EJPPS – European Journal of Parenteral and Pharmaceutical Sciences Volume 24 Issue 4
https://www.ejpps.online/part-1-pharmaceutical-cleanroom-cla
https://doi.org/10.37521/ejpps.24401

Pharmaceutical Cleanroom Classification using ISO 14644-1 and the EU GGMP Annex 1

Part 1: Testing rationale

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Summary

Cleanroom classification is an essential part of the qualification activities in pharmaceutical cleanrooms that confirm the effectiveness of the cleanroom's airborne contamination control system. A review of the classification requirements and principles associated with ISO 14644-1:2015 and the 2008 version of Annex 1 of the EU GGMP is contained in this first article, and a suitable classification test method derived for aseptic manufacturing. A second article will consider the application of the method by means of practical examples.

Key words: Cleanroom classification, ISO 14644-1, EU GGMP Annex 1

1. Introduction

All cleanrooms are classified according to ISO 14644-1 (1) to demonstrate that a specified concentration of airborne particles is not exceeded. Annex 1 of European Union Guide to Good Manufacturing Practice (EU GGMP) (2) specifies the environmental conditions that must be provided for the manufacture of sterile medicinal products and requires that the classification of different grades of cleanrooms and clean zones to be based on ISO 14644-1.

The classification of pharmaceutical cleanrooms or clean zones is an essential part of the qualification process to ensure that appropriate levels of airborne contamination are provided for the type of activities undertaken. In addition, the classification can provide useful reference data if any future modifications to the cleanroom or its ventilation system are completed, or an investigation is undertaken to determine the reasons for any system deterioration. However, the correct interpretation and application of the information given in ISO 14644-1 and Annex 1 of the EU GGMP, as well as consideration of more current expectations from the regulatory authorities, is required and included in this article. Although the classification method is applied to cleanrooms used for aseptic manufacture, the approach can be used for most pharmaceutical and healthcare cleanroom applications with some minor modifications.

2. Origins of the cleanroom classification standard

The standard that most influenced the early establishment of the correct design and operation of cleanrooms was United States' Federal Standard 209 (FS 209), entitled 'Cleanroom and Work Station Requirements, Controlled Environments' which was first published in 1963 and considered both unidirectional airflow (UDAF) and non-UDAF cleanrooms.

The class limits given in FS 209 were established by airborne particle number concentration measurements carried out in a large number of cleanrooms used for many activities, including electronic component and pharmaceutical manufacturing, mainly in the United States. Several revisions of FS 209 were undertaken up to revision D, which was published in 1988. All these revisions included cleanroom class limits based on the number of particles ≥0.5 µm per ft^3 of cleanroom air. The final version E, had class limits that were additionally reported as concentrations per m^3. This standard was withdrawn in 1992, after the International Organization for Standardization (ISO) published ISO 14644-1: 1999 to produce worldwide harmonization. This ISO standard is the basis of the current ISO 14644-1: 2015 standard and also for the airborne particle concentrations included the EU GGMP.

The relationships between the particle number concentration and the threshold particle size(s) that are used to set the class limits in ISO 14644-1 were established in the early 1960s. At that time, conditions in cleanrooms were different from modern cleanrooms and, for example, cotton garments were often used and the methods of counting and measuring particles were in their infancy. Consequently, airborne particle count distributions and class limits that were used, and still used, in ISO 14644-1 and the EU GGMP for cleanrooms and clean zones, are likely to be different from those found in modern healthcare cleanrooms. This difference may cause difficulties when measuring the airborne particle concentrations and applying class limits. The problem is discussed further in the second article (3).

3. Classification of an EU GGMP (2008) cleanroom in accordance with ISO 14644-1: 2015

Annex 1 of the EU GGMP (2008) states that cleanrooms and clean air devices should be classified in accordance with ISO 14644-1. However, since the publication of that guide some additional requirements are expected by the regulatory authorities and may be included in the revised edition of EU GGMP when published.

It should be noted that the ISO 14644-1: 2015 standard refers to cleanrooms and clean zones and Annex 1 of the EU GGMP (2008) refers to cleanrooms and clean air devices. These clean air devices typically include isolators, restricted access barrier systems (RABS), open access safety cabinets etc. and when utilised for critical activities, are required to meet the contamination control requirements of an EU GGMP Grade A environment. For simplicity, this paper often refers to 'cleanrooms', when the term covers both cleanrooms and clean air devices.

The EU GGMP requires the classification of a cleanroom to be carried out in the 'at rest' and 'in operation' states. The 'in operation' classification state relates to the actual manufacturing process and provides the most useful information, and it is therefore the focus of this paper. However, the 'at rest' classification state, which requires a similar approach, is also discussed.

Table 1 summarises the classification requirements and principles contained in ISO 14644-1: 2015 and Annex 1 of the EU GGMP (2008) that is relevant to this article, along with information on the current expectations of the regulators that are additional to EU GGMP (2008). These provide a classification testing rationale for pharmaceutical cleanrooms used for aseptic manufacture.

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Table 1 Classification considerations and derived rationale for pharmaceutical cleanrooms

1. Facility installation status	
ISO 14644-1	"Prior to testing, verify that all relevant aspects of the cleanroom or clean zone that contribute to its integrity are complete and functioning in accordance with its performance specification".
EU GGMP Annex 1	The expectation is that all cleanroom installation activities are completed in an appropriate manner for the occupancy state testing to be undertaken (refer to section 2 of this table for information relating to occupancy states).
Discussion	To provide meaningful data, the cleanroom installation needs to be complete with all fittings and equipment satisfactorily installed. The air ventilation system must be operating in the manner required to provide the correct level of airborne contamination control, with in-situ integrity testing of the cleanroom air supply filters satisfactorily completed. The 'at rest' classification provides essential reference data should the cleanroom or ventilation system be modified, or the manufacturing activities changed, to confirm the effectiveness of the airborne contamination control system relative to the original state. The 'at rest' classification needs to be satisfactorily completed before the 'in operation' classification (refer to Section 2 of this table). The 'in operation' classification needs to be completed before manufacturing can start and prior to other qualification activities, such as microbial testing, as a classification failure may require modifications to the cleanroom structure or ventilation system and may invalidate some of the qualification activities.
Conclusions	1. All installation testing to be fully completed before starting classification. 2. The air conditioning system to be operating in the manner necessary to provide the required level of airborne contamination control. 3. The in-situ integrity testing of all cleanroom terminal air supply filters to be satisfactorily completed. 4. 'At rest' testing to be completed prior to 'in operation' testing.

2. Occupancy state	
ISO 14644-1	"The air cleanliness class by particle concentration of air in a cleanroom or clean zone shall be defined in one or more of three occupancy states, viz. as-built, at-rest or operational".
EU GGMP Annex 1	"The in operation and at rest states should be defined for each clean room or suite of clean rooms". The GGMP also states; "In operation classification may be demonstrated during normal operations, simulated operations or during media fills as worst-case simulation is required for this".
Discussion	The definitions of the occupancy states in both ISO 14644-1: 2015 and the EU GGMP (2008) are shown in Appendix A of this article. These definitions vary a little between the ISO standard and the EU guide but when applied to cleanroom classification, the differences are of no consequence. For 'at rest' testing, no people should be in the cleanroom and all machinery switched off. The dispersion of airborne particles should, therefore, be close to zero. As the air supplied into the cleanroom is particle-free and the cleanroom is pressurised with respect to adjacent areas, the airborne concentration of particles should be effectively zero and with the design, construction, and qualification requirements that are typically required for a pharmaceutical cleanroom, this would be the expectation. However, to confirm this, and provide useful reference data for comparison purposes for any future modifications to the room or ventilation system, or to investigate any system deterioration, 'at rest' classification is required. The 'in operation' classification state will provide the most meaningful information as it relates to the manufacturing process and the time when contamination of the product could occur. The 'worst case' conditions that give the highest airborne particle count should always be tested to ensure the most stringent challenge of the airborne contamination control system. These conditions require all equipment to be fully operational with the maximum occupancy levels present during manufacturing operations. As cleanroom garments are essential to control the dispersal of people contamination into the environment, the cleanroom attire that will be worn routinely during production must be worn by all cleanroom personnel during the operational classification.
Conclusions	1. The 'at rest' and 'in operation' conditions to be tested. 2. For the 'in operation' status, worst-case activities and conditions identical to those used during operation (including maximum occupancy number), to be included in the testing. 3. All personnel to wear the designated cleanroom garments that are used during normal operations.

3. Particle size	
ISO 14644-1	"One, or more than one, threshold (lower limit) particle sizes situated within the range from ≥0,1 µm to ≥5 µm are to be used". The standard also states; "If measurements are made at more than one particle size, each larger particle diameter shall be at least 1.5 times the next smaller particle diameter".
EU GGMP Annex 1	The maximum permitted airborne particle concentrations for each Grade are given in the table in Section 4 of Annex 1 of the GGMP and includes particles at both ≥0.5 µm and ≥5 µm sizes.
Discussion	The airborne cleanliness concentration limits given in Annex 1 are for ≥0.5 µm and ≥5 µm particle sizes. These limits are required for monitoring as well as classification, and to ensure that the limits are not exceeded during monitoring, classification should be carried out at both these particle sizes. A review of the ratios relating ≥0.5 µm to ≥5 µm particle concentrations found at AstraZeneca Macclesfield (UK) (4) reported that average ratios during cleanroom operations were 12:1 and 57:1, for EU GGMP Grade A and Grade B areas respectively. This is similar to other ratios that have also been reported (5) (6). These ratios contrast with those given in Annex 1, which are 171:1 for Grade A and 121:1 for B and C areas, respectively. Consequently, the cleanroom particle concentrations at the ≥5 µm size are likely to be much nearer to the EU GGMP class limit than concentrations at ≥0.5 µm. Therefore, classification is much more likely to fail at the ≥5 µm particle size than the ≥0.5 µm size and this information supports the recommendation to classify the cleanroom at both these particle sizes in anticipation of potential problems with particles ≥5 µm. The use of particle sizes of ≥0.5 µm and ≥5 µm satisfies the ISO standard requirement that, when more than one particle size is utilised, the larger particle diameter is more than 1.5 times the smaller particle diameter. It should be noted that the ISO standard does not include a concentration limit for particles ≥5 µm for an ISO 5 area, which is equivalent to EU GGMP Grade A. This is discussed in Section 4 of this table.
Conclusion	1. Both ≥0.5 µm and ≥5 µm particle sizes to be included in the cleanroom classification.

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4. Particle concentration limits	
ISO 14644-1	The particle concentration limits, for particle sizes within the range ≥0.1 µm to ≥ 5 µm, are shown in Table 1 in the standard.
EU GGMP Annex 1	The particle concentration limits are only given for ≥0.5 µm and ≥5 µm particle sizes and shown in the table in Section 4 of Annex 1 of the GGMP for both the 'at rest' and 'in operation' occupancy states.
Discussion	The 'in operation' particle concentration limits in Annex 1 for ≥0.5 µm and ≥5 µm particle sizes for Grades A, B and C areas correspond approximately to the ISO standard Class numbers 5, 7 and 8, respectively. These limits are compared in Appendix B of this article, where it can be seen that the limits included in the EU GGMP are more stringent at the ≥5 µm size than the corresponding limits in the ISO standard. The EU GGMP concentrations should therefore be applied. It should be noted that the ISO standard does not include a concentration limit for particles ≥5 µm for an ISO 5 area, which is equivalent to EU GGMP Grade A. This is because the sampling and statistical limitations of particles at such low concentrations make classification inappropriate. However, the ISO standard addresses this deficiency by using the 'M descriptor' facility, (refer to the Clause C.7 in Annex C in ISO 14644-1: 2015), which can be used to quantify populations of macroparticles i.e. particles ≥5 µm.
Conclusion	1. The particle concentration limits defined in Annex 1 of the GGMP, for particles ≥0.5 µm and ≥5 µm, to be applied.

5. Sampling volumes and sampling times	
ISO 14644-1	The minimum sample volume for a single sample at each location is calculated by consideration of the class limit of the largest particle size considered. This volume is calculated by use of Formula A.2 in the standard and it should be noted that this formula is also used to calculate the volume required for sampling macroparticles (particles ≥5 µm). In addition, it is stated; "The volume sampled at each location shall be at least 2 litres, with a minimum sampling time of 1 min for each sample at each location. Each single sample volume at each sampling location shall be the same". The standard also requires for the measurement of macroparticles that the sampler "should have a sample flow rate of at least 28.3 l/min".
EU GGMP Annex 1	"For classification purposes EN/ISO 14644-1 methodology defines both the minimum number of sample locations and the sample size based on the class limit of the largest considered particle size". However, the guide states; "For classification purposes in Grade A zones, a minimum sample volume of 1m^3 should be taken per sample location".
Discussion	As the largest considered particle size used in airborne sampling in a pharmaceutical cleanroom is ≥5 µm, this size of particle should be utilised for calculating the minimum sampling volume by use of Formula A.2 in the ISO standard, and for an EU GGMP Grade A clean zone this is 1000 l (1 m^3). Also, for the collection of particles >5 µm, (refer to Section 3 of this table) a particle counter with a minimum sampling rate of 28.3 l/min is required and, typically, particle counters with a greater sampling rate are utilised to reduce the testing duration. For EU GGMP Grade B, C and D cleanrooms, the sample volume calculated using Formula A.2 is likely to be less than the required minimum sample volume of 2 l. This in turn will be less than the volume associated with a minimum sampling time of 1 minute, which, for the required minimum sampling flow rate of 28.3 l/min, will be 28.3 l. However, for Grade A zones, a minimum sample volume of 1 m^3 (1000 l) is stated in the EU GGMP and this is consistent with the minimum sampling volume determined by use of Formula A.2 in the ISO standard. For 'in operation' sampling, it should be noted that the sampling time must be sufficiently large to ensure that important particle-generating activities are included in the measurements. Non-Grade A zones typically utilise non-unidirectional airflow (non-UDAF) that mixes and dilutes the contaminated air with the supplied particle-free air and the cleanroom concentrations are less likely to vary throughout the cleanroom when, compared to a UDAF zone. In this case, the minimum sampling time of 1 minute stated in the ISO standard is likely to be appropriate. However, a 1-minute sample may not be of sufficient duration to capture all particle-generating activities and more than one sample may be required. Within Grade A zones, the EU GGMP and ISO 14644-1: 2015 requires a minimum sample volume of 1 m^3, which needs a sampling time of 36 minutes for a sampling rate of 28.3 l/ min and 20 minutes for sampling rates of 50 l/min. These times should provide an opportunity to capture all particle-generating events.
Conclusions	1. For EU GGMP Grade A zones, the minimum sampling volume to be 1 m^3. 2. For non-EU GGMP Grade A cleanrooms, the minimum sampling time is likely to be at least 1 minute when using a particle counter that has a sampling rate of at least 28.3 l/min. 3. For 'in operation' sampling, all activities to be adequately captured during the sampling period and, therefore, it may be necessary to take more than one sample.

6. Number of sampling locations	
ISO 14644-1	The minimum number of sampling locations is related to the cleanroom floor area and shown in Table A.1 in the standard and included in Appendix C of this article. The cleanroom or clean zone under consideration should be divided into the same number sections of equal area. However, the standard states; "Additional sampling locations may be selected for locations considered critical. Their number and positions shall also be agreed and specified. Additional sections and associated sampling locations may be included to facilitate subdivision into equal sections".
EU GGMP Annex 1	The requirement is to follow the methodology described in the ISO standard to determine the minimum number of sampling locations. However, the regulatory authority expectation is that a higher number of samples are typically required for the critical processing locations for 'in operation' sampling (refer to Section 7 of this table for information relating to a higher number of samples for the critical zones).
Discussion	The minimum number of sampling locations is determined from the cleanroom floor area, and Table A.1 in the ISO standard should be utilised to determine the number of sections required. These should be equal areas but if the cleanroom is of a non-uniform or unusual shape, this may be difficult, and it is appropriate to increase the number of sections to avoid the need for a complex geometrical solution. How this may be accomplished is discussed in Appendix D of this article.
Conclusions	1. The minimum number of sampling locations to be determined from Table A.1 in the ISO standard and the room divided into approximately equal floor area sections where an air sample is taken in each section. 2. If the cleanroom cannot be readily divided into equal area sections, the size of the sections to be decreased and the number of sampling locations increased. 3. If there are any additional difficult to accommodate areas of the cleanroom, such as alcoves or recesses, it is acceptable to simply include additional sampling locations within these areas.

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7. Where to sample in each section	
ISO 14644-1	“Select within each section a sampling location considered to be representative of the characteristics of the section”. The standard states; “a representative location means that features such as cleanroom or clean zone layout, equipment disposition and airflow systems should be considered when selecting sampling locations”. It also states; “For non-unidirectional airflow cleanrooms or clean zones, locations may not be representative if they are located directly beneath non-diffused supply air sources”.
EU GGMP Annex 1	For ‘in operation’ sampling, the regulatory authority expectation is that the sampling locations within each section are based upon a documented risk assessment.
Discussion	The expectation of the EU GGMP is that for ‘in operation’ sampling, activities that present the greatest risk of product contamination are formerly identified by an appropriate risk assessment method, and the associated locations included in the sampling. Suitable risk assessment methods are discussed in Appendix E of this article and their application is demonstrated in the second article. The direction of airflow should also be considered in UDAF zones when determining the exact sampling location, with the sampling completed on the ‘downstream’ side, where the air will contain any released particles, and not on the particle free ‘upstream’ side. If there are no activities that present a high risk of product contamination, it is reasonable to sample in the centre of the section. Sampling in a representative position in each section is typically appropriate for ‘at rest’ testing. This is also required for cleanrooms outside the aseptic processing room. It is necessary to ensure that sampling locations are not directly under a non-diffused air supply source as this location will have low concentrations of particles and for classification purposes these locations should be avoided. Some cleanrooms and clean zones may have a relatively small floors or base areas and the minimum number of sampling locations determined by the ISO standard may be less than the number of sampling locations identified by the documented risk assessment method. In this case, extra sampling locations in the same section can be utilised for each of the different risk location.
Conclusions	1. For ‘at rest’ testing and for cleanrooms outside the aseptic processing room, sampling within the centre of each section is typically appropriate. 2. For ‘in operation’ testing, a formal risk assessment process should be used to establish where air sampling should be carried out. 3. Consideration to be given to the direction of airflow in UDAF and avoiding sampling under air inlets with no diffuser. 4. If the number of sampling locations identified by the risk assessment method is more than determined by the ISO standard method, additional sampling locations in the same sampling section can be utilised.

8. Sampling probe and tubing	
ISO 14644-1	“The sampling probe shall be positioned pointing into the airflow. If the direction of the airflow being sampled is not controlled or predictable (e.g. non-unidirectional airflow), the inlet of the sampling probe shall be directed vertically upward”. The standard also states that the particle counter “should be fitted with an inlet probe sized for isokinetic sampling in unidirectional flow zones” and “the transit tube from the sampling probe inlet to the LSAPC [light scattering airborne particle counter] sensor should be as short as possible. For sampling of particles larger than and equal to 1 µm, the transit tube length should not exceed the manufacturer’s recommended length and diameter and will typically be no longer than 1 m in length”.
EU GGMP Annex 1	“Portable particle counters with a short length of sample tubing should be used for classification purposes because of the relatively higher rate of precipitation of particles ≥5.0µm in remote sampling systems with long lengths of tubing. Isokinetic sample heads shall be used in unidirectional airflow systems”.
Discussion	For UDAF locations where a true sample of macroparticles (particles ≥5 µm) is required, an isokinetic sampling head is required to ensure the velocity of air entering the sampler is equal to the velocity of the air passing by the sampling head. This is achieved by adjusting the diameter of the sampling head. For a UDAF velocity of 0.45 m/s, the probe diameter for an air sampling rate of a LSAPC of 28.3 l/min should be 3.7 cm. In Grade A locations, where access may be limited due to impediment by equipment or operational activities, tubes may be required to transport air from sampling locations to a LSAPC. As macroparticles pass along the tube they may sediment by gravity onto the walls. To avoid this, a sampling tube should not be used but, if required, it will be impossible to avoid some losses of macroparticles, but these losses will be acceptable if the tube is not too long. It has been shown that about 20% of particles ≥5µm are lost after being transported in 3 metres of tubing (7), and ASTM F50-12: 2015 (8) recommends that sampling tubes should be no longer than 3 metres. However, the ISO standard recommends that the tube should not be longer than 1 m. Losses of macroparticles can occur on bends when their inertia may throw them from air onto the inner surface of the tube. Inertial impaction can be reduced to an acceptable level by ensuring the bends in tubes are not too tight, and ASTM F50-12 (2015) recommends that the radius of curvature of the sampling tube should be no greater than 15 cm. Particles may be attracted to the tubing surface by electrostatic charge, and tubing made from a material that is a good conductor of electricity should be used. ‘Bev-A-Line XX’, stainless steel, or electrically conductive polyurethane, are examples of such materials. Within the cleanroom, when air is sampled directly into the air sampler, or if a sample probe with connecting tubing is utilised, the air intake should be at a height which is representative of the working environment (typically 1 m from the floor). At critical locations within UDAF, the sampling probe should be as close as possible to the identified location, and at similar height. The US FDA Guidance (9) suggests that all such sampling should be within 1 foot (approximately 30 cm) of the critical location.
Conclusions	1. For UDAF locations, isokinetic sampling probes to be utilised that are specific to the rate of sampling and the UDAF velocity, with the probe pointing into the direction of the airflow. 2. For non-UDAF locations, the probe to be directed vertically upwards and does not need to be isokinetic. 3. Connecting tubing from sampling point to particle sampler to be of minimal length (no more than 1 m), with no kinks, or bends of less than 15 cm radius, and made of a material that minimises electrostatic attraction. 4. For non-UDAF locations, the intake to the air sampler, or sampling tube, to be located at working height that is typically 1 m above the floor, and for critical locations, to be as close as possible to the sampling location (within 30 cm).

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9. Particle counter	
ISO 14644-1	"Light scattering (discrete) airborne particle counters (LSAPC) are commonly used when undertaking air cleanliness classification". The standard also states that for sampling macroparticles "The LSAPC should have a sample flow rate of at least 28.3 l/min". The particle counter "shall have a valid calibration certificate: the frequency and method of calibration should be based upon current accepted practice as specified in ISO 21501-4 (10). Some particle counters cannot be calibrated to all of the required tests in ISO 21501-4. If this is the case, record the decision to use the counter in the test report".
EU GGMP Annex 1	The expectation is that a fit-for-purpose and calibrated particle counter is utilised that can record the resulting particle concentrations with a high level of precision.
Discussion	A LSAPC that can measure and count individual particles for the chosen particle sizes of ≥0.5 µm and ≥5 µm (refer to Section 3 in this table) is appropriate. Cumulative and not discrete particle size sampling should be used. Because of their age, many particle counters cannot be calibrated according to the ISO 21501-4 standard. However, if the counter has been supplied by a well-known manufacturer, and calibrated by a competent body, it is reasonable to assume that it will be fit for purpose.
Conclusions	1. A LSAPC to be used to count and size cumulative particle sizes at ≥0.5 µm and ≥5 µm. 2. The instrument to be fit-for-purpose and calibrated by a competent body. If it cannot be calibrated as specified in ISO 21501-4, this should be evaluated and stated accordingly.

10. Interpretation of air sampling counts	
ISO 14644-1	"The cleanroom or clean zone is deemed to have met the specified air cleanliness classification requirements if the average of the particle concentrations (expressed as number of particles per cubic metre) measured at each of the sampling locations does not exceed the concentration limits determined from Table 1" [in the standard].
EU GGMP Annex 1	The air cleanliness classification requirements are met if all of the considered particle concentrations do not exceed the (per m^3) concentration limits for the ≥0.5 µm and ≥5 µm particle sizes shown in the table in Section 4 of Annex 1 of the EU GGMP.
Discussion	If several samples are taken from the same location within a section, the ISO standard states that the average concentration /m^3 needs to be determined. However, the GMP expectation is that if several samples are taken, the sample with the highest count is selected. The concentrations/m^3 should then be compared with the concentrations in Annex 1 to determine if the air cleanliness classification requirements have been met. For ≥5 µm concentrations measured within EU GGMP Grade A areas, where there is no corresponding concentration limit for an ISO 5 area, the classification can be reported using the 'M descriptor' notation.
Conclusions	1. Calculate the particle concentrations per m^3 at ≥0.5 µm and ≥5 µm, at each location. If several samples are taken from the same location, or at several locations within the same sampling section, select the sample with the highest recorded concentration. 2. Ensure that no concentration per m^3 exceeds the concentrations specified in the EU GGMP for ≥0.5 µm and ≥5 µm particle sizes, for the chosen cleanroom Grade.

11. Out-of-Specification result	
ISO 14644-1	"In the event of an out-of-specification count, an investigation shall be undertaken. The result of the investigation and remedial action shall be noted in the test report". The standard also states; "If an out-of-specification count is found at a location due to an identified abnormal occurrence, then that count can be discarded and noted as such on the test report and a new sample taken". Additionally, it is stated; "If an out-of-specification count found at a location is attributed to a technical failure of the cleanroom or equipment, then the cause should be identified, remedial action taken, and retesting performed of the failed sampling location, the immediate surrounding locations and any other locations affected. The choice shall be clearly documented and justified".
EU GGMP Annex 1	The expectation is that the cleanroom is satisfactorily classified with appropriate investigation and re-certification to address any out-of-specification results.
Discussion	In the event of an out-of-specification result, an investigation must be completed. This may involve identification of machines or personnel activities that are the source of the airborne particles, or an investigation of the ventilation system to ensure that it is able to provide the correct airborne particle concentration. Airflow visualisation (smoke) studies may be used to determine the effectiveness of the ventilation system at the out-of-specification location by determining the airflow direction and time taken for the smoke to clear. Information can also be gathered about the ventilation effectiveness by measuring the Performance Index and Air Change Effectiveness index at the out-of-specification location. Information on how this is done is described elsewhere (11) and is discussed in the second article (3). If actions taken to address the failure are relatively simple and do not impact on other areas of the cleanroom, retesting at the failed sampling location, the immediate surrounding locations and any other locations affected is appropriate. The chosen sampling locations should be justified and documented. If significant modifications to equipment, process or to the air supply and extract system are needed, classification of the whole cleanroom is likely to be required.
Conclusions	1. In the event of an out-of-specification result, an investigation to be completed, the cause identified, and the remedial actions taken to rectify the issue recorded. 2. If the remedial actions are relatively simple and do not impact on other areas of the cleanroom, retesting at the failed sampling location, the immediate surrounding locations, and any other locations affected is appropriate, and needs to be justified and documented. 3. If significant modifications to equipment, process, or the air supply and extract system are needed, classification of the whole cleanroom is likely to be required.

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12. Re-classification frequency	
ISO 14644-1 (and ISO 14644-2) (12)	"At-rest, or operational, classification may be performed periodically based upon risk assessment of the application, typically on an annual basis. Where the installation is equipped with instrumentation for continuous or frequent monitoring of air cleanliness by particle concentration and other parameters of performance, as applicable, the time intervals between classifications may be extended provided that the results of the monitoring remain within the specified limits".
EU GGMP Annex 1	The expectation is that appropriate monitoring and testing is carried out to ensure the cleanroom continues to maintain its classified status.
Discussion	The ISO standards suggest an annual re-classification but accepts that cleanrooms equipped with instrumentation for continuous or frequent monitoring of test parameters, may have the maximum time interval between re-classification extended. A schedule for periodic testing that includes re-classification ('airborne particle concentration' test) and other test methods is included in BS EN ISO 14644-2:2015 (12) (National Annex section). It should be noted that this information is only given in the BS EN ISO 14644-2:2015. This information recommends a maximum time interval for cleanrooms that carry out periodic testing of 6 months for ≤ ISO 5 areas and 12 months for > ISO 5 areas. However, this schedule applies to testing of a periodic nature but a typical pharmaceutical cleanroom is likely to monitor the following parameters to provide an indication of the ongoing state of control; a. non-viable particles, likely to be measured continuously in Grade A and B areas, and periodically in other areas b. microbiological contamination, throughout manufacture (and during periods when there is no manufacture) c. pressure differentials, continuously d. air supply velocity (UDAF), continuously e. air volume supply (non-UDAF), continuously Additionally, periodic cleanroom testing may be also completed to provide further information regarding the state of airborne contamination control. Typical tests that may be carried out are as follows; f. air supply filter integrity g. air supply velocities at several locations across the filter face (UDAF) h. air volume supply at each air inlet (non-UDAF) i. airflow visualisation (UDAF) j. air volume extract rate (or velocity measurement) The frequency of any re-classification should be determined by assessment of the extent of the monitoring and periodic testing activities listed above. Where these are comprehensive and full environmental control is maintained and demonstrated, re-classification may only be required when there are modifications to the facility, air conditioning system, or to cleanroom activities and room occupancies.
Conclusions	1. The frequency of re-classification of the cleanroom to be determined by assessment of the extent of the monitoring and periodic testing. 2. Re-classification is likely to be required when there are significant modifications to the cleanroom, air conditioning system, or changes to cleanroom activities and occupancy numbers.

4. Discussion and conclusions

Classification is an essential part of the cleanroom qualification activities in pharmaceutical cleanrooms to provide information regarding appropriate control of airborne contamination. A review of the classification requirements and principles associated with Annex 1 of the EU GGMP (2008) and ISO 14644-1 (2015) has been discussed in this paper as well as some more recent regulatory authority expectations. With an understanding and interpretation of these requirements, and the cleanroom operational activities and type of airflow utilised to achieve control of airborne contamination, the correct approach for pharmaceutical cleanrooms classification has been derived. If this approach is followed, it will provide meaningful reference information regarding the effectiveness of the cleanroom's airborne contamination control system under worst case operational conditions. This information will also provide essential reference data should the cleanroom or ventilation system be modified, or the manufacturing activities changed, and to confirm the effectiveness of the airborne contamination control system relative to the original state.

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Appendix A: ISO 14644-1 and EU GGMP Annex 1 room occupancy definitions

Table A1 Cleanroom occupancy definitions given by ISO 14644-1:2015 and EU GGMP Annex 1 (2008)

Document reference	Occupancy state		
	As-Built	At-Rest	Operational [a]
EU GGMP Annex 1: 2008	No definition included.	The condition where the installation is installed and operating, complete with production equipment but with no operating personnel present.	The condition where the installation is functioning in the defined operating mode with the specified number of personnel working.
ISO14644-1: 2015	The condition where the cleanroom or clean zone is complete with all services connected and functioning but with no equipment, furniture, materials or personnel present.	The condition where the cleanroom or clean zone is complete with equipment installed and operating in a manner agreed upon, but with no personnel present.	The agreed condition where the cleanroom or clean zone is functioning in the specified manner, with equipment operating and with the specified number of personnel present.

Note:
a. Annex 1 of the EU GGMP refers to 'in operation' and the ISO 14644-1 standard refers to 'operational' but these two terms are considered to be equivalent.

Appendix B: ISO 14644-1:2015 and EU GGMP Annex 1 (2008) airborne cleanliness concentrations

Shown in Table B1 are the airborne cleanliness concentrations for particles ≥0.5 µm and ≥5 µm given in ISO 14644-1 and the EU GGMP Annex 1 (2008). It should be noted that ISO standard 14644-1 allows airborne classification in three occupancy states and the associated occupancy state must be stated. Annex 1 of the EU GGMP only consider two occupational states. Shown in the table are the ISO 14644-1 concentrations considered to correspond with the EU GGMP Annex 1 concentrations for the 'at rest' and 'in operation' occupancy states.

Table B1 Comparative airborne cleanliness concentrations of ≥0.5 µm and ≥5 µm particle sizes given in ISO 14644-1: 2015 and EU GGMP Annex 1 (2008).

Document reference	Classification designation	Maximum permitted number of particles per m^3 equal to or greater than the tabulated size			
		At Rest		In Operation	
		≥0.5µm	≥5µm	≥0.5µm	≥5µm
EU GGMP, Annex 1	Grade A	3 520	20	3 520	20
ISO 14644-1	ISO 5	3 520	[a]	3 520	[a]
EU GGMP Annex 1	Grade B	3 520	29	352 000	2 900
ISO 14644-1	ISO 5	3 520	[a]	-	-
	ISO 7	-	-	352 000	2 930
EU GGMP Annex 1	Grade C	352 000	2 900	3 520 000	29 000
ISO 14644-1	ISO 7 [b]	352 000	2 930	-	-
	ISO 8 [b]	3 520 000	29 300	3 520 000	29 300
EU GGMP Annex 1	Grade D	3 520 000	29 000	Not defined[b]	Not defined[b]
ISO 14644-1	ISO 8	3 520 000	29 300	-	-

Notes:
a. Sample collection limitations for both sizes of particles in low concentrations and sizes greater than 1 µm make classification at this particle size inappropriate, due to potential particle losses in the system.
b. The 'in operation' concentrations for EU GGMP Grade D areas are not defined, and the user is expected to set their own limits. As the 'at rest' limits are typically easily attainable for 'in operation' conditions, the 'at rest' limits are often also applied to the 'in operation' state.

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Appendix C: Table in ISO 14644-1:2015 used to obtain minimum number of samples in a cleanroom

Table C1 Number of sampling locations required according to the size of the cleanroom given in ISO 14644-1.

Area of cleanroom (m^2) less than or equal to	Minimum number of sampling locations to be tested (N_L)
2	1
4	2
6	3
8	4
10	5
24	6
28	7
32	8
36	9
52	10
56	11
64	12
68	13
72	14
76	15
104	16
108	17
116	18
148	19
156	20
192	21
232	22
276	23
352	24
436	25
636	26
1000	27
>1 000	See Note 3

Note 1 If the considered area falls between two values in the table, the greater of the two should be selected.

Note 2 In the case of unidirectional airflow, the area may be considered as the cross section of the moving air perpendicular to the direction of the airflow. In all other cases the area may be considered as the horizontal plan area of the cleanroom or clean zone.

Note 3 When the area of the cleanroom or clean zone is greater than $1000m^2$, apply the following formula to determine the minimum number of locations required:

$$N_L = 27 \times \left[\frac{A}{1000}\right]$$

Where N_L is the minimum number of sampling locations to be evaluated, rounded up to the next whole number, and A is the area of the cleanroom in m^2.

Appendix D: Calculation of number of air sampling locations required for cleanroom classification

To classify a cleanroom according to ISO 14644-1:2015, airborne particle sampling must be carried out across the cleanroom. The number of sampling locations is related to the surface area of the floor and given in Table A1 of ISO 14644-1 (reproduced in Appendix C of this article). ISO 14644-1 suggests that the floor area should be divided into equal sized areas where sampling is carried out. This is relatively simple to achieve in a cleanroom that is square or rectangle but difficult where the floor area is asymmetrical. However, ISO allows additional sections to be added to facilitate subdivision into equal sections. The ISO standard does not give information about how this sub-division can be carried out but the following method can be used.

1. Divide the asymmetric floor or base area of the cleanroom or clean zone into suitable sizes of rectangular sub-area. Start with the largest rectangle that can be accommodated and work towards the smallest.

2. Add together the floor surface areas (m^2) of the sub-areas to obtain the total floor area of the cleanroom.

3. Calculate the number of sampling sections in each sub-area using the following equation;

$$\text{Number sections in sub-area} = \frac{\text{floor area of sub-area}}{\text{total floor area}} \times \text{minimum no. sampling locations}$$

Where, the 'minimum no. of sampling locations' is equal to the number of equal-sized sampling sections required for total floor area that is obtained from Table A1 of ISO 14644-1 (reproduced in Appendix C of this article).

These results should be rounded up to whole numbers (any number less than 1 should be assumed to be 1). Ensure that the total of these results is greater that the number required by ISO 14644-1.

4. Starting with the largest sub-area, divide its cleanroom floor area by the number of sampling sections required in its area. This will give the surface area of the sampling section and, taking account of the sampling requirements, the length and width of the sampling sections should be decided.

5. An example of the above method is given in the second part of this article[3].

Using the formal risk assessment methods discussed in Appendix E of this article, the sampling locations within each section can be identified, and particle sampling carried out.

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Appendix E: Use of risk assessment to select sampling locations

Annex 1 of the EU GGMP (2008) requires the classification of a cleanroom or clean zone to be carried out according to the method given in ISO 14644-1. ISO 14644-1: 2015 recommends that the floor area of a cleanroom or clean zone is divided into sections, and airborne sampling carried out at locations representative of the conditions in the sections. However, the current expectations of the regulatory authorities is for sampling to be carried out where the risks from airborne contamination are highest. In clean air devices (EU GGMP Grade A, hereafter referred to as workstations), the chosen locations should be in proximity to critical surfaces, such as where product, components or product contacting surfaces, are exposed to airborne contamination. In cleanrooms that contain the clean air device, the sampling positions should be where the highest particle concentrations associated with personnel activity are located. In cleanrooms outside the aseptic processing room, the sampling should be carried out in representative locations without the need for a risk assessment.

Selection of sampling locations by risk assessment in critical workstation
Information about various risk assessment methods used in cleanrooms is available elsewhere (13). It is explained that the level of risk from contamination can be calculated by the following equation:

Risk = Severity x Occurrence Equation E.1

Severity; The importance, or seriousness, of an event. In the situation where the level of risk of airborne contamination to vulnerable surfaces, such as product, is required, the risk can be calculated by use of the following risk factors.

1. The likely particle airborne concentrations at a critical surface; However, this will not be known, but descriptors can be used as surrogates for the airborne concentration. These descriptors are (a) personal activity and, therefore, the dispersion rate of particles, and (b) the effectiveness of the ventilation system in reducing the airborne particle concentration.

2. The surface area of the critical surface that is exposed to airborne deposition.

It should be noted that only the airborne contamination dispersed from personnel is considered in this risk assessment, and not that from machinery or equipment. This will simplify the risk assessment and is consistent with the demonstrated fact (14) (15) that the most important contaminant in pharmaceutical cleanrooms is microbial, and not small inert particles. However, large sources of particles emitted from machines may influence the actual particle concentrations measured during classification and this should be considered when the sampling results are collected.

Occurrence; The frequency that the event occurs. In the case of airborne contamination, it is the time that the critical surface, such as product or product contacting surface, is exposed to airborne contamination.

An example of the descriptors of the risk factors, and the risk scores that can be assigned to the descriptors when assessing the risk of contamination, are given in Table E1.

Table E.1 Risk factors and scoring system for a risk assessment for critical workstation.

Severity					Occurrence
Personnel activity	**Score**	**Ventilation type**	**Score**	**Surface exposed**	**Time exposed**
Some activity	Proportion of manipulations e.g. manipulations for 50% of the time gives a score of 1.5	Open operation RABS or open access UDAF workstation	2	Area (cm^2)	Time (mins)
Continuous activity	2	Non-UDAF cleanroom	3	Area (cm^2)	Time (mins)

The level of risk at each location can then be obtained by the equation E.1;

Risk = Severity x Occurrence

= (Personnel activity score x Ventilation type score x Surface exposed) x Time exposed

An example of the use of this method is given in Appendix B in the second article (3).

Selection of sampling locations in the cleanroom containing a workstation
For cleanrooms in which the clean air device is located and, therefore, critical surfaces are not directly exposed to airborne contamination, the sampling locations should be where the highest airborne concentrations of particles are found. As discussed previously, the emission of particles from machinery and equipment is likely to affect the particle concentration but only personnel need to be considered. An example of the use of this method is given in Appendix A in the second article.

Cleanrooms adjacent to the aseptic processing room
For lower Grades of cleanrooms that are adjacent to the aseptic processing room, samples should be taken at locations that are representative of conditions in each of the sections obtained by the procedure discussed in Appendix D. There is no need for a risk assessment.

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