination, and changes recently announced by Annex 1 focus more on strategic control than on measurement of quality. This new revision also better aligns the manufacturing principles contained in the Annex 1 to those presented by the World Health Organization (WHO), Pharmaceutical Inspection Cooperation Scheme (PIC/S), and US Food and Drug Administration (FDA).

The new revision is a complete rewrite of the existing Annex 1 from 2008 and almost quadruples the length. It divides the document into 10 newly defined sections. One major sectioning change is the separation and differentiation of Certification (Section 4) and Monitoring (Section 9), which allows for expanded guidance and distinction between premise design/qualification and ongoing routine monitoring. There is a new section that discusses the concept of contamination control strategy (CCS). This section shifts to a new paradigm of incorporating CCS as a central holistic approach to how each aspect of contamination interacts with the facility as a whole. There is also a new section that discusses and identifies Quality Risk Management (QRM) as a central principle to defining processes, operations, and limits, and it ties to CCS to balance process against risk. Additionally, as laid out in the new revision, regulations for Environmental Monitoring is essentially the same with a few enhanced descriptions to better align with QRM.

## Sections of the Annex 1 Document

**Sections 4, 5, and 9 are covered in this paper in the most detail**

1. **Scope**
   Includes additional areas (other than sterile products) where the general principles of the annex can be applied.
2. **Principle**
   General principles as applied to the manufacture of sterile products.
3. **Pharmaceutical Quality System (PQS)**
   Highlights the specific requirements of the PQS when applied to sterile products.

### 4. Premises

General guidance regarding the specific needs for premises design and guidance on the qualification of premises including the use of Barrier Technology.

### 5. Equipment

General guidance on the design and operation of equipment.

6. **Utilities**
   Guidance with regards to the special requirements of utilities such as water, gas and vacuum.

7. **Personnel**
   Guidance on the requirements for specific training, knowledge and skills. Also gives guidance to the qualification of personnel.

8. **Production and Specific Technologies**
   Discusses the approaches to be taken with regards to aseptic and terminal sterilization processes. Discusses approaches to sterilization of products, equipment and packaging components. Also discusses different technologies such as lyophilization and Form-Fill-Seal where specific requirements apply.

## 9. Viable and Nonviable Environmental and Process Monitoring

This section differs from guidance given in section 4 in that the guidance here applies to ongoing routine monitoring with regards to the design of systems and setting of action limits alert levels and reviewing trend data. The section also gives guidance on the requirements of Aseptic Process Simulation (APS).

10. **Quality Control (QC)**
    Gives guidance on some of the specific Quality Control requirements relating to sterile products.

11. **Glossary**
    Explanation of specific terminology.

## Annex 1 Sections and Interpretation

| Section Number | Section | Interpretation |
|---|---|---|
| 2.1 | The manufacture of sterile products is subject to special requirements in order to minimize risks of microbial, particulate and pyrogen contamination. | The importance of contamination by pathogens as well as microbiological and particle is underlined |
| 2.2 | QRM priorities should include appropriate design of the facility, equipment and processes, followed by the implementation of well-designed procedures, and finally application of monitoring systems as the element that demonstrates that the design and procedures have been correctly implemented and continue to perform in line with expectations.<br>*Monitoring or testing alone does not give assurance of sterility.* | QRM becomes the key to reading and applying the new Annex |
| 2.3 | A **Contamination Control Strategy** (CCS) should be implemented across the 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 associated with contamination. | General introduction of the CCS concept. |
| 2.5 | The development of the CCS requires thorough technical and process knowledge. Potential sources of contamination are attributable to microbial and cellular debris (e.g., pyrogen, endotoxins) as well as particulate matter (e.g., glass and other visible and sub-visible particulates). | |

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| 2.6 | The CCS should consider all aspects of contamination control and its life cycle with ongoing and periodic review resulting in updates within the quality system as appropriate | General introduction of the CCS concept. |
| 3.1 | PQS for sterile product manufacture should also ensure that:<br>• An effective **risk management** system is integrated into all areas of the product life cycle to minimize microbial contamination and to ensure the quality of sterile products manufactured.<br>• **Risk management is applied in the development and maintenance of the CCS**, to identify, assess, reduce/ eliminate (where applicable) and control contamination risks. | Issue and management of the CCS via QRM |
| 3.2 | All **non-conformities**, such as sterility test failures, **environmental monitoring excursions** or deviations from established procedures should be adequately investigated. | It is stressed that all non-conformities must be investigated, also when it comes to monitoring in routine, not related only to grade A. |
| 4.25 | Cleanroom and clean air equipment qualification is the overall process of assessing the level of compliance of a classified cleanroom or clean air equipment with its intended use.<br>• Installed filter system leakage and integrity testing.<br>• Airflow tests - volume and velocity.<br>• Air pressure difference test.<br>• Airflow direction test and visualization.<br>• Microbial airborne and surface contamination.<br>• Temperature measurement test.<br>• Relative humidity test.<br>• Recovery test.<br>• Containment leak test. | Room Classification required and qualification to determine risk points |

<table>
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<td>4.27</td>
<td>For cleanroom classification, the total of particles equal to or greater than 0.5 and 5.0 µm should be measured. This measurement should be performed both at rest and in simulated operations in accordance with the limits specified in Table 1.<br><br>TABLE 1 Maximum permitted airborne particulate concentration during classification<br>
<table>
<tr><th rowspan="2">Grade</th><th colspan="2">Maximum limits for particulates<br>≥ 0.5 µm/m³</th><th colspan="2">Maximum limits for particulates<br>≥ 5 µm/m³</th></tr>
<tr><th>at rest</th><th>in operation</th><th>at rest</th><th>in operation</th></tr>
<tr><td>A</td><td>3 520</td><td>3 520</td><td>Not applicable</td><td>Not applicable</td></tr>
<tr><td>B</td><td>3 520</td><td>352 000</td><td>Not applicable</td><td>2 900</td></tr>
<tr><td>C</td><td>352 000</td><td>3 520 000</td><td>2 900</td><td>29 000</td></tr>
<tr><td>D</td><td>3 520 000</td><td>Not defined[a]</td><td>29 000</td><td>Not defined[a]</td></tr>
</table>
[a] For Grade D, in operation limits are not defined. The company should establish in operation limits based on a risk assessment and historical data where applicable.</td>
<td>Classification at 0.5 and 5.0 µm required. As ISO dropped the 5.0 µm value in the table the limits should be set by historical trend – or ‘m’ description.<br><br>There was initially a 1.0 µm potential requirement here– a misunderstanding of the ISO14644-1 standard – and dropped in final release</td>
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<td>4.30</td>
<td>The speed of air supplied by unidirectional airflow systems should be clearly justified in the qualification protocol … Unidirectional airflow systems should provide a homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance value) at the working position, unless otherwise scientifically justified in the CCS. Airflow visualization studies should correlate with the air speed measurement.</td>
<td>The air speed is subject to a new ISO working standard and is not based on scientific data. Speeds as low as 0.23 m/s can be justified with airflow visualization studies</td>
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<td>4.31</td>
<td>The microbial contamination level of the cleanrooms should be determined as part of the cleanroom qualification.<br>The number of sampling locations should be based on a documented risk assessment and the results obtained from room classification, air visualization studies and knowledge of the process and operations to be performed in the area.<br>The maximum limits for microbial contamination during qualification for each grade are given in Table 2. Qualification should include both “at rest” and “in operation” states.<br><br>TABLE 2 Limits for microbial contamination during qualification
<table>
<tr><th>Grade</th><th>Air sample cfu/m$^3$</th><th>Settle plates (diameter 90 mm) cfu/4 hours [a]</th><th>Contact plates (diameter 55 mm) cfu/plate</th></tr>
<tr><td>A[b]</td><td colspan="3">No growth[b]</td></tr>
<tr><td>B</td><td>10</td><td>5</td><td>5</td></tr>
<tr><td>C</td><td>100</td><td>50</td><td>25</td></tr>
<tr><td>D</td><td>200</td><td>100</td><td>50</td></tr>
</table>
[a] Settle plates should be exposed for the duration of operations and changed as required after 4 hours. Exposure time should be based on recovery studies and should not allow desiccation of the media used.<br>[b] It should be noted that for Grade A, the expected result should be no growth.</td>
<td>Microbial Qualification at same time as particle based on Risk</td>
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<td>5.9</td>
<td>Particle counters, including sampling tubing, should be qualified. The manufacturer’s recommended specifications should be considered for tube diameter and bend radius. Tube length should typically be no longer than 1 m unless justified and the number of bends should be minimized. Portable particle counters with a short length of sample tubing should be used for classification purposes. Isokinetic sampling heads should be used in unidirectional airflow systems. They should be oriented appropriately and positioned as close as possible to the critical location to ensure that samples are representative.<br><br>5.9 Particle counters, including sampling tubing, should be qualified. The tubing length should be no greater than 1 meter with a minimum number of bends and bend radius should be greater than 15 cm. Portable particle counters with a short length of sample tubing should be used for classification purpose. Isokinetic sampling heads should be used in unidirectional airflow systems and should be positioned as close as possible to sample air representative of the critical location.</td>
<td>Misinterpretation of ISO 14644-1: 2015 Annex C regarding tubing lengths<br>• ISO TC 209 working group 15 are releasing a document of best practices for particle counting.</td>
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| 9.1 | The site's environmental and process monitoring program forms part of the overall CCS and is used to monitor the controls designed to minimize the risk of microbial and particulate contamination. It should be noted that the reliability of each of the elements of the monitoring system (viable, nonviable and APS) when taken in isolation is limited and should not be considered individually to be an indicator of asepsis. When considered together, their reliability is dependent on the design, validation and operation of the system that they are monitoring. | Correlation with CCS |
| 9.2 | This program is typically comprised of the following elements:<br>**1.** Environmental monitoring – non-viable particles.<br>**2.** Environmental and personnel monitoring – viable particles.<br>**3.** Aseptic process simulation (aseptically manufactured product only). | APS becomes an integral part of the monitoring system |
| 9.4 | An environmental monitoring program should be established and documented.<br>• Risk assessments should be performed in order to establish this comprehensive environmental monitoring program, i.e. sampling locations, frequency of monitoring, monitoring methods, and incubation conditions.<br>• These risk assessments should be conducted based on detailed knowledge of; the process inputs and final product, the facility, equipment, the criticality of specific processes and steps, the operations involved, routine monitoring data, monitoring data obtained during qualification and knowledge of typical microbial flora isolated from the environment.<br>• The risk assessment should include the determination of critical monitoring locations, those locations where the presence of microorganisms during processing may have an impact upon product quality, (e.g., grade A, aseptic processing areas and the grade B areas that directly interface with the grade A area).<br>• Consideration of other information such as air visualization studies should also be included. These risk assessments should be reviewed regularly in order to confirm the effectiveness of the site's environmental monitoring program.<br>• The monitoring program should be considered in the overall context of the trend analysis and the CCS for the site | An Environmental Monitoring program should be implemented and documented<br>• Based upon a formal / documented Risk Assessment, with knowledge of:<br>  • Process inputs<br>  • Facility equipment<br>  • Criticality of a process<br>  • EM Data<br>• Risk Assessments should contain:<br>  • Monitoring locations<br>  • Frequency<br>  • Method of sampling<br>  • Incubation conditions |

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| 9.5 | Routine monitoring of cleanrooms, clean air equipment and personnel should be performed in **operation throughout all critical stages, including equipment *set-up*.** | The criticality of the set-up phases is underlined |
| 9.7 | The monitoring of grade A should demonstrate the maintenance of aseptic processing conditions during critical operations. Monitoring should be performed at locations posing the highest risk of contamination to the sterile equipment surfaces, containers, closures and product. The selection of monitoring locations and the orientation and positioning of sampling devices should be justified and appropriate to obtain reliable data from the critical zones. | It is required to specify the orientation of the sampling point (such as orientation towards the air flow or heights ...) |
| 9.8 | Sampling methods should not pose a risk of contamination to the manufacturing operations. | Sampling methods must be chosen so as not to interfere with production activities |
| 9.9 | Appropriate alert levels and action limits should be set for the results of viable and total particle monitoring. The maximum total particle action limits are described in Table 5 and the maximum viable particle action limits are described in Table 6. However, more **stringent action limits** may be applied based on data trending, the nature of the process or as determined within the CCS. **Both viable and total particle alert levels should be established based on results of cleanroom qualification tests and periodically reviewed based on ongoing trend data.** | The approach to analyzing trends is highlighted and described, it should be noted that the alert and action limits must be distinguished from those shown in the table. |
| 9.10 | **Alert levels** for grade A (total particle only), grade B, grade C, and grade D should be set such that adverse trends (e.g., a numbers of events or individual events that indicate a deterioration of environmental control) are detected and addressed.<br><br>9.15 NOTE 2: The occasional indication of macro particulate counts, especially ≥ 5.0 µm, may be considered to be false counts due to electronic noise, stray light, coincidence, etc. However, consecutive or regular counting of low levels may be indicative of a possible contamination event and should be investigated. Such events may indicate early failure of the room air supply filtration system, filling equipment failure, or may also be diagnostic of poor practices during machine set-up and routine operation. | Trend analysis must take into consideration the microbial flora |

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| 9.11 | Monitoring procedures should define the approach to trending. Trends should include, but are not limited to:<br>• Increasing numbers of excursions from action limits or alert levels.<br>• Consecutive excursions from alert levels.<br>• Regular but isolated excursion from action limits that may have a common cause, (e.g., single excursions that always follow planned preventative maintenance).<br>• Changes in microbial flora type and numbers and predominance of specific organisms. | The short, medium and long term trending of data is now defined as a requirement. |
| 9.12 | The monitoring of grade C and D cleanrooms in operation should be performed based on data collected during qualification and routine data to allow effective trend analysis. The requirements of alert levels and action limits will depend on the nature of the operations carried out. Action limits may be more stringent than those listed in Table 5 and Table 6. | Grade C/D areas monitoring based upon Risk Assessment |
| 9.13 | If action limits are exceeded, operating procedures should prescribe a root cause investigation, an assessment of the potential impact to product (including batches produced between the monitoring and reporting) and requirements for corrective and preventive actions. If alert levels are exceeded, operating procedures should prescribe assessment and follow-up, which should include consideration of an investigation and/or corrective actions to avoid any further deterioration of the environment | Action limit excursions should have a Root Cause follow up. Alert excursions should have a process within the SOP to follow |

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<td>9.15</td>
<td>The limits for environmental monitoring of airborne particulate concentrations for each graded area are given in Table 6.<br><br>TABLE 3 Limits for airborne particulate concentration for the monitoring of non-viable contamination (Table 6 in the Annex1 document)<br><br>
<table>
<tr><th rowspan="2">Grade</th><th colspan="2">Maximum limits for particulates<br>≥ 0.5 µm/m³</th><th colspan="2">Maximum limits for particulates<br>≥ 5 µm/m³</th></tr>
<tr><th>at rest</th><th>in operation</th><th>at rest</th><th>in operation</th></tr>
<tr><td>A</td><td>3 520</td><td>3 520</td><td>29</td><td>29</td></tr>
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</table>
<br>[a] For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and on routine data, where applicable.<br><br>Note 1: The particle limits given in the table for the “at rest” state should be achieved after a short “clean up” period defined during qualification (guidance value of less than 20 minutes) in an unmanned state, after the completion of operations (see paragraph 4.29).<br><br>Note 2: The occasional indication of macro particle counts, especially ≥ 5.0 µm, within grade A may be considered to be false counts due to electronic noise, stray light, coincidence loss etc. However, consecutive or regular counting of low levels may be indicative of a possible contamination event and should be investigated. Such events may indicate early failure of the room air supply filtration system, equipment failure, or may also be diagnostic of poor practices during machine set-up and routine operation.</td>
<td>5.0 µm measurement returns for monitoring as an early indication of system deterioration</td>
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<td>9.17</td>
<td>The grade A area should be monitored continuously (for particles ≥0.5 and ≥5 µm) and with a suitable sample flow rate (at least 28 litres (1ft3 ) per minute) so that all interventions, transient events and any system deterioration is captured. The system should frequently correlate each individual sample result with alert levels and action limits at such a frequency that any potential excursion can be identified and responded to in a timely manner. Alarms should be triggered if alert levels are exceeded. Procedures should define the actions to be taken in response to alarms including the consideration of additional microbial monitoring.</td>
<td>Continuous monitoring for Grade A as previously noted. Due to statistical sampling, a 28.3 LPM instrument should be used - statistics is a vital part of continuous monitoring and sufficient sample must be taken.<br><br>• System based data</td>
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| 9.18 | It is recommended that a similar system be used for the grade B area although the sample frequency may be decreased. The grade B area should be monitored at such a frequency and with 45 suitable sample size that the program captures any increase in levels of contamination and system deterioration. If alert levels are exceeded, alarms should be triggered. | Immediate area surrounding Grade A should be continuous monitoring. Backgrounds areas beyond this zone can be Portable instruments or automated? |
| 9.20 | In the case where contaminants are present due to the processes involved and would potentially damage the particle counter or present a hazard (e.g., live organisms, powdery products and radiation hazards), the frequency and strategy employed should be such as to assure the environmental classification both prior to and post exposure to the risk. An increase in viable particle monitoring should be considered to ensure comprehensive monitoring of the process. **Additionally, monitoring should be performed during simulated operations. Such operations should be performed at appropriate intervals.** The approach should be defined in the CCS. | For hazardous contaminants that are present (and powders) a different approach is required. Simulated filling with no powder to identify baseline. Monitoring before and after filling during normal production |
| 9.21 | The size of monitoring samples taken using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of cleanrooms and clean air equipment. Monitoring sample volumes should be justified | A rolling cubic meter is not required – the data should fit with CCS |
| 9.22 | Where aseptic operations are performed, microbial monitoring should be frequent using a combination of methods such as settle plates, volumetric air sampling, glove, gown and surface sampling (e.g., swabs and contact plates). The method of sampling used should be justified within the CCS and should be demonstrated not to have a detrimental impact on grade A and B airflow patterns. Cleanroom and equipment surfaces should be monitored at the end of an operation. | Frequent monitoring that is justified in the CCS, but does not have a detrimental effect on the environment. |
| 9.23 | Viable particle monitoring should also be performed within the cleanrooms when normal manufacturing operations are not occurring, and in associated rooms that have not been used, in order to detect potential incidents of contamination which may affect the controls within the cleanrooms. In case of an incident, additional sample locations may be used as a verification of the effectiveness of a corrective action (e.g., cleaning and disinfection). | **"Monitoring not in operational state ONLY"**: The purpose of this monitoring is different from the operational state, because the final purpose is to verify the environment after determinate activities. This data is not related to the process but to the environment and for this reason the data came from this monitoring must not be confused with the routine monitoring data. |

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| 9.24 | **Continuous viable air monitoring in the Grade A zone (e.g., air sampling or settle plates)** should be undertaken for the full duration of critical processing, including equipment (aseptic set-up) assembly and filling operations. **A similar approach should be considered for Grade B cleanrooms based on the risk of impact** on the aseptic processing. The monitoring should be performed in such a way that all interventions, transient events and any system deterioration would be captured and any risk caused by interventions of the monitoring operations is avoided. | Continuous viable air monitoring in the Grade A zone and critical grade B (e.g., surrounding). There are to possible methods to choose from: settle plates or air sampling |
| 9.28 | The adoption of suitable rapid or automated monitoring systems should be considered by manufacturers in order to expedite the detection of microbiological contamination issues and to reduce the risk to product. **These rapid and automated microbial monitoring methods may be adopted after validation has demonstrated their equivalency or superiority to the established methodology.** | Promotion of Rapid Microbial Methods (RMM). Still a new technology which is difficult to implement against traditional techniques |
| 9.29 | Sampling methods and equipment used should be fully understood and procedures should be in place for the correct operation and interpretation of results obtained. Supporting data for the recovery efficiency of the sampling methods chosen should be available. | **Recovery efficiency** is defined as the growth promotion test before and after the use of settle plates or agar system used in active air sampling.<br>• Contact plate: Recovery from the surfaces<br>• Swabs: Release efficiency |

9.30

Action limits for viable particle contamination are shown in Table 7.

**TABLE 4** Maximum action limits for viable particle contamination (Table 7 in the Annex 1 document)

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

[a] Settle plates should be exposed in grade A and B areas for the duration of operations (including equipment set-up) and changed as required after a maximum of 4 hours (exposure time should be based on validation including recovery studies and it should not have any negative effect on the suitability of the media used).

- For grade C and D areas, exposure time (with a maximum of 4 hours) and frequency should be based on QRM
- Individual settle plates may be exposed for less than 4 hours.

Grade C & D gowning sampling based on Risk (QRM and CCS)

[b] Contact plate limits apply to equipment, room and gown surfaces within the grade A and grade B areas. Routine gown monitoring is not normally required for grade C and D areas, depending on their function.

[c] It should be noted that for grade A, any growth should result in an investigation.

[c] Any Growth in Grade A – MUST be investigated

**Note 1:** It should be noted that the types of monitoring methods listed in the table above are examples and other methods can be used provided they meet the intent of providing information across the whole of the critical process where product may be contaminated (e.g., aseptic line set-up, aseptic processing, filling and lyophilizer loading).

**Note 2:** Limits are applied using CFU throughout the document. If different or new technologies are used that present results in a manner different from CFU, the manufacturer should scientifically justify the limits applied and where possible correlate them to CFU

**Note 2** – BioCounts / Auto Fluorescing Units (AFU) can be used if validated.

## References


1. Annex 1 2022, "Manufacture of Sterile Products". EU GMP Guide for *Good manufacturing practice for drug products and drug substances.*
2. Eudralex Volume 4 – Annex 1 2008


## Author


**Mark Hallworth**

Senior GMP Scientis, Life Sciences, Particle Measuring Systems


Mark Hallworth is the Life Sciences Strategic Senior GMP Scientist. Mark has managed the design, installation, and validation of over 200 environmental monitoring system projects worldwide. He has designed several products specific for pharmaceutical environmental monitoring, including particle counters for explosive and corrosive areas and 21 CFR part 11 compliant software for batch test and release. He currently lectures for the PDA, ISPE and other international pharmaceutical societies on environmental monitoring and GMP compliance design and validation. Mark has written over 100 technical papers on environmental monitoring and contributed to several books specific to this field, he was awarded the IEST James Mildon award for "significant contributions to the advancement and increase of knowledge in the field of contamination control".

## Editor


**Noelle Boyton**

Technical Writer, Particle Measuring Systems