EJPPS – European Journal of Parenteral and Pharmaceutical Sciences Volume 27 Issue 4

https://www.ejpps.online/post/vol27-4-eu-ggmp-annex-1-2022-and-pharmaceutical-cleanroom-classification-consideration

https://doi.org/10.37521/ejpps.27402

EU GGMP Annex 1 2022 and Pharmaceutical Cleanroom Classification - Consideration of the changes from EU GGMP Annex 1 2008

Corresponding Author: Tim Eaton, Sterile Manufacturing Specialist

AstraZeneca,
UK Operations,
Silk Road Business Park,
Macclesfield
Cheshire. SK10 2NA
England

N D Lenegan Energy & Carbon Reduction Solutions Ltd, Manchester, UK

Email: tim.eaton@astrazeneca.com

Telephone: +44(0) 1625 514916

EU GGMP Annex 1 2022 and Pharmaceutical Cleanroom Classification - Consideration of the changes from EU GGMP Annex 1 2008.

T Eaton[1] , N D Lenegan [2]

[1] AstraZeneca, Macclesfield, UK
[2] Energy & Carbon Reduction Solutions Ltd, Manchester, UK

Summary

Classification is an essential part of the qualification activities for pharmaceutical cleanrooms to confirm the effectiveness of the airborne contamination control system. A review of the classification requirements and principles associated with ISO 14644-1:2015 and Annex 1 2008 of the European Union Guide to Good Manufacturing Practice (GGMP) has previously been reported. As this version has now been superseded by the 2022 edition, review of the relevant updates has been completed to assess the impact of the classification process for a cleanroom used for aseptic processing. With some additions and text updates, many of the requirements and expectations for classification can be considered to remain effectively unchanged from the 2008 version. However, there are items of more significant change for consideration and this article summarises those elements that have minimal impact and focuses upon the significant changes and provides recommendations, and options, to ensure continued meaningful classification.

Key words: Cleanroom classification, ISO 14644-1, EU GGMP Annex 1 2022

1. Introduction

All cleanrooms are classified according to ISO 14644-1 [1] to demonstrate that a specified concentration of airborne particles is not exceeded. For the manufacture of sterile medicinal products, Annex 1 of EU GGMP [2] specifies the required environmental airborne particle concentrations, and for classification, references ISO 14644-1. For meaningful classification, the correct interpretation and application of the information given in both ISO 14644-1 and Annex 1 of the EU GGMP is required and, with reference to the 2008 version of Annex 1, this has been previously published [3,4]. However, with the introduction of the 2022 edition of the Annex (published 22.08.22), the identification and assessment of the amendments relating to cleanroom classification are required to ensure meaningful classification is continued. A summary of the approaches to classification that had previously been completed and are considered to remain effectively unchanged are included in this article. Changes relating to the optional inclusion of the ≥5 µm particle size for Grade A ('at rest' and 'in operation') and Grade B ('at rest') areas and increases in the associated airborne concentration limits at this particle size require assessment and they also have impact to the minimum required sampling volumes. The inclusion of a statement for the requirement for periodic re-classification may also impact the testing and all of these aspects are collectively considered for the classification of an EU GGMP cleanroom used for aseptic processing. With some further considerations, this approach can be applied to most pharmaceutical and healthcare cleanrooms.

2. Annex 1 2022 and 2008, for classification of an EU GGMP cleanroom in accordance with ISO 14644-1: 2015

In addition to confirming the effectiveness of the cleanroom's airborne contamination control system, cleanroom classification should provide meaningful baseline information and data that can be referenced 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.
It should be noted that Annex 1 of the EU GGMP (2022) refers to cleanrooms and now clean air equipment (previously referred to as clean air devices), which typically still includes unidirectional airflow units (UDAFs), RABS (restricted access barrier systems) and isolators that the 2008 version referred to. When utilised for critical activities, they are required to meet the contamination control requirements of an EU GGMP Grade A environment. For simplicity, this article mainly refers to 'cleanrooms', where the term covers both cleanrooms and clean air equipment.

3. No significant changes, Annex 1 2022

Summarised in table 1 are the recommendations for cleanroom classification, that are considered to remain effectively unchanged in the 2022 Annex 1, with the same intent as the 2008 version, although there are additions and changes to some of the text.

Table 1 Recommendations for cleanroom classification considered to be unchanged, 2022 Annex 1 compared with Annex 1 2008

Item	Continued Recommendations
a. Facility installation status	All installation testing to be fully completed with the air conditioning system to be operating in the defined manner to provide the specified level of airborne contamination control. The in-situ integrity testing of all cleanroom terminal air supply filters to be satisfactorily completed.
b. Occupancy state	Classification of a cleanroom to be carried out firstly in the 'at rest' state and then the 'in operation' state. The 'in operation' testing provides the most useful information, but the 'at rest' classification requires a similar approach. The 'in operation' classification state relates to the actual manufacturing process, under worst-case (typically simulated and not actual manufacture) operational conditions that includes the maximum cleanroom personnel occupancy number, with all personnel wearing the designated cleanroom garments.
c. Number of sampling locations	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 (workstation base) area sections where at least one air sample is taken in each section (refer to section d below - Where to sample in each section).
d. Where to sample in each section	For 'in operation' testing within critical and background to critical locations, an understanding of the process and operations to be performed are utilised to provide a documented risk assessment to establish where air sampling should be carried out. This recommendation is now formerly included in the 2022 Annex 1 guide. 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 to ensure all critical positions are included. For 'at rest' testing and for cleanrooms outside the aseptic processing room, sampling within the centre of each section is typically appropriate. It is not appropriate to sample directly below a non-diffused air supply source where the airborne particle concentrations are likely to be unrepresentatively low.
e. Particle counter	A light scattering (discrete) airborne particle counter (LSAPC) to be used which can count and size cumulative particles ≥0.5 µm and ≥5 µm. The instrument to be fit-for-purpose and calibrated by a competent body. If it cannot be calibrated as specified in ISO 21501-4 [5] and it is to be utilised, the rationale to support the use to be clearly recorded.
f. Sampling probe and tubing	Connecting tubing from sampling point to particle sampler to be of minimal length but no more than 1 m, with no kinks, minimal number of bends and no bends of less than 15 cm radius, and made of a material that minimises electrostatic attraction. A maximum tube length of 1 m, for monitoring, is now specified in the 2022 Annex 1 guide. 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 to ensure that samples are representative. For unidirectional airflow (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. For non-UDAF locations, the probe to be directed vertically upwards and does not need to be isokinetic, but an isokinetic sampling head can be utilised if preferred.
g. Interpretation	The measured particle concentrations are normalised to present concentrations per m^3 for each sample. If several samples are taken from the same location, or

of air sampling counts	at several locations within the same sampling section, the sample with the highest recorded concentration is selected. It must be then ensured that no per m^3 concentration exceeds the concentrations specified in the EU GGMP for the chosen Grade of cleanroom.
h. Out-of-Specification result	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. 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 is to be justified and documented. 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.

4. Significant changes for consideration, Annex 1 2022

Table 2 summarises the significant changes in the wording and additions associated with the classification requirements for Annex 1 2022 compared with the 2008 edition and also includes the relevant principles contained in ISO 14644-1: 2015, along with information on the expectations of the regulators. This provides an assessment of the updates and includes recommendations for the classification testing for aseptic manufacturing cleanrooms and complements the approach that has been previously published [3,4]. The changes included in table 2 affect each other and are assessed with consideration for these influences. A convenient and simplified summary of the overall approach to classification that collectively considers these changes and provides various classification options, is shown in table A1 in Appendix A.

Table 2 Classification considerations and derived rationale for pharmaceutical cleanrooms

1. Particle sizes to sample	
ISO 14644-1	This standard is applicable to all cleanrooms used for many applications, including pharmaceutical, and as such states; '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 2008	Airborne particle concentrations for particles at both ≥0.5 µm and ≥5 µm sizes for each Grade of cleanroom were given in the table shown in Section 4 of this version of the guide. These limits, for both of these particle sizes, were applicable for classification and for monitoring.
EU GGMP Annex 1 2022	Two tables, one for classification (table 1, section 4) and one for monitoring (table 5, section 9), are included in the guide. Both tables include the maximum permitted airborne particle concentrations for particles ≥0.5 µm and ≥5 µm. The limits are the same in each table except that for classification, the limits for particles ≥5 µm for Grade A areas ('at rest' and 'in operation') and Grade B areas ('at rest') are 'not specified' but 'can be considered where indicated by the CCS [contamination control strategy] or historical trends'. Tables 1 and 5 of the Annex are shown in Appendix B as tables B1 and B2 respectively.
Discussion	Annex 1 requires monitoring of airborne particles at both ≥0.5 µm and ≥5 µm sizes for all Grade areas, but for classification, limits are not specified for Grade A areas ('at rest' and 'in operation') and Grade B areas ('at rest') for particles ≥5 µm. This is unusual and contrary to Grade B ('at rest') and Grade C and D ('at rest' and 'in operation' - if specified for Grade D) cleanrooms where both sizes of particles are required to be sampled for classification and also for monitoring. The reason for this relates to the associated calculation of the required minimum sampling volume, using Formula A.2 in ISO 14644-1. Removal of the ≥5 µm particle size results in a smaller required minimum sample volume, and hence sampling time, with the expectation of the regulators that an increased number of samples could be taken to provide a more comprehensive understanding of the control throughout the entire area. This is explained further in section 2 (Sampling volumes and sampling times) of this table. Review of the ratios relating ≥0.5 µm to ≥5 µm particle concentrations found at AstraZeneca, Macclesfield (UK) [6] 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 [7,8] and contrast with the ratios stated in Annex 1 2022, which is 121:1 for the same Grade areas. 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 and classification is therefore much more likely to fail at the ≥5 µm particle size. Additionally, the data may also be used to derive initial alert values that are subsequently to be used during monitoring and so overall, initial classification at both the ≥0.5 µm and ≥5 µm particle sizes is recommended. For re-classification, if it has been demonstrated that control has been maintained, no significant changes implemented, and the correlation of ≥5 µm with ≥0.5 µm particles is understood, it may be appropriate to perform sampling only at the ≥0.5 µm particle size, for Grade A ('at rest' and 'in operation') and for Grade B ('at rest') areas. This is consistent with the Annex 1 approach and should be evaluated

	in conjunction with the sampling volume to be utilised, (discussed in the section 2 of this table), as the size of the particle to be sampled is a consideration for the determination of the required minimum sample volume and if ≥5 µm particles are not included, with the exception of Grade A areas, the determined minimum sample volume is reduced. It should also be noted that these two particle sizes satisfy the ISO standard requirement that, when more than one particle size is utilised, the larger particle diameter is more than 1.5 times the size of the smaller particle diameter.
Conclusion	1. For initial classification, or classification following significant change, it is recommended that both ≥0.5 µm and ≥5 µm particle sizes are considered for all Grades of cleanroom, including Grade A ('at rest' and 'in operation') and also Grade B ('at rest'). 2. For re-classification, if it has been demonstrated that control has been maintained, assessment has confirmed that there have been no significant changes and the correlation of ≥5 µm particles with ≥0.5 µm particles is understood, it may be appropriate to perform sampling only at the ≥0.5 µm particle size, for Grade A ('at rest' and 'in operation') and also for Grade B ('at rest') areas. This should however be evaluated in conjunction with the required minimum sample volume, which is determined from consideration of the particle sizes to be sampled. (Refer to section 2 in this table for sampling volume considerations). 3. For re-classification, for Grade B ('in operation') and Grade C and D cleanrooms (both occupancy states), both ≥0.5 µm and ≥5 µm particle sizes to be included.
2. 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 is the minimum air volume to be sampled to ensure a count of ≥ 20 particles. Formula A.2 is as follows; $V_s = (20/C_{n,m}) \times 1000$ $C_{n,m}$ = Class limit for largest particle size considered V_s = Minimum sample volume (liters) 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 that for the measurement of macroparticles [particles ≥5 µm] the sampler should have a sample flow rate of 'at least 28.3 l/min'.
EU GGMP Annex 1 2008	It was stated; '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 also stated; 'For classification purposes in Grade A zones, a minimum sample volume of $1m^3$ should be taken per sample location'.
EU GGMP Annex 1 2022	The guide states; 'For classification of the cleanroom, the minimum number of sampling locations and their positioning can be found in ISO 14644 Part 1' but there is no reference to this standard to determine the sample size. For Grade A zones, the minimum sample volume of $1m^3$, stated in the 2008 version, has been removed. It also however states; 'Reference for the qualification [of which classification is a part] of the cleanrooms and clean air equipment can be found in the ISO 14644 series of standards'. For monitoring, the guide states; '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 ($1ft^3$) per minute).'

Discussion	For the collection of particles ≥5 µm, a particle counter with a minimum sampling rate of 28.3 l/min is required by the ISO standard and Annex 1 states that this same minimum sampling rate is to be utilised for monitoring. As it is recommended that both ≥0.5 µm and ≥5 µm particle sizes are sampled for classification, the largest considered particle size (≥5 µm) is utilised for calculating the sampling volume by use of Formula A.2 in the ISO standard. These calculated volumes are shown in table C1 in Appendix C for each Grade of cleanroom where the limits for particles ≥5 µm applied for monitoring are shown where not included in Annex 1 for classification testing. For EU GGMP cleanrooms required to meet Grade B ('in operation'), Grade C ('at rest' and 'in operation') and Grade D ('at rest' and, where limits have been defined, for 'in operation'), this calculated sample volume is less than the required minimum sample volume of 2 l stated in the ISO standard. This in turn will be less than the volume associated with a minimum sampling time of 1 minute that is also stated in the standard, which, for the required minimum sampling flow rate (28.3 l/min), will be 28.3 l. However, for Grade A ('at rest' and 'in operation') and Grade B ('at rest') cleanrooms, a sample volume of 690 l (0.69 m^3) is determined using Formula A.2. when considering particles ≥5 µm. This volume is more than both the 2 l minimum sample volume stated in the ISO standard and also that associated with a minimum sample time of 1 minute, even for an air sampler with a sampling rate as high as 100 l/min. The reason for the exclusion in Annex 1 of the ≥5 µm particle concentrations for these two Grades (and states) of cleanroom was to reduce the minimum required sample volume, which when calculated from the ISO equation by consideration of only ≥0.5 µm particles, is extremely small and less than that relating to a 1 minute sample time. With this reduced sampling time, the expectation is that an increased number of samples could be taken to provide a more comprehensive understanding of the control throughout the entire area. Although there is no specific reference to ISO 14644-1 in Annex 1 for determining the sample volume, it does however refer to the 'ISO 14644 series of standards' for qualification and it is therefore reasonable to assume that the ISO 14644-1 Formula A.2 is to utilised. For 'in operation' sampling, the expectation is that the sampling time must be sufficient to ensure that all particle-generating activities are captured or several, same volume samples, are to be taken. Grade A areas require the most stringent control and typically utilise UDAF, and with this, the airborne particle concentrations are more localised and more likely to vary throughout the zone, compared to non-UDAF areas. As these Grade A areas are the most important, where the greatest risk of product or process contamination occurs, a minimum sample volume of 1 m^3 is recommended for initial classification or following any significant changes. This requires sampling times of 36 minutes, 20 minutes or 10 minutes for sampling rates of 28.3 l/ min, 50 l/min and 100 l/min respectively and should allow capture of all particle-generating events. If the qualified status of the area has been maintained and there have been no changes, a reduced sampling volume of 0.69 m^3 may be a consideration for re-classification activities when measurement of the ≥5 µm particle size is retained. If the correlation of ≥5 µm with ≥0.5 µm particles is understood then the ≥5 µm size may not to be sampled for re-classification. A reduced sample volume relating to a 1 minute sampling time may then be considered, but the worst case particle generating activities would need to be captured and so more than one sample may be required, with all samples being the same volume. Background to Grade A environments, such as L-UDAF zones,

	that meet Grade A airborne cleanliness levels, should be similarly classified although an initial sampling volume of 0.69 m^3 may be appropriate with consideration for the reduced risk of product contamination. Grade B areas are considered by Annex 1 to be the 'background environment' to Grade A ('where it is not an isolator') and are likely to have a significant influence to the control of contamination in the Grade A area and typically utilise non-UDAF that is reasonably well mixed and less localised than UDAF. With consideration for included ≥5 µm particles, the minimum 'at rest' sample volume is calculated to be 0.69 m^3, whereas for the 'in operation' occupancy state, the volume relates to a 1 minute sample, which, even for a 100 l/min sampler, is significantly less than this. This is inappropriate as the 'in operation' testing provides the most useful information to confirm control during manufacturing activities and should not have a lesser sample volume than used for the 'at rest' testing. Therefore, for initial classification, a minimum volume of 0.69 m^3 is recommended, for both 'at rest' and 'in operation' testing. For re-classification activities, as before, if the correlation of particles ≥5 µm with ≥0.5 µm particles is understood, then the ≥5 µm size may not to be sampled and a reduced sample volume relating to a 1 minute sampling time may be considered. If it was assessed to complete only worst case 'in operation' testing for re-classification, where there is a higher limit for ≥5 µm particles, this issue of the larger sampling volume for Grade B cleanrooms 'at rest' is avoided and both ≥5 µm and ≥0.5 µm particles can be sampled with a reduced volume relating only to a 1 minute sampling time although more than one sample may be required to capture all particle generating events. For Grade C and D cleanrooms, these are typically support areas and utilise non-UDAF and for both the 'at rest' and 'in operation (where limits are specified for Grade D)' classification, the calculated sample volume is less than the minimum sampling time of 1 minute and this is likely to be appropriate. If a 1-minute sample is not of sufficient duration to capture all particle-generating activities, then more than one sample may be required but each sample should have the same volume.
Conclusions	1. All particle counters to have a minimum flow rate of 28.3 l/min 2. For Grade A zones, an appropriate sampling volume for initial 'at rest' and 'in operation' classification is recommended to be 1 m^3. This meets the minimum volume (0.69 m^3) based on sampling particles ≥5 µm and is also likely to capture all particle generating events in a single sample. For re-classification, if it has been demonstrated that control has been maintained and assessment has confirmed that there have been no significant changes, a reduced volume of 0.69 m^3 is recommended. If however, the correlation of ≥5 µm particles with ≥0.5 µm particles is also understood, it may be appropriate to perform sampling only at the ≥0.5 µm particle size, with a minimum 1 minute sample time but more than one sample is likely to be required to capture all particle-generating activities (each sample to be the same volume). 2. For background environments that meet Grade A airborne cleanliness levels, a reduced sampling volume for initial 'at rest' and 'in operation' classification of 0.69 m^3 is recommended. For reclassification, the same approach utilised for Grade A areas should be considered. 3. For Grade B cleanrooms, a sampling volume for initial 'at rest' and 'in operation' classification of 0.69 m^3 is recommended. This meets the minimum volume based on sampling particles ≥5 µm and is also likely to capture all particle generating events in a single sample. For re-classification, if it has been demonstrated that

	control has been maintained, assessment has confirmed that there have been no significant changes and the correlation of ≥5 µm particles with ≥0.5 µm particles is understood, it may be appropriate to perform sampling only at the ≥0.5 µm particle size, with a minimum 1 minute sample time but more than one sample may be required to capture all particle-generating activities (each sample to be the same volume). This 1 minute sampling time would also be a similar consideration if only the worst case 'in operation' occupancy state, where there is a higher limit for ≥5 µm particles, is assessed to be appropriate for re-classification. 4. For Grade Cand D cleanrooms, a minimum 1 minute sample time is appropriate but more than one sample may be required to capture all particle-generating activities (each sample to be the same volume).
3. 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 2008	The maximum permitted airborne particle concentrations for each Grade of cleanroom were given in the table shown in Section 4 of this version of the guide. These concentration limits, for particles ≥0.5 µm and ≥5 µm, were applicable for classification and monitoring.
EU GGMP Annex 1 2022	For classification and monitoring, the maximum permitted airborne particle concentrations at the ≥0.5 µm size remain unchanged from the 2008 edition but for the ≥5 µm size, the limits have been increased for all Grade areas. However, for classification, limits are not specified for ≥5 µm particles for Grade A ('at rest' and 'in operation') and Grade B ('at rest') areas. The concentration tables for classification and monitoring are included in Appendix B as tables B1 and B2 respectively.
Discussion	With the increase to the limits for concentrations of ≥5 µm particles associated with the 2022 EU GGMP, the limits for both ≥0.5 µm and ≥5 µm particle sizes are now fully aligned with the limits in the ISO standard. Consistent with the recommendation of this article that both ≥0.5 µm and ≥5 µm particle sizes should be included for EU cleanroom classification, the 'in operation' Annex 1 concentration limits for monitoring, for Grades A, B and C areas, now correspond exactly with the ISO standard Class numbers 5, 7 and 8, respectively. These are shown in table D1 in Appendix D for the Annex 1 monitoring concentrations which also includes 'at rest' limits and the corresponding ISO standard Class numbers. 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. 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. However, as the EU GGMP concentrations are the prime reference for pharmaceutical cleanrooms, compliance with the relevant Grade of the EU Annex 1 guide should simply be stated.
Conclusion	1. The particle concentration limits for monitoring that are defined in table 5 in Annex 1 of the GGMP, for ≥0.5 µm particles and ≥5 µm particles (where utilised), to be applied.
4. Re-classification occupancy state and frequency	
ISO 14644-1 (and ISO 14644-2 [9])	'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 2008	The expectation was that appropriate monitoring and testing is carried out to ensure the cleanroom continues to maintain its classified status. There was no reference to the occupancy state for any re-classification activities
EU GGMP Annex 1 2022	Periodic requalification is stated and cleanroom classification (total particle concentration) is included as one of the tests to be completed. 'The maximum time interval for requalification of grade A & B areas, is 6 months. The maximum time interval for requalification of grade C & D areas, is 12 months'. There is no specific reference to the occupancy state to be included for any re-classification. The Annex also states; 'Processes, equipment, facilities and manufacturing activities should be managed in accordance with QRM [Quality Risk Management] principles to provide a proactive means of identifying, scientifically evaluating and controlling potential risks to quality. Where alternative approaches are used, these should be supported by appropriate rationale, risk assessment and mitigation, and should meet the intent of this Annex'.
Discussion	Cleanroom re-classification is likely to be required when there are significant modifications to the cleanroom, air conditioning system, or changes to cleanroom activities, occupancy numbers and the type of garments worn. For routine operations, Annex 1 states maximum time intervals of 6 months for Grade A and B areas and 12 months for C and D areas. The ISO standards has similar time intervals for 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 [9] (National Annex section). This information recommends a maximum time interval for cleanrooms that carry out periodic testing of 6 months for $\leq$ ISO 5 areas and 12 months for > ISO 5 areas. However, this schedule includes facilities with limited monitoring programmes whereas a typical pharmaceutical cleanroom is likely to provide comprehensively monitoring of the following parameters to provide an indication of the ongoing state of control; a. total airborne 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 rate, or air changes per hour (non-UDAF), continuously Additionally, periodic cleanroom testing may be also be 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 rate at each air inlet (non-UDAF) i. airflow visualisation (UDAF) j. air volume extract rate Consistent with the recommendations of Annex 1 to proactively engage with the principles of QRM, the frequency of any re-classification should be determined by

	consideration of the extent of the monitoring and periodic testing activities. This should be supported by appropriate risk assessment, scientific rationale, and adequate mitigations to control potential product airborne contamination. Where controls 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. In cases where monitoring and periodic testing is less comprehensive, re-classification at the intervals defined in the Annex 1 guide may be more appropriate. The ISO standard suggests that 'at-rest', or 'operational' (considered to be equivalent to Annex 1 'in operation' status) occupancy be utilised for re-classification. The Annex 1 guide does not suggest any occupancy state and testing may include both 'at rest' and 'in operation' states. However, if control has been maintained and demonstrated, risk assessment may determine that only the worst case 'in operation' testing is completed as this would provide the most meaningful information that relates to the actual cleanroom processing activities and the effectiveness of the airborne contamination control system. If this approach is utilised, then for Grade B areas, the greater required sampling volume associated with the 'at rest' occupancy is avoided and the minimum sampling volume will relate to a 1 minute sampling time.
Conclusions	1. Re-classification is likely to be required when there are significant modifications to the cleanroom, air conditioning system, or changes to cleanroom activities, occupancy numbers and the type of garments worn. 2. The frequency of re-classification of the cleanroom is defined in the Annex 1 guide as 6 monthly for Grade A and B areas and 12 monthly for C and D areas. Assessment of the extent of the cleanroom monitoring, periodic testing and the overall effectiveness of the contamination controls is recommended be to determine if these frequencies are suitable or if alternative, scientifically valid, frequencies can be utilised. 3. Re-classification may be completed in both, or either, of the 'at rest' or 'in operation' occupancy states. If the worst case 'in operation' occupancy only is utilised, then for Grade B areas, the larger minimum required sampling volume associated with the 'at rest' occupancy is avoided and a minimum 1 minute sampling time can be considered.

5. Discussion and Conclusions

With the publication of the 2022 EU GGMP Annex 1, the changes relating to cleanroom classification, relative to the succeeded 2008 version, have been considered and assessed. Many of the requirements and expectations can be considered to remain effectively unchanged but with some minor additions and text changes. However, more significant are changes relating to the optional inclusion of the ≥5 µm particle size for Grade A ('at rest' and 'in operation') and Grade B ('in operation') areas and increases in the associated concentration limits, the minimum sampling volumes and statement for periodic re-classification. These aspects are interrelated and require careful consideration to ensure meaningful classification is completed.

With reference to these changes, and the unchanged requirements that are included in this article and detailed in previous publications [3,4], an appropriate approach for pharmaceutical cleanrooms classification can be continued. A convenient summary of this approach is shown in Appendix A.

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 is useful should the cleanroom or ventilation system be modified, or the manufacturing activities changed, to confirm the performance relative to the original state.

References

1. ISO 14644-1: 2015 Cleanrooms and associated controlled environments - Part 1: Classification of air cleanliness. Geneva, Switzerland, International Organization for Standardization, 2015.
2. The rules governing medicinal products in the European Union –Volume 4 EU guidelines to good manufacturing practice - medicinal products for human and veterinary use - Annex 1 - Manufacture of sterile medicinal products. European Commission, Brussels, 2022.
3. Eaton T. Pharmaceutical Cleanroom Classification using ISO 14644 and the EU GGMP Annex 1; Part 1: Testing rationale. *European Journal of Parenteral and Pharmaceutical Sciences* 2019; 24(4).
4. Eaton T. Pharmaceutical Cleanroom Classification using ISO 14644 and the EU GGMP Annex 1; Part 2: Practical application. *European Journal of Parenteral and Pharmaceutical Sciences* 2019; 24(4).
5. ISO 21501-4: 2018 Determination of particle size distribution - Single particle light interaction methods - Part 4: Light scattering airborne particle counter for clean spaces. Geneva, Switzerland, International Organization for Standardization, 2018.
6. Eaton T. Annex 1 of the EC Guide to Good Manufacturing Practice (EC GGMP) and continuous particle monitoring - help or hindrance for cleanroom manufacturing? *European Journal of Parenteral & Pharmaceutical Sciences* 2007; 12(2): 29-37.
7. Whyte W. and Hejab M. Dispersion of particles and microorganisms from people. *European Journal of Parenteral and Pharmaceutical Sciences* 2007; 12 (2): 39- 46.
8. Ljungqvist B and Reinmüller B. Cleanroom clothing systems. In: Practical safety ventilation in pharmaceutical and biotech cleanrooms. Parenteral Drug Association, Bethesda, USA. 2006; 57-76.
9. BS EN ISO 14644-2: 2015 Cleanrooms and associated controlled environments - Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration (ISO 14644-2:2015). London, England, British Standards Institution, 2015.

Appendix A: Recommendations and options for cleanroom classification testing

Table A1 Recommendations for initial cleanroom classification and options for re-classification testing, EU Grade A and Grade B areas

EU GGMP Grade A			
Initial Classification [a]	**Options for Re-classification (level to be determined by the user [b])**		
Comprehensive data required	**Continued inclusive data required**	**Basic data required**	**Minimal data required**
All pre-classification activities have been satisfactorily completed	Control demonstrated to have been maintained and assessment confirms that no changes have occurred but limited operational data available	Effective control demonstrated to have been maintained, assessment confirms that no changes have occurred and correlation of ≥5 µm with ≥0.5 µm particles established	Extensive control demonstrated to have been maintained, assessment confirms that no changes have occurred and correlation of ≥5 µm with ≥0.5 µm particles established
No existing data	Initial classification (and any previous re-classification) data available	Initial classification (and any previous re-classification) data available	Initial classification (and any previous re-classification) data available
'At rest' and 'in operation' testing	'At rest' and 'in operation' testing	'At rest' and 'in operation' testing	'In operation' testing only
Sample particles ≥0.5 µm and ≥5 µm	Sample particles ≥0.5 µm and ≥5 µm	Sample particles ≥0.5 µm only	Sample particles ≥0.5 µm only
Minimum sample volume 1 m^3 at each location	Minimum sample volume 0.69 m^3 at each location	Minimum 1 minute sample at each location (ensure all, or worst case, particle generating events are captured - more than one sample may be required).	Minimum 1 minute sample at each location (ensure all, or worst case, particle generating events are captured - more than one sample may be required).
EU GGMP Grade B			
All pre-classification activities have been satisfactorily completed	Control demonstrated to have been maintained and assessment confirms that no changes have occurred but limited operational data available	Effective control demonstrated to have been maintained, assessment confirms that no changes have occurred and correlation of ≥5 µm with ≥0.5 µm particles established	Extensive control demonstrated to have been maintained and assessment confirms that no changes have occurred
No existing data	Initial classification (and any previous re-classification) data available	Initial classification (and any previous re-classification) data available	Initial classification (and any previous re-classification) data available
'At rest' and 'in operation' testing	'At rest' and 'in operation' testing	'At rest' and 'in operation' testing	'In operation' testing only
Sample particles ≥0.5 µm and ≥5 µm	Sample particles ≥0.5 µm and ≥5 µm	Sample particles ≥0.5 µm only for 'at rest' but ≥0.5 µm and ≥5 µm for 'in operation'	Sample ≥0.5 µm and ≥5 µm particles
Minimum sample volume 0.69 m^3 at each location	Minimum sample volume 0.69 m^3 at each location	Minimum 1 minute sample at each location (ensure all, or worst case, particle generating events are captured - more than one sample may be required).	Minimum 1 minute sample at each location (ensure all, or worst case, particle generating events are captured - more than one sample may be required).

Notes;
a. Initial classification also includes significant modifications to the cleanroom, air conditioning system, or changes to cleanroom activities, occupancy numbers and the type of garments worn.
b. If it has been assessed that re-classification is to be completed, the user to determine the most appropriate testing to be undertaken.

For EU Grade C and Grade D cleanrooms, minimum 1minute sampling times for particles ≥0.5 µm and ≥5 µm are recommended, with considerations to ensure all particle generating events are included and more than one sample maybe required to ensure this.

Appendix B: EU GGMP Annex 1 2022 - maximum airborne particle concentrations for classification and monitoring.

Table B1 Maximum permitted total particle concentration for classification (table 1 in Annex 1 2022)

Grade	Maximum limits for total particle ≥ 0.5 µm/m³		Maximum limits for total particle ≥ 5 µm/m³	
	at rest	in operation	at rest	in operation
A	3 520	3 520	Not specified [a]	Not specified [a]
B	3 520	352 000	Not specified [a]	2 930
C	352 000	3 520 000	2 930	29 300
D	3 520 000	Not predetermined [b]	29 300	Not predetermined [b]

(a) Classification including 5µm particles may be considered where indicated by the CCS or historical trends.
(b) For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and routine data where applicable.

Table B2 Maximum permitted total particle concentration for monitoring (table 5 in Annex 1 2022)

Grade	Maximum limits for total particle ≥ 0.5 µm/m³		Maximum limits for total particle ≥ 5 µm/m³	
	at rest	in operation	at rest	in operation
A	3 520	3 520	29	29
B	3 520	352 000	29	2 930
C	352 000	3 520 000	2 930	29 300
D	3 520 000	Not predetermined [(a)]	29 300	Not predetermined [(a)]

(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.

Appendix C: EU GGMP Annex 1 (2022) minimum sampling volumes for classification calculated using ISO 14644-1:2015

Shown in Table C1 are the airborne sampling volumes calculated using Formula A.2 in ISO 14644-1. These are the minimum air volumes to be sampled to ensure a count of ≥ 20 particles, for the largest considered particle size. Formula A.2 is as follows;

$V_s = (20/C_{n,m}) \times 1000$

$C_{n,m}$ = Class limit for largest particle size considered

V_s = Minimum sample volume (liters)

Table C1 Minimum sampling volumes for airborne cleanliness concentrations of ≥0.5 µm and ≥5 µm particle sizes given EU GGMP Annex 1 (2022) calculated from in ISO 14644-1: 2015.

EU GGMP Annex 1 Grade	Maximum permitted number of particles per m³ equal to or greater than the tabulated size			
	At Rest		In Operation	
	≥0.5µm	≥5µm	≥0.5µm	≥5µm
A	3 520	(29) [a]	3 520	(29) [a]
Calculated volume (l)	5.68×10^{-3}	(690) [a]	5.68×10^{-3}	(690) [a]
B	3 520	(29) [a]	352 000	2 930
Calculated volume (l)	5.68×10^{-3}	(690) [a]	- [b]	6.83×10^{-3}
C	352 000	2 930	3 520 000	29 300
Calculated volume (l)	-	6.83×10^{-3}	- [b]	6.83×10^{-4}
D	3 520 000	29 300	Not defined	Not defined
Calculated volume (l)	-	6.83×10^{-4}	-	-

Notes:

a. For classification, Annex 1 does not include limits for ≥0.5µm particles but the limits defined for monitoring have been included and the associated minimum sample volumes have been calculated and are shown in parenthesis.

b. Volumes not calculated for these particle sizes as they are not the largest considered size

Appendix D: ISO 14644-1:2015 and EU GGMP Annex 1 (2022) airborne particle cleanliness concentrations

Shown in Table D1 are the airborne cleanliness concentrations for particles ≥0.5 µm and ≥5 µm given in ISO 14644-1 and the EU GGMP Annex 1 (2022) for monitoring (table A2 in Appendix A). 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 considers 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 D1 Comparative airborne cleanliness concentrations of ≥0.5 µm and ≥5 µm particle sizes given in ISO 14644-1: 2015 and EU GGMP Annex 1 (2022).

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	29	3 520	29
ISO 14644-1	ISO 5	3 520	[a]	3 520	[a]
EU GGMP Annex 1	Grade B	3 520	29	352 000	2 930
ISO 14644-1	ISO 5	3 520	[a]	-	-
	ISO 7	-	-	352 000	2 930
EU GGMP Annex 1	Grade C	352 000	2 930	3 520 000	29 300
ISO 14644-1	ISO 7	352 000	2 930	-	-
	ISO 8	-	-	3 520 000	29 300
EU GGMP Annex 1	Grade D	3 520 000	29 300	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.