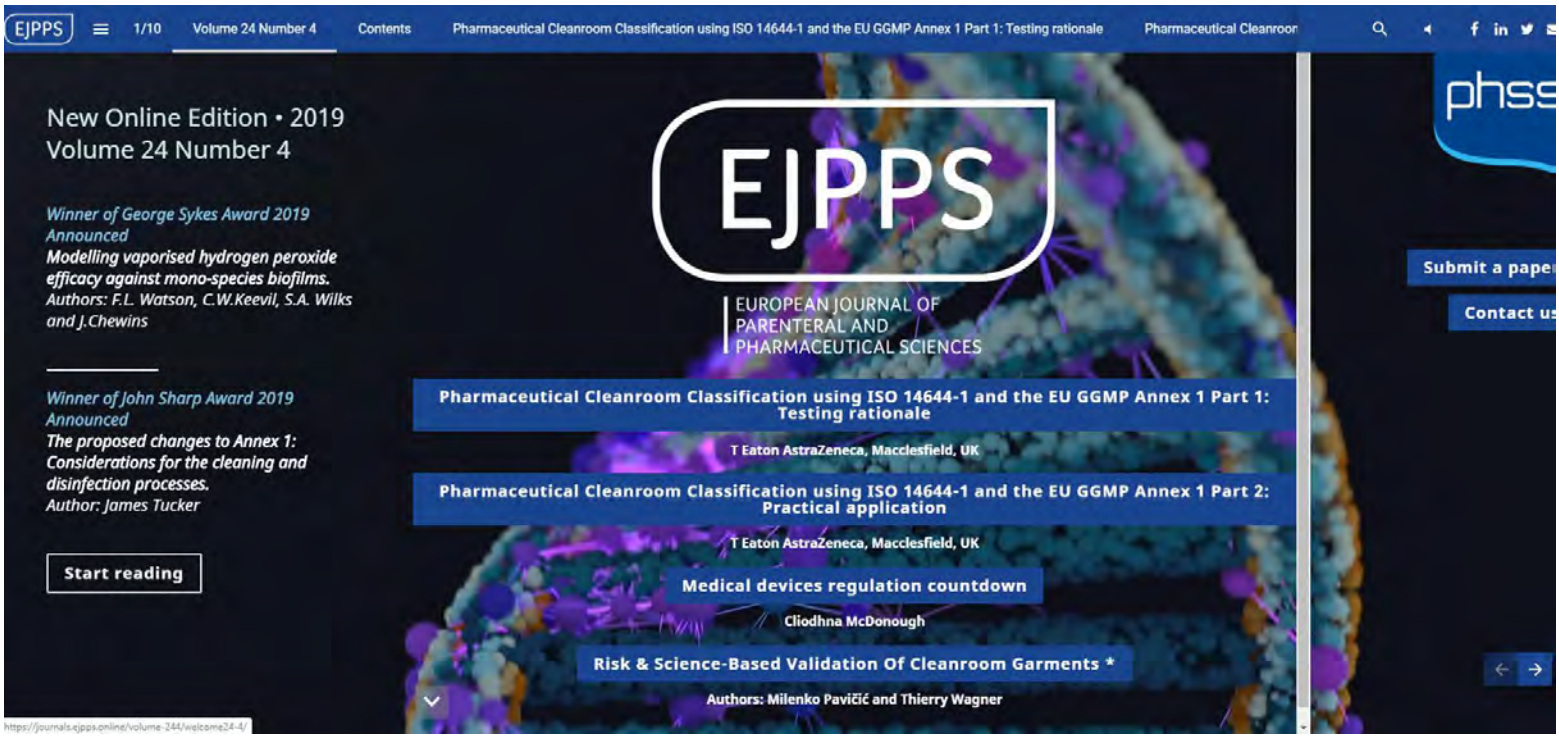
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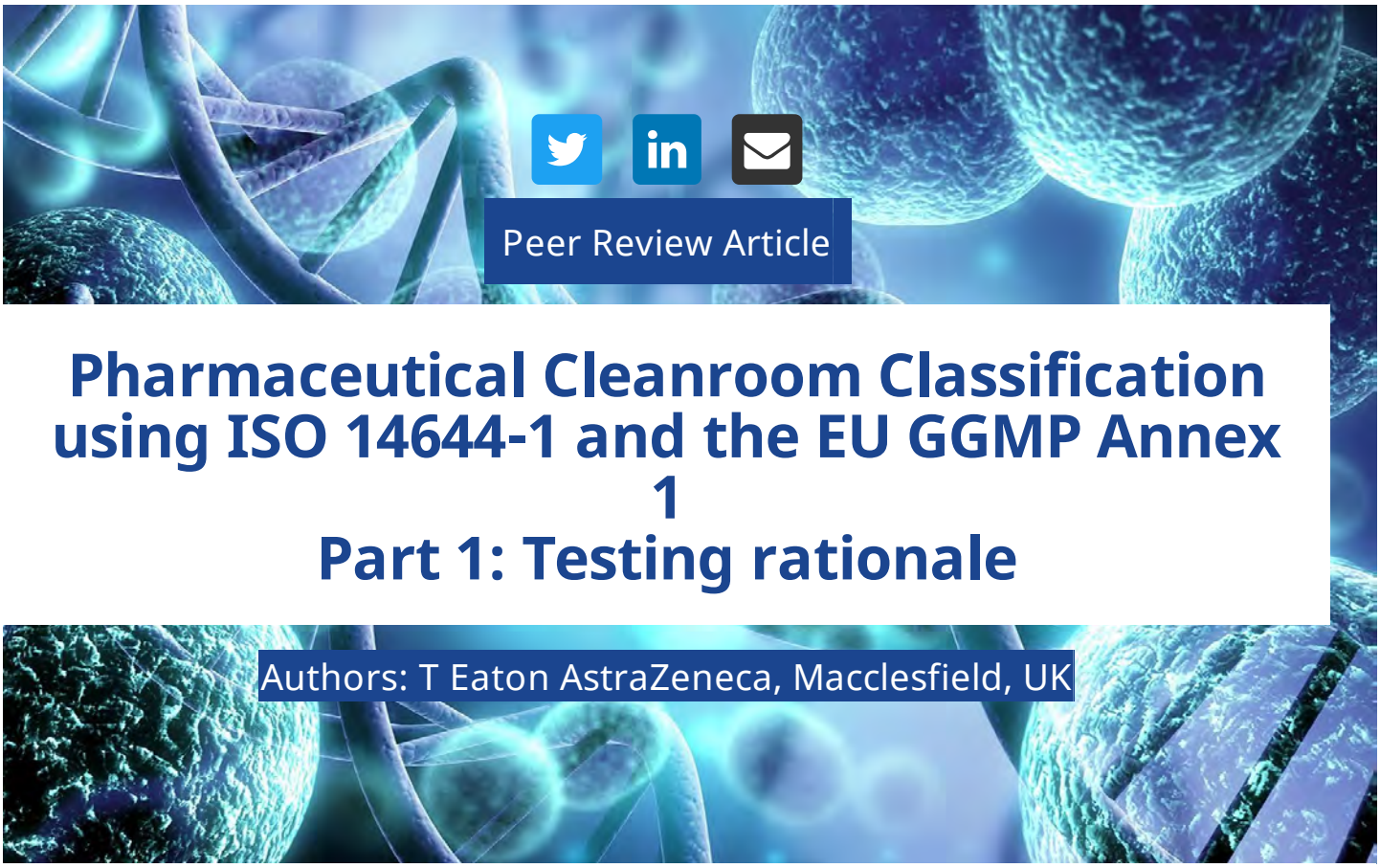

Peer Review Article

# 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 the 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 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 $\geq$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 (200 ). These provide a classification testing rationale for pharmaceutical cleanrooms used for aseptic manufacture.

**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.<br>The 'at rest' classification needs to be satisfactorily completed before the 'in operation' classification (refer to Section 2 of this table).<br>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.<br>2. The air conditioning system to be operating in the manner necessary to provide the required level of airborne contamination control.<br>3. The in-situ integrity testing of all cleanroom terminal air supply filters to be satisfactorily completed.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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.<br>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. |

| 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.<br>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<br>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.<br>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.<br>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$^3$.<br>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.<br>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.<br>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.<br>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. |

| 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<br>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.<br>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.<br>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.<br>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.<br>2. For 'in operation' testing, a formal risk assessment process should be used to establish where air sampling should be carried out.<br>3. Consideration to be given to the direction of airflow in UDAF and avoiding sampling under air inlets with no diffuser.<br>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.<br>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.<br>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.<br>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.<br>2. For non-UDAF locations, the probe to be directed vertically upwards and does not need to be isokinetic.<br>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 <15cm radius, and made of a material that minimises electrostatic attraction.<br>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). |

| 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".<br>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.<br>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.<br>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<br>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 actions shall be recorded in the report". The standard states "If any out-of-specification count is found at a location due to an identified abnormal occurrence, then that event can be documented 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 and the immediate surrounding locations and any other locations affected. The choice shall be clearly documented and justified". |
| EU GGMP Annex 1 | The air cleanliness classification requirements are met in all of the considered particle concentrations do not exceed the (per m³) 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/m³ 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/m³ should then be compared with the concentrations in Annex 1 to determine if the air cleanliness classification requirements have been met. |
| 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 machine or personnel activities that are the source of the airborne particles, or investigation of the ventilation system to ensure that it is able to provide the correct airborne particle concentration.<br>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). |
| Conclusions | 1. Calculate the particle concentrations per m³ at ≥0.5 µm and ≥0.5 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.<br>2. Ensure that no concentration per m³ exceeds the concentrations specified in the EU GGMP for ≥0.5 µm and ≥5.0 µm particle sizes, for the chosen cleanroom Grade. |
| | 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.<br>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.<br>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.<br>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. |

| 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.<br>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;<br><br>a. non-viable particles, likely to be measured continuously in Grade A and B areas, and periodically in other areas<br>b. microbiological contamination, throughout manufacture (and during periods when there is no manufacture)<br>c. pressure differentials, continuously<br>d. air supply velocity (UDAF), continuously<br>e. air volume supply (non-UDAF), continuously<br><br>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;<br><br>f. air supply filter integrity<br>g. air supply velocities at several locations across the filter face (UDAF)<br>h. air volume supply at each air inlet (non-UDAF)<br>i. airflow visualisation (UDAF)<br>j. air volume extract rate (or velocity measurement)<br><br>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.<br>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 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 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.

## 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.

## 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 E1:

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 |
| No activity | 1 | UDAF isolator or closed operation RABS | 1 | Area ($cm^2$) | Time (mins) |
| 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) |

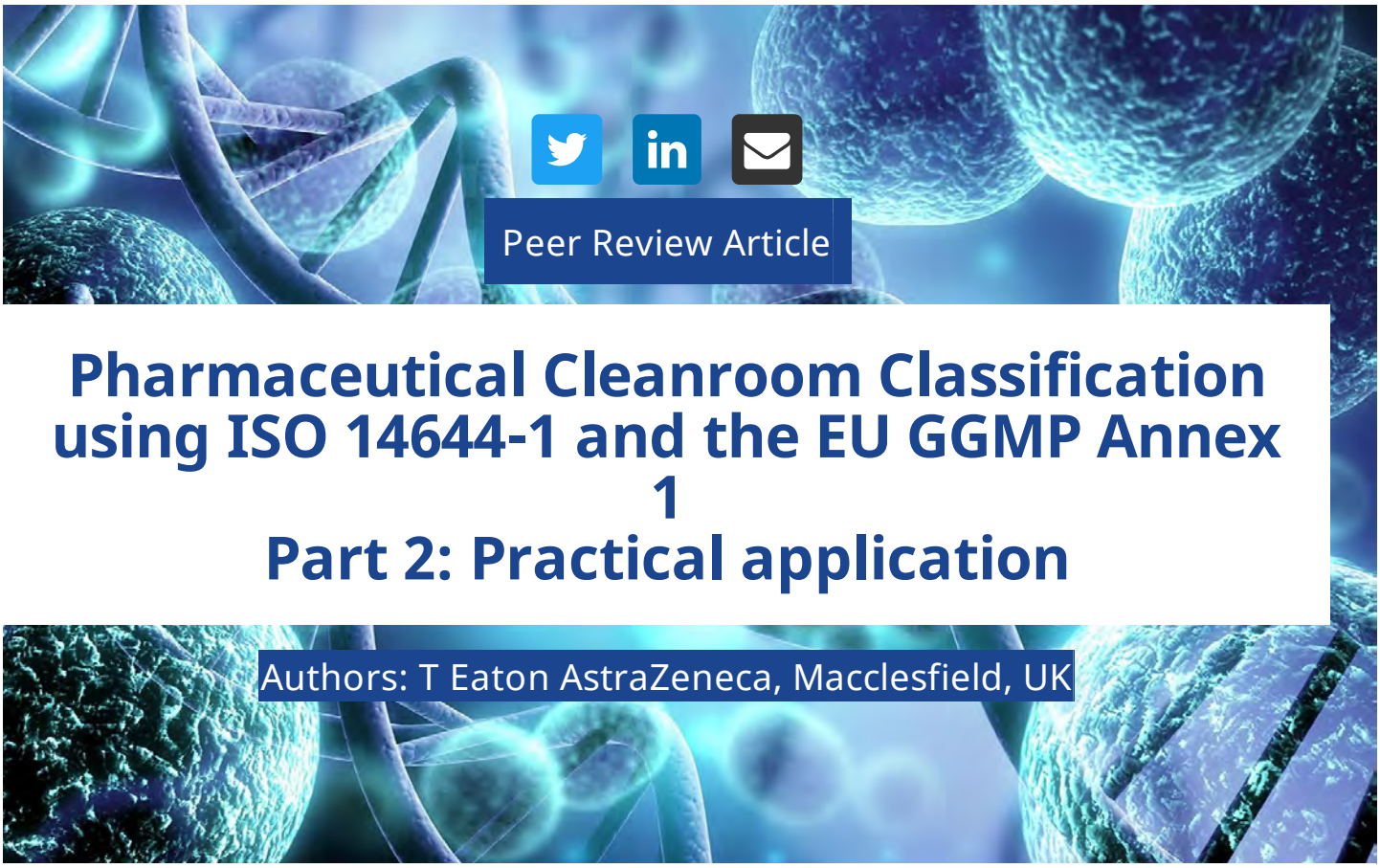
The level of risk at each location can then be obtained by the equation E.1;

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

**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.



Peer Review Article

# Pharmaceutical Cleanroom Classification using ISO 14644-1 and the EU GGMP Annex 1
# Part 2: Practical application

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**Summary**

**Classification of cleanrooms and clean zones associated with the manufacture of medicinal products has been assessed in two articles. The first article discussed the classification requirements and principles associated with ISO 14644-1and Annex 1 of the EU GGMP, and a suitable classification test method for aseptic manufacturing was derived. This second article considers the practical application of the method for the classification of a pharmaceutical cleanroom, and isolator located within it.**

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

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## 1. Introduction

Annex 1 of European Union Guidance to Good Manufacturing Practice (EU GGMP) (1) specifies the environmental conditions that must be provided for the manufacture of sterile medicinal products and requires the classification of different grades of cleanrooms and clean zones to be carried out in accordance with ISO 14644-1 (2). With the correct interpretation and application of the information given in ISO 14644-1 and Annex 1 of the EU GGMP, as well as more current expectations of the regulatory authorities, an appropriate testing regime has been established in our first article that is suitable for the classification of a pharmaceutical cleanroom and clean zone utilised for aseptic manufacture (3). The testing regime method is applied to the classification of a pharmaceutical cleanroom and isolator but the same approach can be used, with some modifications, for most types of healthcare cleanrooms.

## 2. Description of cleanroom and isolator

The classification method described in the first article is applied to a cleanroom and isolator used for the aseptic filling of a liquid formulation into vials, which are then sealed with sterile closures. Shown in Figure 1 is a schematic diagram of the cleanroom and isolator.

The cleanroom (EU GGMP Grade C) that contains the isolator is a non-unidirectional airflow (non-UDAF) cleanroom with ceiling-mounted HEPA filters, that supply air via swirl diffusers, and low-level air extracts that return the cleanroom air to a central air conditioning system. The cleanroom is operated at positive pressure with respect to a surrounding EU GGMP Grade C corridor. Typically, 4 people work in the cleanroom but a maximum of 6 people may be present, wearing a polyester smock with full hood, overboots, mask, and sterile, double sets of latex gloves.

The isolator (EU GGMP Grade A) is of rigid construction and supplied with UDAF through terminal HEPA filters that cover the entire isolator ceiling area and is maintained at a positive differential pressure with respect to the cleanroom. It interfaces with a vial cooling zone linked to a washing, depyrogenation and cooling tunnel located within an adjacent preparation cleanroom that provides prepared vials for the filling operation. This preparation cleanroom is also used for the formulation of the product in a stainless-steel vessel that then supplies the liquid product directly into the isolator through pre-sterilised single use, sterilising grade product filters and tubing, connected to 4 filling needles. The tubing is transferred into the isolator via a secure liquid transfer port following decontamination of the closed isolator with vaporised hydrogen peroxide. A peristaltic pump, located next to the preparation vessel, is used to transfer the liquid from the vessel to the needles for the subsequent filling operation. The product outfeed (exit mousehole) is used for the transfer of the filled vials secured with rubber stoppers directly into a UDAF workstation in an adjacent cleanroom, where capping and crimping of the closures is completed. Sterilised rubber closures are transferred into the isolator from the cleanroom using a secure split butterfly transfer device that is connected to the isolator. It is changed four times during the course of the filling in order to supply the required number of closures. Vials with an internal neck area of 2 cm² are aseptically filled with 2 ml of product solution in batches of 4 000, which takes approximately 4 hours.

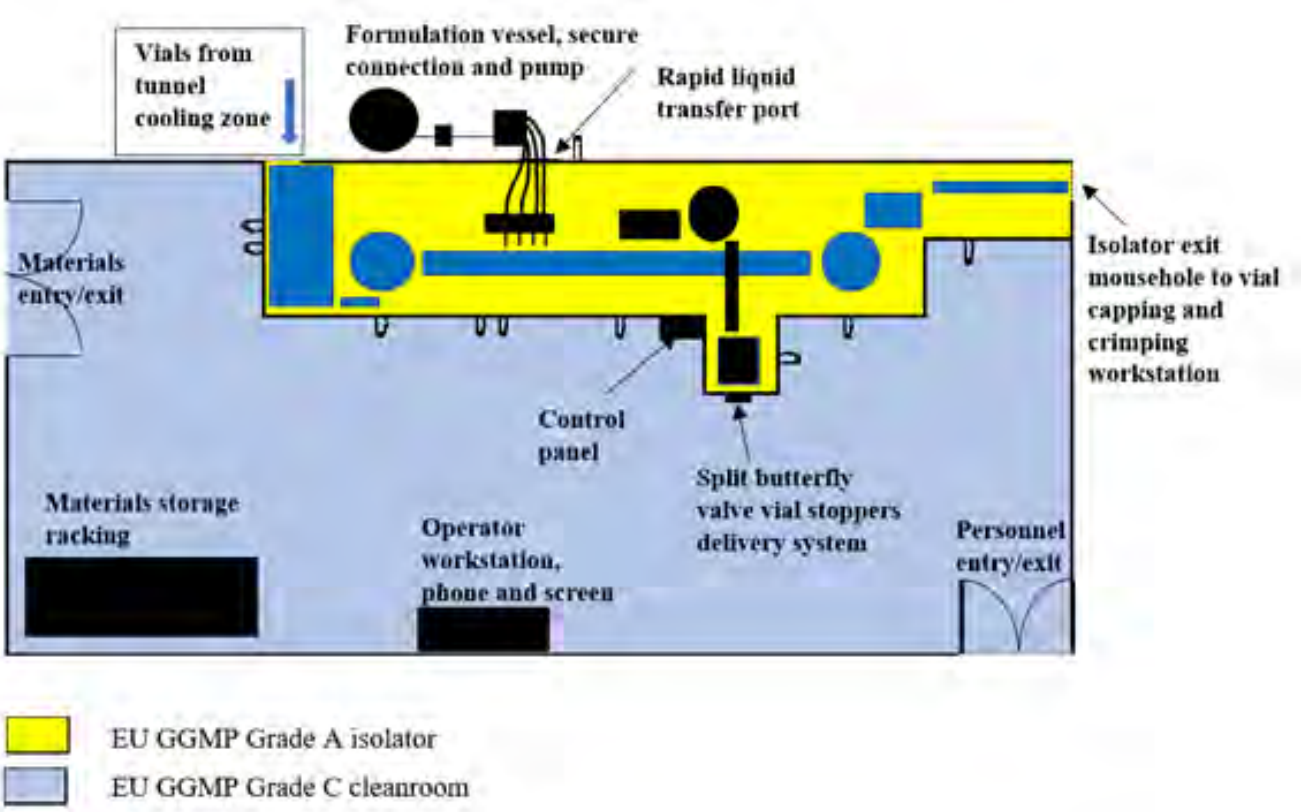



Figure 1 Cleanroom and isolator schematic

### 3. Classification of the EU GGMP (2008) cleanroom and isolator in accordance with ISO 14644-1: 2015

A review of the EU GGMP (2008) and ISO 14644-1: 2015 classification requirements and principles has been undertaken in the first article[3] and used to provide a classification testing rationale for pharmaceutical cleanrooms used for aseptic manufacture. This is applied to the example described above for the classification of the cleanroom that contains the isolator, and then to the isolator.

The EU GGMP requires classification to be carried out 'at rest' and 'in operation'. The information given in this article is for the 'in operation' classification, and it is assumed that a successful 'at rest' classification has already been completed. The 'at rest' classification is very similar to the 'in operation' classification, but no production is carried out during the testing and has been discussed in the first article.

### 3.1 Cleanroom classification

The testing rationale and the associated considerations for the 'in operation' classification of the isolator cleanrooms are shown in Table 1. This is an abbreviated version of Table 1 given in the first article (3), and if more comprehensive information is required, that table should be consulted.

**Table 1** In operation classification considerations for the cleanroom containing the isolator

| Classification Parameter | Cleanroom 'in operation' classification considerations |
|---|---|
| 1. Facility installation status | **1.1 Installation testing**<br>All cleanroom installation and equipment testing to be satisfactorily completed. |
| | **1.2 Air conditioning system**<br>The cleanroom ventilation system to be operating in the established manner to provide the required level of airborne contamination control. |
| | **1.3 Cleanroom terminal HEPA filters**<br>The in-situ integrity testing of all cleanroom terminal air supply filters to be satisfactorily completed. |
| | **1.4 'At rest' testing**<br>The 'at rest' testing of the cleanroom to be satisfactorily completed prior to the commencement of the 'in operation' testing. |
| 2. Occupancy State | **2.1 Operational conditions**<br>The worst-case maximum number of 6 people to be present and performing the established cleanroom manufacturing activities. |
| | **2.2 Personnel cleanroom garments**<br>All cleanroom personnel to be wearing the designated cleanroom garments, as described in Section 2. |
| 3. Particle size | **3.1 EU GGMP Annex 1 particle sizes**<br>Both ≥0.5 µm and ≥5 µm particle sizes are included in the EU GGMP and are to be used for the cleanroom classification. The rationale for sampling at both these sizes has been explained in the first article [3] and in the discussions and conclusions section of this article.<br>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. |
| 4. Particle concentrations | **4.1 EU GGMP Annex 1 particle sizes particle concentrations**<br>The 'in operation' limit for ≥0.5µm particles in an EU Grade C cleanroom is 3 520 000/$m^3$, which is equivalent to an ISO Class 8. The 'in operation' limit for ≥5µm particles in an EU Grade C cleanroom is 29 000/$m^3$, which is slightly less than the limit for an ISO Class 8 cleanroom of 29 300/$m^3$. The EU GGMP concentration for the ≥5µm particle size of 29 000/$m^3$ is applied. |
| 5. Sampling volumes and sampling times | **5.1 Sample volume**<br>The minimum sample volume for a single sample at each location is calculated using the class limit for the largest particle size considered, using Formula A.2 in the ISO standard. For a largest considered particle size of ≥5 µm, with an associated class limit ($C_{n,m}$) of 29 000 per $m^3$, the minimum sample volume ($V_S$), in litres, is calculated from the formula to be;<br><br>$V_S = (20/ C_{n,m}) \times 1000 = (20/29000) \times 1000 = 0.69$ l<br><br>In addition, there is a requirement stated in the ISO standard to sample at least 2 l and to take a minimum 1-minute sample at each location. Also, for measuring macroparticles (particles with an equivalent diameter greater than 5 µm), the sampler should have a sample flow rate of at least 28.3 l/min. The sampler utilised had a flow rate of 50 l/min, and satisfies these criteria, and with a minimum sampling time of 1 minute, will provide an associated sample volume of 50 l. |
| | **5.2. Number of samples at each sampling location**<br>Continuous activities are to be undertaken throughout the sampling period, and with the exception of the activity associated with the connection of the container closures split butterfly valve transfer device to the isolator, it is assessed that with a sampling time of 1 minute, all particle generating activities would be adequately included in a 1 minute sample. For the addition of the split |

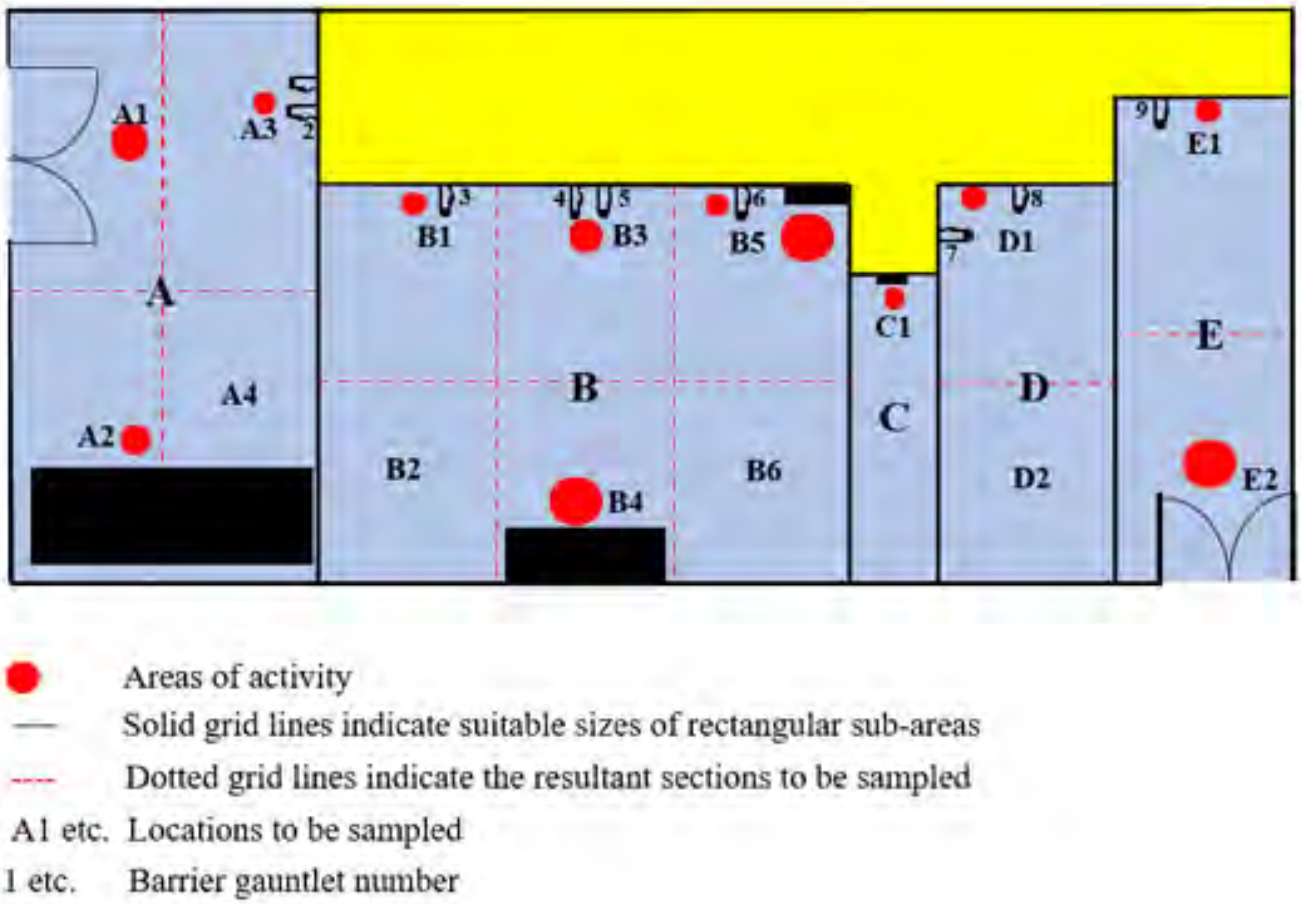
| | |
|---|---|
| | butterfly valve transfer device, the activity was assessed to require two 1 minute samples. |
| 6. Number of sampling locations | **6.1. Division of cleanroom into sampling sections**<br>The total floor area of the cleanroom is 56.2 $m^2$ and according to Table A.1 in the ISO standard, a minimum of 12 sampling locations are to be tested by dividing the cleanroom into equal area sections. As the cleanroom contains the isolator, the cleanroom floor area is asymmetrical and cannot be readily divided into equal area sections and so the method previously described[3] to determine the dimensions of each section is utilised. This approach is summarised in section 3.2, and detailed in Appendix A, which concluded that a total of 15 sections require to be tested. |
| 7. Where to sample in each section | **7.1. Risk assessment**<br>A formal risk assessment is recommended by the EU GGMP to determine the position of the sampling locations within the 15 sections of the cleanroom floor area. Suitable risk assessment methods have been discussed in the first article [3]. In the cleanroom there is no product exposure and the risk assessment used is that which determines locations where operator activities give the highest levels of airborne contamination. This method is explained in section 3.2, and detailed in Appendix A.<br>**7.2 Airflow considerations**<br>The isolator cleanroom is supplied with non-UDAF via ceiling-mounted swirl diffusers, and the direction of airflow associated with UDAF, or avoidance of sampling under non-diffuser air inlets, are not a consideration.<br>**7.3 Sampling locations**<br>The final sampling locations are shown in Figure 2 and detailed in Table 2. |
| 8. Sampling probe and tubing | **8.1 Air sampler tubing**<br>The cleanroom is non-UDAF and the particle counter probe must be directed vertically upwards but the sampling head does not need to be isokinetic. Sampling can be achieved directly by the air sampler at each identified sampling location without the use of any connecting tubing, and this method is used.<br>**8.2 Sampling height**<br>The air sampler is located at working height, (1 m above the floor) using a suitable trolley, or similar method. There are no large vessels or objects to prevent or inhibit sampling at each of the identified locations. |
| 9. Particle counter | **9.1 Particle counter and calibration**<br>A light scattering airborne particle counter (LSAPC) is the most appropriate instrument for counting and sizing cumulative particle sizes at ≥0.5 µm and<br>≥5 µm. For sampling macroparticles, the LSAPC should have a sample flow rate of at least 28.3 l/min. The instrument used has a sampling rate of 50 l/min and set for cumulative and not discrete particle sizes sampling. It is several years old and cannot be fully calibrated as specified in ISO 21501-4 (4) but is a proprietary instrument that has a valid calibration certificate from a competent body and considered to be fit-for-purpose. |
| 10. Interpretation of air sampling counts | **10.1 Calculated particle concentrations**<br>The resultant particle concentrations per $m^3$ at each location at sizes ≥0.5 µm and ≥5 µm are calculated from the sampling results. These are shown in Table 2. With the exception of one out-of-specification sampling count at the ≥5 µm particle size at a single location, all of the in-operation concentrations specified in the EU GGMP for a Grade C cleanroom (shown in Section 4 in this table) were achieved. |
| 11. Out-of-Specification result | **11.1 Investigation and remedial actions**<br>An investigation at the location that recorded the out-of-specification result was completed and this is considered in Section 3.4 in this article. |

### 3.2 Determination of cleanroom sampling sections and sampling positions

Is necessary to divide the cleanroom into the number of sampling sections given in Table A1 of ISO 14644-1. As the cleanroom contains an isolator, the floor area is not a simple square or rectangle but is asymmetrical, and the method discussed in Appendix D of the first article (3) should be used to divide the floor area of the cleanroom. The application of the method is explained in Appendix A of this article, where it was found that the cleanroom needed to be divided into 15 sections, and these are shown in Figure 2.

As there is no product exposure in the cleanroom, 'hot spots' of operator activity were identified to determine the position in each of these sections where air sampling should be carried out. These are lettered and shown in Figure 2 at the identified sampling locations. Sections A4, B2, B6 and D2 had no 'hot spots' and sampling was carried out in the centre of the sections as these were considered to be representative of the characteristics of the sections.

Figure 2 Cleanroom activity areas, sampling sections and sampling locations




### 3.3 Results of airborne sampling in cleanroom

Using the information given in the previous section, sampling was carried out at each of the locations, and the results shown in Table 2. It can be seen that, with one e ception at location A2, all the samples had air concentrations below the EU GGMP Grade C limits set for particles 0.5 m and 5 m.

Table 2 **Cleanroom 'in operation' sampling data for particles ≥0.5 µm and ≥5 µm**

| Sampling Location Number | Location and Activities | Sample Results (no./50 l) | Location concentration (no./m³) [sample result ×20] | Pass/Fail EU Grade C limit Particles /m³ ≥ 0.5 µm; 3 520 000 ≥ 5 µm; 29 000 | Ventilation Performance Index |
|---|---|---|---|---|---|
| A1 | **Location;** Adjacent to materials entry/hatch doors<br>**Activity;** Entry of transfer trolley with equipment and operator using gauntlets 1 and 2 | **≥0.5 µm** 2170 | 43 400 | Pass | 2.3 |
| | | **≥5 µm** 156 | 3 120 | Pass | 2.1 |
| A2 | **Location;** Adjacent to materials storage racking<br>**Activity;** Operator loading equipment onto racking | **≥0.5 µm** 22981 | 459 620 | Pass | 0.2 |
| | | **≥5 µm** 1532 | 30 640 | Fail | 0.2 |
| A3 | **Location;** Adjacent to barrier gauntlets 1 and 2<br>**Activity;** Operator using gauntlets and entry of transfer trolley with equipment | **≥0.5 µm** 2930 | 58 600 | Pass | 1.7 |
| | | **≥5 µm** 147 | 2 940 | Pass | 2.3 |
| A4 | **Location;** Centre of section<br>**Activity;** Operator loading equipment onto storage racking | **≥0.5 µm** 3302 | 66 040 | Pass | 1.5 |
| | | **≥5 µm** 221 | 4 420 | Pass | 1.5 |
| B1 | **Location;** Adjacent to barrier gauntlet 3<br>**Activity;** Operator using gauntlet | **≥0.5 µm** 696 | 13 920 | Pass | 7.3 |
| | | **≥5 µm** 133 | 2 660 | Pass | 2.5 |
| B2 | **Location;** Centre of section<br>**Activity;** Operator at the operator workstation | **≥0.5 µm** 2892 | 57 840 | Pass | 1.8 |
| | | **≥5 µm** 700 | 3 220 | Pass | 2.1 |
| B3 | **Location;** Adjacent to barrier gauntlets 4 and 5<br>**Activity;** Operator using both gauntlets | **≥0.5 µm** 3310 | 66 200 | Pass | 1.5 |
| | | **≥5 µm** 201 | 4 020 | Pass | 1.7 |
| B4 | **Location;** Adjacent to operator workstation<br>**Activity;** Operator at the operator workstation | **≥0.5 µm** 5914 | 118 280 | Pass | 0.9 |
| | | **≥5 µm** 236 | 5 260 | Pass | 1.3 |
| B5 | **Location;** Between barrier gauntlet 6 and control panel<br>**Activity;** Two operators present, one using gauntlet 5 and the other operating the control panel | **≥0.5 µm** 1865 | 37 300 | Pass | 2.7 |
| | | **≥5 µm** 233 | 4 660 | Pass | 1.4 |
| B6 | **Location;** Centre of section<br>**Activity;** Operator at the operator workstation | **≥0.5 µm** 5518 | 110 360 | Pass | 0.9 |
| | | **≥5 µm** 263 | 5 260 | Pass | 1.3 |
| C1 | **Location;** Adjacent to vial closures delivery system<br>**Activity;** Operator adding the closures split butterfly delivery system to isolator | **≥0.5 µm** 4998<br>4251 | 99 960* | Pass | 1.0 |
| | | **≥5 µm** 455<br>472 | 9 440* | Pass | 0.7 |
| D1 | **Location;** Between barrier gauntlets 6 and 7<br>**Activity;** Two operators present, one using gauntlet 7 and the other using gauntlet 8 | **≥0.5 µm** 6430 | 128 600 | Pass | 0.8 |
| | | **≥5 µm** 215 | 4 300 | Pass | 1.5 |
| D2 | **Location;** Centre of section<br>**Activity;** Operator entering via personnel entry/exit door | **≥0.5 µm** 3189 | 63 780 | Pass | 1.6 |
| | | **≥5 µm** 146 | 2 920 | Pass | 2.3 |
| E1 | **Location;** Adjacent to barrier gauntlet 9<br>**Activity;** Operator using gauntlet | **≥0.5 µm** 4982 | 99 640 | Pass | 1.0 |
| | | **≥5 µm** 333 | 6 660 | Pass | 1.0 |
| E2 | **Location;** Adjacent to personnel entry/exit door<br>**Activity;** Operator entering via personnel entry/exit door | **≥0.5 µm** 4973 | 99 460 | Pass | 1.0 |
| | | **≥5 µm** 498 | 9 960 | Pass | 0.7 |
| **Average counts /m³** | | **≥0.5 µm** | 101 533 | | |
| | | **≥5 µm** | 6 632 | | |

* These concentrations have been calculated from the worst case (highest) result recorded from two separate samples taken at the same location.

### 3.4 Investigation and rectification of the high airborne particle concentration

As shown in Table 2, the concentration of particles ≥5 µm at location A2 exceeds the classification limit of an EU GGMP Grade C cleanroom. ISO 14644-1 allows out-of-specification counts to be retested but on retesting, similar high counts were obtained. The reason for high particle counts in a cleanroom is caused by either an unusually high dispersion rate of particles, or insufficient ventilation. An investigation was carried out to determine the cause of the high count, and to rectify the problem.
The high concentration of particles appeared to be connected with the ventilation effectiveness of the air supply system. The overall average concentrations in the cleanroom of particles ≥0.5µm and ≥5µm were 101 533 /m³ and 6 632 /m³ respectively, which are well below the airborne concentrations specified for an EU GGMP Grade C cleanroom. Had the average count been above the Grade limit, it would indicate that there was insufficient air for the given amount of particle dispersion, and that the air supply to the cleanroom needed to be increased, or the dispersion of particles from machinery or personnel reduced. These particle concentration results suggest that the problem did not lie with an insufficient supply of filtered air.

Table 2 shows that the airborne concentrations at locations in the cleanroom were very variable and the ventilation effectiveness was investigated. The Performance Indexes (PI) was calculated for each sampling location using the following equation[6].

$$\text{Performance Index} = \frac{\text{Average particle airborne concentration / m}^3}{\text{Airborne particle concentration/m}^3 \text{ at location}}$$

The PI results included in Table 2 confirm that the airborne concentrations to be very variable. If the supply air was consistently well mixed with cleanroom air, all the PI values would be close to 1, and this would have been the ideal situation. However, it can be seen that at location A2 the PI was 0.2, which suggests that the location had 5 times less clean air than the average in the room, and further ventilation effectiveness tests were carried out.

The recovery rate was measured at location A2 according to the method given in ISO 14644-3 (5) and explained elsewhere (6). Test particles were released and then discontinued, and the rate of decay of the particles measured and the recovery rate calculated. Using hours as the units of measurement, the recovery rate was found to be 5 /hour. The recovery rate at a location is known to be the same as the air change rate at that location (6), and if this recovery rate (local air change rate) is compared to the overall air change rate in the room, the Air Change Effectiveness (ACE) index is obtained. This index shows how much clean air a specified location receives, compared to the average in a cleanroom. The overall (average) air change rate in the cleanroom was 30 per hour and therefore the ACE index at the location A2 was 0.17 (5/30). As expected, the PI and ACE indexes were similar. This showed that location A2 receives about 5 times less air that the average in the room, and this was most likely to be the reason for the high concentration of particles at that location.

The problem of uneven distribution of clean air was likely to be solved by improving the air distribution by either installing another ceiling air supply inlet, or air return/extract, close to location A2. It was decided to install an additional exhaust outlet but not increase the overall extract air rate. The air extract system was then rebalanced. The recovery rate was then re-measured at location A2 and found to have increased to 15 /hour, and the ACE index to 0.5. This was considered acceptable but, if it had not been so, an additional air supply could have been installed close to location A2 and, with the same overall room rate of supply volume retained, the air supply system rebalanced. Had neither of these remedial actions been adequate, an increase in the air supply rate and rebalancing to give more air supply around location A2 would have been the next consideration. Reducing the dispersion from machinery, or improvement of the cleanroom garments to reduce the dispersion from personnel, would have been another possibility. However, a new measurement of the airborne particle concentration at location A2 gave a concentration below that required.

ISO 14644-1 allows remedial action to be taken in a cleanroom where an out-of-specification count found at a location is attributed to a technical failure of the cleanroom or equipment. 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. However, in this case, it was decided that the remedial action was substantial, and required a full reclassification of the cleanroom. This was carried out and the cleanroom passed the EU GGMP Grade C classification.

### 3.5 Isolator classification

The testing rationale and associated considerations for the 'in operation' classification of the isolator is shown in Table 3.

**Table 3** In operation classification considerations for the isolator

| Classification Parameter | Isolator 'in operation' classification considerations |
|---|---|
| 1. Facility installation status | **1.1 Installation testing**<br>All isolator installation and equipment testing to be satisfactorily completed. |
| | **1.2 Air conditioning system**<br>The isolator ventilation system to be operating in the established manner to provide the required level of airborne contamination control. |
| | **1.3 Isolator terminal HEPA filters**<br>The in-situ integrity testing of all isolator terminal air supply filters to be satisfactorily completed. |
| | **1.4 'At rest' testing**<br>The 'at rest' testing of the isolator to be satisfactorily completed prior to the commencement of the 'in operation' testing. |
| 2. Occupancy State | **2.1 Operational conditions**<br>The worst case was utilised of the maximum number of 4 people accessing the isolator via the gauntlets nearest to the sampling location and performing the established isolator manipulations. |
| | **2.2 Personnel cleanroom garments**<br>All cleanroom personnel were required to wear the designated cleanroom garments. |
| 3. Particle size | **3.1 EU GGMP Annex 1 particle sizes**<br>Both ≥0.5 µm and ≥5 µm particle sizes are included in the EU GGMP and included in the cleanroom classification. The rationale for sampling at both these sizes has been explained in the first article[3] and in the discussions and conclusions section of this article.<br>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. |
| 4. Particle concentrations | **4.1 EU GGMP Annex 1 particle concentrations**<br>The 'in operation' particle limits for an EU GGMP Grade A zone are the same at the ≥0.5 µm size as ISO Class 5, which is 3 520/$m^3$. It should be noted that ISO 14644-1:2015 does not include a concentration limit for particles ≥5 µm for ISO Class 5 because the sampling and statistical limitations of particles at such low concentrations make classification inappropriate. However, for the reasons explained in the first article[3], the EU GGMP requirement for a concentration of 20/$m^3$ is applied at the ≥5 µm particle size. |
| 5. Sampling volumes and sampling times | **5.1 Sample volume**<br>The minimum sample volume for a single sample at each location is calculated using the class limit for the largest particle size considered and using Formula A.2 in the ISO standard. For a largest considered particle size of ≥5 µm, with an associated class limit ($C_{n,m}$) of 20 per $m^3$, the minimum sample volume ($V_S$), in litres, is calculated from the formula to be;<br><br>$V_S = (20/ C_{n,m}) \times 1000 = (20/20) \times 1000 = 1000$ l<br><br>In addition, there is a requirement stated in the ISO standard to sample at least 2 l and to take a minimum 1-minute sample at each location. Also, for measuring macroparticles (particles ≥5 µm), the sampler should have a sample flow rate of at least 28.3 litres per minute. The sampler used had a flow rate of 50 l/min and satisfies these criteria. For Grade A zones, the EU GGMP states that a minimum sample volume of 1 $m^3$ should be taken at each sample location, and so a minimum sampling time of 20 minutes is used. |
| | **5.2. Number of samples at each sampling location**<br>It is assessed that with a sampling time of 20 minute, all particle generating activities at each sampling location could be adequately included in a single sample. |
| 6. Number of sampling locations | **6.1. Division of isolator into sampling sections**<br>The base area of the isolator is 15.83 $m^2$. According to Table A.1 in the ISO standard, a minimum of 6 equal area sampling sections are required to be tested during the classification. However, the isolator base area is asymmetrical and cannot be readily divided into equal area sections. The method described in the first article [3] is, therefore, used to determine the dimensions of the sampling sections. This approach is detailed in Appendix B, where it is concluded that 8 sections should be tested. However, a higher number of samples (and air sample volumes) are typically required for this type of environment, and this is considered in the next section of this table. |
| 7. Where to sample in each section | **7.1. Risk assessment**<br>The expectation of the regulatory authorities is that the choice of sampling locations should be obtained by a documented formal risk assessment based on knowledge of the ventilation system, process, and manufacturing activities. A risk assessment method that identifies locations with the greatest risk of product and component contamination is described in the first article [3] and its application to the isolator classification detailed in Appendix B. This risk assessment identified 10 locations and at each of 2 of these locations, 2 different activities that may present risk were identified and need to be included in the sampling. Therefore, 12 activities were identified for sampling.<br>**7.2 Airflow and other considerations**<br>Outflow of air from the isolator at the vial exit is utilised to prevent the ingress of air from the interfacing areas, and this is an additional location where a risk exists and should be sampled. Also, owing to the nature of UDAF, airborne particle concentrations within the isolator are likely to be localised, and it was considered that 2 further sampling locations associated with areas where there are no manufacturing activities, should be included to ensure all zones of the isolator are included. These 3 locations are also included for sampling.<br>**7.3 Overall number of sampling locations**<br>A total of 13 locations should be sampled and due to the two locations which each have 2 different activities, 15 activities are to be sampled. The 13 sampling locations are shown in Figure 3 and the associated 15 activities are detailed in Table 4. |
| 8. Sampling probe and tubing | **8.1 Air sampler tubing**<br>The isolator has UDAF and the particle counter probe should be directed vertically upwards into the airflow and the sampling head must be isokinetic. For some of the identified sampling locations, the particle counter can be placed in the isolator. However, in other locations, the sampler cannot be accommodated within the isolator, and connecting tubing from sampling point to the particle sampler outside of the isolator is required. The most appropriate barrier gauntlet, or other penetration locations into the isolator, such as a particle monitoring probe access point, should be used to access the tubing into the isolator and sealed in place. All such tubing must be of minimal length (no more than 1 m) [2], with no kinks or bends of less than 15cm radius [7] and the intake as close as possible to the identified sampling location (within 1 foot, 30 cm) [8]. |
| 9. Particle counter | **9.1 Particle counter and calibration**<br>A light scattering airborne particle counter (LSAPC) is the most appropriate instrument for counting and sizing cumulative particle sizes at ≥0.5 µm and ≥5 µm. The sampler is several years old and cannot be fully calibrated as specified in ISO 21501-4 [4] but is a proprietary instrument that has a valid calibration certificate from a competent body and considered to be fit-for-purpose. |
| 10. Interpretation of air sampling counts | **10.1 Calculated particle concentrations**<br>The resultant particle concentrations per $m^3$ at each location at sizes ≥0.5 µm and ≥5 µm are shown in Table 3. All the in-operation concentrations specified in the EU GGMP for a Grade A clean zone were achieved. |
| 11. Out-of-Specification result | **11.1 Investigation and remedial actions**<br>No out-of-specification results were recorded, and no investigation and remedial actions were required. |

### 3.6 Determination of cleanroom sampling sections and sampling positions

The 13 sampling locations are shown in Figure 3 and the associated 15 sampling activities are detailed in Table 4.

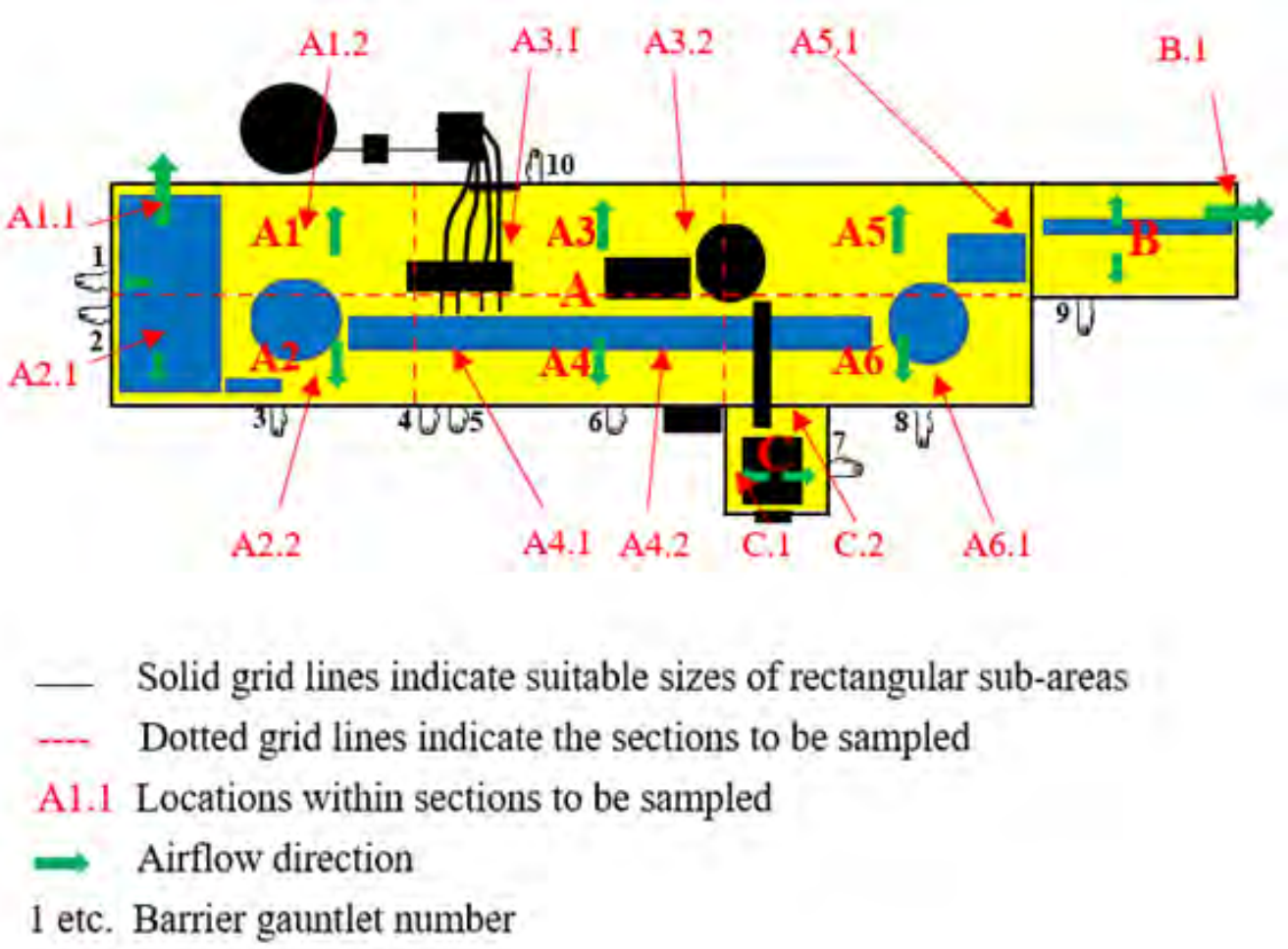



Figure 3 Isolator sampling sections and sampling locations

### 3.7 Results of airborne sampling in isolator

Sampling was carried out at each of the identified sampling locations, and the results shown in Table 4. It can be seen that all the samples had air concentrations below the Grade A limits set for particles ≥5 µm and ≥0.5 µm in the EU GGMP.

**Table 4** Isolator 'in operation' sampling data for particles ≥0.5 µm and ≥5 µm

| Sampling Location | Risk Score | Isolator Location and Activities | Sample Results (no./ $m^3$) | Pass/Fail EU Grade A Particles /$m^3$ ≥ 0.5 µm; 3 520 ≥ 5 µm; 20 |
|---|---|---|---|---|
| A1.1 Vial entry into isolator from cooling zone | 1 | **Location;** Centrally in vial entry zone from cooling tunnel **Activity;** Entry of vials into isolator and operator working in gauntlets 1 and 2 | **≥0.5 µm** 54 | Pass |
| | | | **≥5 µm** 5 | Pass |
| A1.2 No activity | 0 | **Location;** Midway between isolator barrier and vial supply turntable **Activity;** Full operational filling | **≥0.5 µm** 3 | Pass |
| | | | **≥5 µm** 0 | Pass |
| A2.1 Vial entry, accumulation and corrections | 21 | **Location;** Centrally in vial accumulation outlet zone **Activity;** Full operational filling and operator working in gauntlets 1 and 2 | **≥0.5 µm** 20 | Pass |
| | | | **≥5 µm** 1 | Pass |
| A2.2 Vial feed to filling head and corrections | 4.2 | **Location;** Centre of section in front of vial supply turntable **Activity;** Full operational filling and operator working in gauntlet 10 | **≥0.5 µm** 10 | Pass |
| | | | **≥5 µm** 1 | Pass |
| A3.1 Needles and tubing entry via rapid liquid transfer port | 0.004 | **Location;** Adjacent to rear of filling head manifold **Activity;** Set up with and operator working in gauntlets 4 and 5 and using rapid liquid transfer port | **≥0.5 µm** 22 | Pass |
| | | | **≥5 µm** 6 | Pass |
| A3.2 No activity | 0 | **Location;** Adjacent to closures workstation **Activity;** Full operational filling | **≥0.5 µm** 6 | Pass |
| | | | **≥5 µm** 1 | Pass |
| A4.1* Assembly of needles onto filling head | 0.004 | **Location;** Adjacent to front of filling head manifold in front of filling line **Activity;** Set up with and operator working in gauntlets 5 and 6 and placement of needles onto filling head manifold | **≥0.5 µm** 18 | Pass |
| | | | **≥5 µm** 3 | Pass |
| A4.1* Vial filling | 0.5 | **Location;** Adjacent to front of filling head manifold in front of filling line **Activity;** Full operational filling and operator working in gauntlets 5 and 6 | **≥0.5 µm** 44 | Pass |
| | | | **≥5 µm** 4 | Pass |
| A4.2* Filled vial stopper addition | 0.1 | **Location;** Adjacent to front of stoppering workstation in front of filling line **Activity;** Full operational filling and operator working in gauntlet 6 | **≥0.5 µm** 30 | Pass |
| | | | **≥5 µm** 1 | Pass |
| A4.2* Filled vial feed to stopper station and | 1.05 | **Location;** Adjacent to front of stoppering workstation in front of filling line **Activity;** Full operational filling and operator | **≥0.5 µm** 20 | Pass |
| | | | **≥5 µm** | Pass |

| | | | | |
|---|---|---|---|---|
| corrections | | working in gauntlet 6 | 0 | |
| A5.1 Vial exit and correction | 0 | **Location;** Rear of filled vial exit accumulation zone<br>**Activity;** Full operational filling and operator working in gauntlet 9 | **≥0.5 µm** 15 | Pass |
| | | | **≥5 µm** 1 | Pass |
| A6.1 Vial exit accumulation and correction | 0 | **Location;** Rear of filled vial exit turntable<br>**Activity;** Full operational filling and operator working in gauntlet 8 | **≥0.5 µm** 28 | Pass |
| | | | **≥5 µm** 0 | Pass |
| B.1 Vial exit from isolator | 0 | **Location;** Adjacent to vial exit, central<br>**Activity;** Full operational filling and operator working in gauntlet 9 | **≥0.5 µm** 20 | Pass |
| | | | **≥5 µm** 3 | Pass |
| C.1 Addition of closures to hopper | 0.25 | **Location;** Left hand side of closure hopper<br>**Activity;** Addition of closures to hopper | **≥0.5 µm** 101 | Pass |
| | | | **≥5 µm** 9 | Pass |
| C.2 Closures in hopper and feed to closures station corrections | 16.5 | **Location;** At interface with outfeed from hopper<br>**Activity;** Full operational filling and operator working in gauntlet 7 | **≥0.5 µm** 31 | Pass |
| | | | **≥5 µm** 3 | Pass |

*Locations with 2 different activities and both activities are sampled.

## 4. Discussion and conclusions

A method for carrying out the operational classification of cleanrooms and clean zones used in aseptic pharmaceutical manufacturing has been discussed in the first part (3) of a two-part article. The method was derived from the classification requirements and principles given in ISO 14644-1:2015 and Annex 1 of the EU GGMP but included more current expectations of the regulatory authorities. To demonstrate how this method can be used, an example is given in this second article, of a cleanroom and isolator used for aseptic filling of a liquid formulation into vials.

For the classification of the EU GGMP Grade C cleanroom, a method explained in the first article(3) to divide up the cleanroom into sampling sections, as required by ISO 14644-1, is explained. Also described is the method to identify the locations within each sampling section where the airborne concentration has to be measured. This is carried out using a formal risk assessment process that locates where the highest concentrations of particles caused by personnel activity are likely to be. The particle concentrations measured at these sampling locations are given and it was found that one result at the ≥5µm particle size exceeded the limit. This result was used to illustrate two problems that may be encountered during classification.

Firstly, although the air sampling identified a location that failed to meet the airborne concentration limit for particles ≥5 µm, the corresponding concentrations at the ≥0.5 µm particle size passed. The distribution of particle sizes within pharmaceutical cleanrooms has previously been investigated (9) and it was shown that the limit allocated to particles ≥5 µm of 29 000 per m³ in the EU GGMP is too stringent when compared to the corresponding concentrations of ≥0.5 µm particles and airborne microbial contamination. For a Grade C area, with a stated concentration limit for particles ≥5 µm of 29 000 per m³, a more appropriate limit would be 88 000 per m³. The origin of the particle concentration limits in ISO 14644-1, and hence Annex 1 of the EU GGMP, and the reasons for these discrepancies are discussed in the first article (3). It is more likely that the classification (and the subsequent monitoring) would fail at the ≥5 µm size than at the ≥0.5 µm size. It is therefore important that classification is undertaken at the ≥5 µm size as well as the ≥0.5 µm size in order to avoid failures during monitoring.

Secondly, although the cleanroom is non-UDAF, and the airborne particle concentrations are expected to be reasonably even throughout the cleanroom, this was not the case in the cleanroom investigated, and the airborne concentration at the location that failed was much higher than the average in the cleanroom. An investigation was carried out into the ventilation effectiveness of the location that failed. This was firstly undertaken by calculating the Performance Index (PI) using the airborne concentrations obtained during the classification. Further experimental work was also carried out with test particles to determine the Air Change Effectiveness (ACE) index. With both of these indexes providing similar values, the high concentration of particles at the failed location was considered to be caused by the location receiving significantly less clean air than the average in the rest of the room. This was successfully addressed by the installation of an additional air extract at this location.

The classification method derived in the first article was also illustrated by its application to an isolator. The same method used for the cleanroom was used to divide up the isolator base area into the sampling sections, as required by ISO 14644-1. Also, a formal risk assessment method is the expectation of the regulatory authorities to identify the sampling positions to be used during classification. A risk assessment method described in the first article (3) considers risk factors that relate to product or critical surfaces exposure area, time of exposure, type of ventilation, and associated operator activities, to identify locations where the risks of product contamination might occur. This approach was used and illustrated for the isolator. It should be noted that the number of sampling locations identified by risk assessment was greater than calculated by the ISO 14644-1: 2015 method, but in line with regulatory authority expectations.

It should be noted that the risk assessments methods employed in this article for non-UDAF cleanrooms and UDAF clean zones can also be used as the bases of determining the locations for environmental monitoring (microbial and non-viable) of both cleanrooms and clean zones during manufacturing.

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**Appendix A: Determination of sampling sections and sampling locations in non-UDAF cleanroom**

1. The cleanroom floor area (not including the isolator) should be divided into suitable sizes of rectangular sub-areas, starting with the largest area and working towards the smallest. These divisions are shown in Figure A1 by the black full lines, and the sub areas lettered A to E.

2. The floor surface areas ($m^2$) of sub-areas A to E are added together to obtain the total area of the cleanroom.
Total floor area of cleanroom = A(2.8 x 6) + B(4.2 x 5) + C(0.8 x 3.2) + D(1.7 x 4.2) + E(1.7 x 5.1)
= A(16.8) + B(21.0) + C(2.6) +D(7.1) + E(8.7)
= 56.2 $m^2$

3. Table A1 of ISO 14644-1 gives 12 as the minimum number of sampling locations for a cleanroom with a floor area of 56.2 $m^2$. If all sections are equal, this requires a minimum area of 4.7 $m^2$ per sub-area, and it is therefore assumed that any additional sub-areas should not be larger than 4.7 $m^2$.
4. The required number of sections in each rectangular sub-area of the floor is calculated using the following equation;

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

Where, 'minimum no. of sampling locations' is given in Table A1 of ISO 14644-1 and is 12.
Taking the sub-area B in the cleanroom, with the largest floor area of 21.0 $m^2$, as an example;

Number of sections in sub-area B = 21.0/56.2 x 12 = 4.5

This calculation should be repeated for all the 5 sub-areas A to E and the numbers rounded up to whole numbers (any number under 1 is assumed to be 1). These sampling sections total 15, and as this number exceeds the minimum number of 12 required by ISO 14644-1, it is acceptable. The dimensions of these divisions are shown in Figure A1 by use of red dash lines for the 15 sampling sections.

**Table A1** Number of cleanroom sampling sections

| Rectangle sub-section | Calculated number of sections | Specified number of sections | Dimensions of each section (m) |
|---|---|---|---|
| A | 3.6 | 4 | 1.4 x 3.0 |
| B | 4.5 | 6 | 1.67 x 2.1 |
| C | 0.6 | 1 | 0.8 x 3.2 |
| D | 1.5 | 2 | 1.7 x 2.1 |
| E | 1.9 | 2 | 1.7 x 2.55 |

5. Starting with the largest rectangle sub-area B (4.2 m x 5 m), this floor area (21 $m^2$) is divided by the rounded number of sections (5) to obtain the area of each section. However, with consideration for the rectangular shape of sub-area B, it would be most appropriate to utilise 6 sections which would readily fit the sub-area, each with an area of 3.5 $m^2$. This can be achieved by two options, namely, sections of either 1.67 m x 2.1 m, or 2.5 m x 1.4 m. With knowledge of the cleanroom activities associated with the generation of contamination, the most appropriate dimensions can be chosen that will place the activity locations closest to the centre.

The locations where personnel activities are carried out are shown as red spots in Figure 2. Also assessed is the amount of activity, and this is shown by the size of the red spot. Using this information, the most appropriate dimensions for each sub-area is considered to be the 1.67 m x 2.1 m configuration. This process is repeated for

the other sub-areas, with the exception of sub area A where there is only one possible configuration, and for sub-area C where there is only a single section. The resultant sampling sections within each rectangle sub-areas are shown by dashed red lines in Figure A1 as A1, A2, etc. and detailed in Table A1.

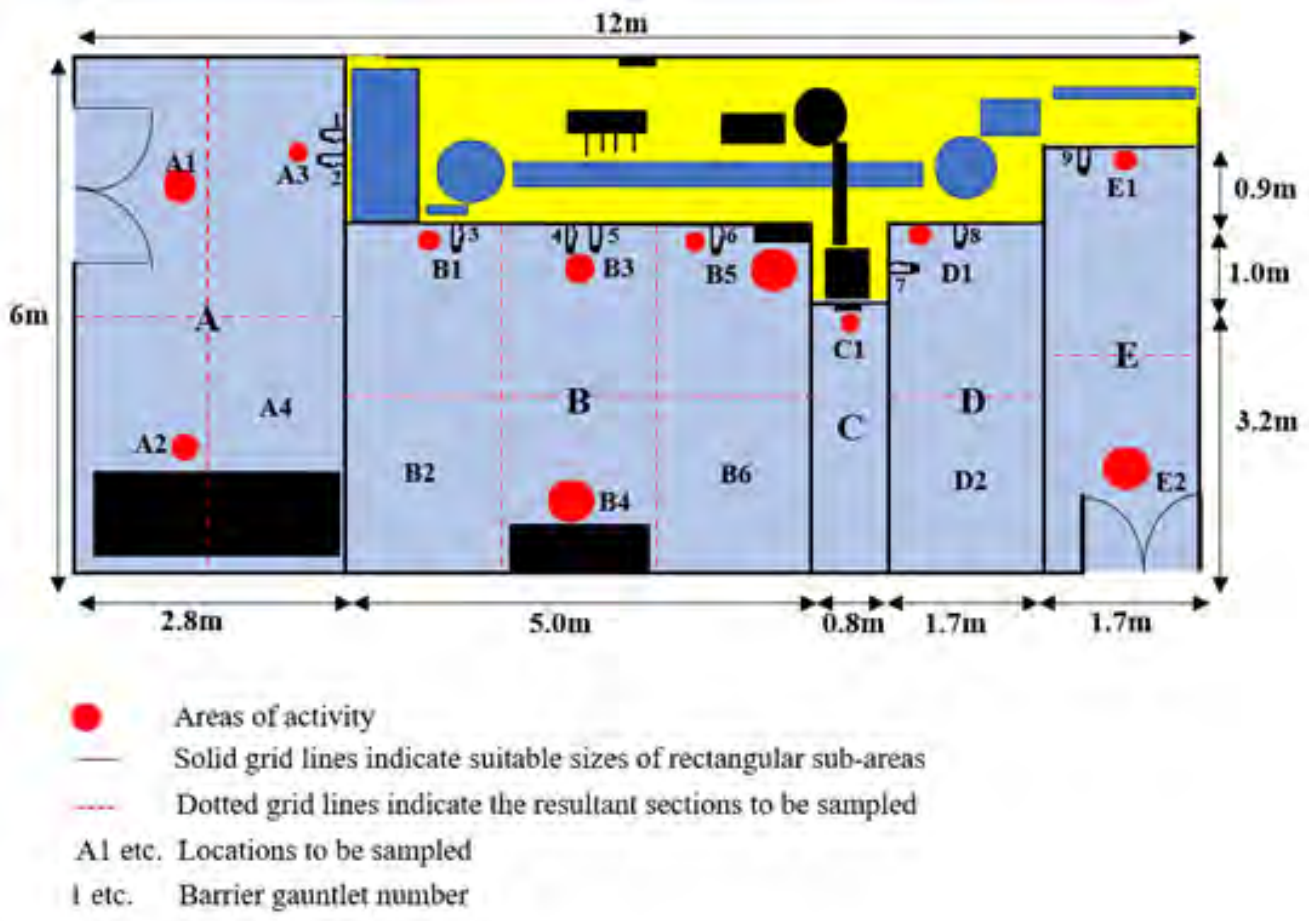



Figure A1 Cleanroom activity areas, sub-areas and sampling locations

**Appendix B: Determination of sampling sections and sampling locations in UDAF isolator**

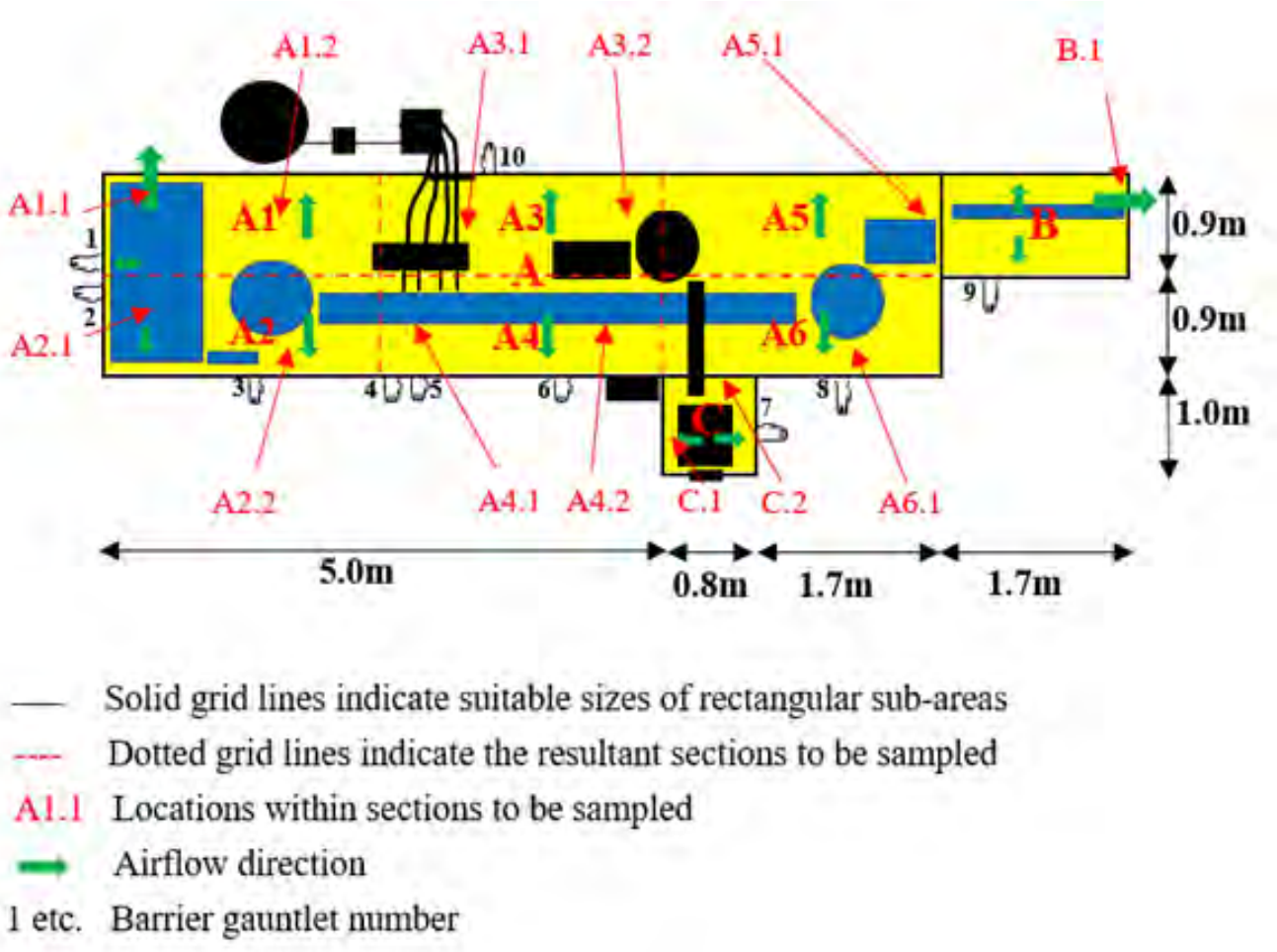
**Division of isolator base area into sampling sections**

The process described in Appendix A to determine the number of sampling sections for the cleanroom is similarly applied to the isolator. The isolator base area (15.83 m²) is divided into 3 rectangular sub-areas, A (13.5 m²), B (1.53 m²) and C (0.8 m²) as shown by black solid lines in Figure B1. The resultant dimensions of each section associated with each of the 3 rectangle sub-areas are shown in Table B1 and also included in Figure B1 by the dashed red lines. These total 8, and as this number exceeds the minimum number of 6 determined by ISO 14644-1, it is acceptable.

**Table B1** Number of sampling sections determined for the isolator

| Rectangle sub-area | Calculated number of sections | Specified number of sections | Dimensions of each section (m) |
|---|---|---|---|
| A | 5.1 | 6 | 2.5 x 1.4 |
| B | 0.6 | 1 | 1.7 x 0.9 |
| C | 0.3 | 1 | 0.8 x 1.0 |

The sampling locations within each of the 8 sections should be derived by further considerations of the activities within the isolators and use of a risk assessment. The risk assessment method is discussed in the next section and the resultant locations shown in Figure B1 as A1.1, etc. Also included are the other sampling locations discussed in section 3.5.



Selection of sampling locations in sections by risk assessment
The current expectation of the regulatory authorities is for sampling to be carried out in the isolator where the risks from airborne contamination are highest. The chosen locations should be in proximity to critical surfaces, such as where product, components or product contacting surfaces, are exposed to airborne contamination. An appropriate risk assessment method is described in the first article (3), which uses the risk factors and scoring system shown in Table B2.

Table B2 **Risk factors and scoring system for a risk assessment of a critical workstation.**

| Severity | | | | | Occurrence |
|---|---|---|---|---|---|
| **Personnel activity** | **Score** | **Ventilation type** | **Score** | **Surface exposed** | **Time exposed** |
| No activity | 1 | UDAF isolator or closed operation RABS | 1 | Area ($cm^2$) | Time (mins) |
| 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 following equation;
Risk = Severity x Occurrence
= (Personnel activity score x Ventilation type score x Surface exposed) x Time exposed

For the isolator manufacturing activities, the risk scores are calculated using this equation and are shown in Table B3.

**Table B3** Isolator manufacturing activities and risk scores

| Sampling location and manufacturing activity | Severity | | | Occurrence | Risk Score |
|---|---|---|---|---|---|
| | Personnel activity score | Ventilation type score | Surface area exposed ($cm^2$) | Time exposed (mins) | |
| A1.1 Vial entry into isolator from cooling zone | 1 (no activity) | UDAF 1 | 2 | 0.5 | 1 [a] |
| A1.2 No activity | 1 (no activity) | UDAF 1 | 0 | 0 | 0 |
| A2.1 Vial entry, accumulation and corrections | 1.05 (5% activity) | UDAF 1 | 2 | 10 | 21 [a] |
| A2.2 Vial feed to filling head and corrections | 1.05 (5% activity) | UDAF 1 | 2 | 2 | 4.2 [a] |
| A3.1 Needles and tubing entry via rapid liquid transfer port | 2 (continuous) | UDAF 1 | 2.1 [b] | 1 | 0.004 [c] |
| A3.2 No activity | 1 (no activity) | UDAF 1 | 0 | 0 | 0 |
| A4.1 Assembly of needles onto filling head | 2 (continuous) | UDAF 1 | 2.1 [b] | 1 | 0.004 [c] |
| A4.1 Vial filling | 1 (no activity) | UDAF 1 | 2 | 0.25 | 0.5 |
| A4.2 Filled vial stopper addition | 1 (no activity) | UDAF 1 | 2 | 0.05 | 0.1 |
| A4.2 Filled vial feed to stopper station and corrections | 1.05 (5% activity) | UDAF 1 | 2 | 0.5 | 1.05 |
| A5.1 Vial exit and correction | 1.05 (5% activity) | UDAF 1 | 0 | 1 | 0 |
| A6.1 Vial exit accumulation and correction | 1.05 (5% activity) | UDAF 1 | 0 | 10 | 0 |
| B.1 Vial exit from isolator | 1 (no activity) | UDAF 1 | 0 | 1 | 0 |
| C.1 Addition of stoppers to hopper | 1 (no activity) | UDAF 1 | 0.5 [d] | 0.5 | 0.25 [e] |
| C.2 Stoppers in hopper and feed to stopper workstation corrections | 1.1 (10% activity) | UDAF 1 | 0.55 | 30 | 16.5 [f] |

Notes
a. It is assumed that all contamination on the internal vial surface is subsequently transferred to the product solution.
b. Each of the 4 needles have a diameter of 0.3 cm and length 7 cm and so a worst-case horizontal area that is exposed is assumed to be 2.1 $cm^2$.

c. All contamination on the external needles surface is assumed to be subsequently transferred to the product solution during the filling when the needles external surface contacts the solution and is distributed equally into the 1000 vials associated with each needle.
d. It is assumed that the stopper internal area is 2 $cm^2$ but only 25% are actually exposed on the upper most layer of the hopper and approximately 50% of these will be exposed with the internal surface facing upwards. Therefore, an average surface area of 0.5 $cm^2$ is assumed.
e. All manipulations from cleanroom without intrusion into the isolator.
f. All contamination on the internal stopper surface is assumed to be subsequently transferred to the product solution.

Science & Technology Article

# Medical devices regulation countdown

Cliodhna McDonough
PHSS Management Committee

**This regulatory topic has become increasingly pertinent, ahead of the countdown to the EU's new medical device and in-vitro diagnostic rules, set to take effect on May 26, 2020 and 2022, respectively.**

Currently, there are more than 500,000 types of medical devices and in-vitro diagnostic medical devices on the EU market. Robust regulation that ensures a supply of safe devices, and allows monitoring of the introduction and use of medical devices, is essential.

The new Medical Device Regulation (EU) 2017/745 was published in the Official Journal of the European Union and came into force on May 5, 2017, replacing two existing Directives:
1. Regulation (EU) 2017/745 of the European Parliament and of the Council of April 5, 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC
2. Regulation (EU) 2017/746 of the European Parliament and of the Council of April 5, 2017 on in-vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU

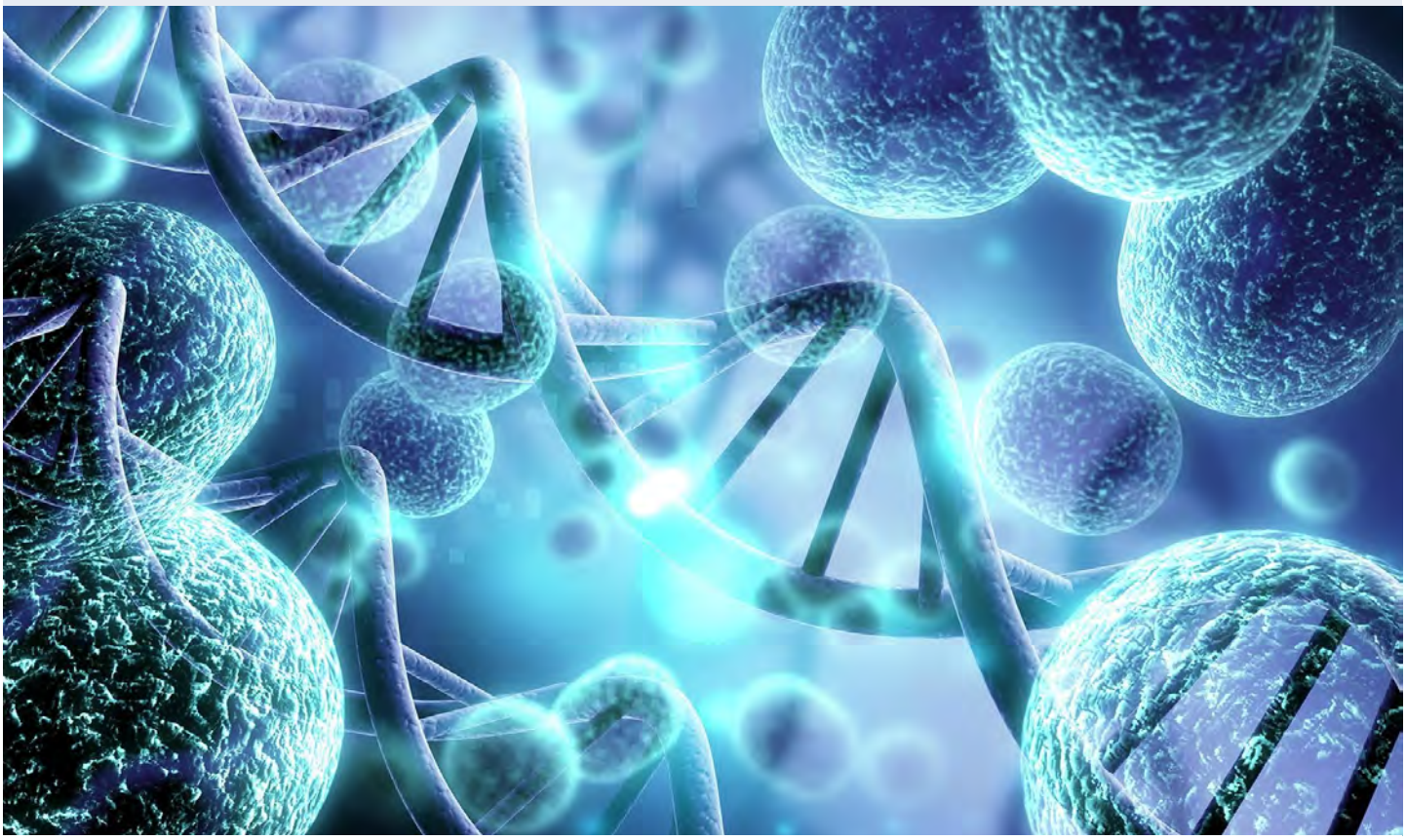
The new regulations apply after a transitional period. Namely, three years after entry into force of Regulation (EU) 2017/745 on medical devices (May 26, 2020), and five years after entry into force (May 26, 2022) of Regulation (EU) 2017/746 on in-vitro diagnostic medical devices.

Existing devices that have been CE marked under the current Medical Device Directive (MDD) must be recertified to abide by the new MDR.
The new regulations aim to enhance patient safety and modernise public health, by introducing an enhanced governance framework around the definition, supervision, traceability and risk-based classification system for medical device equipment

# Some key elements of the new legislation include:

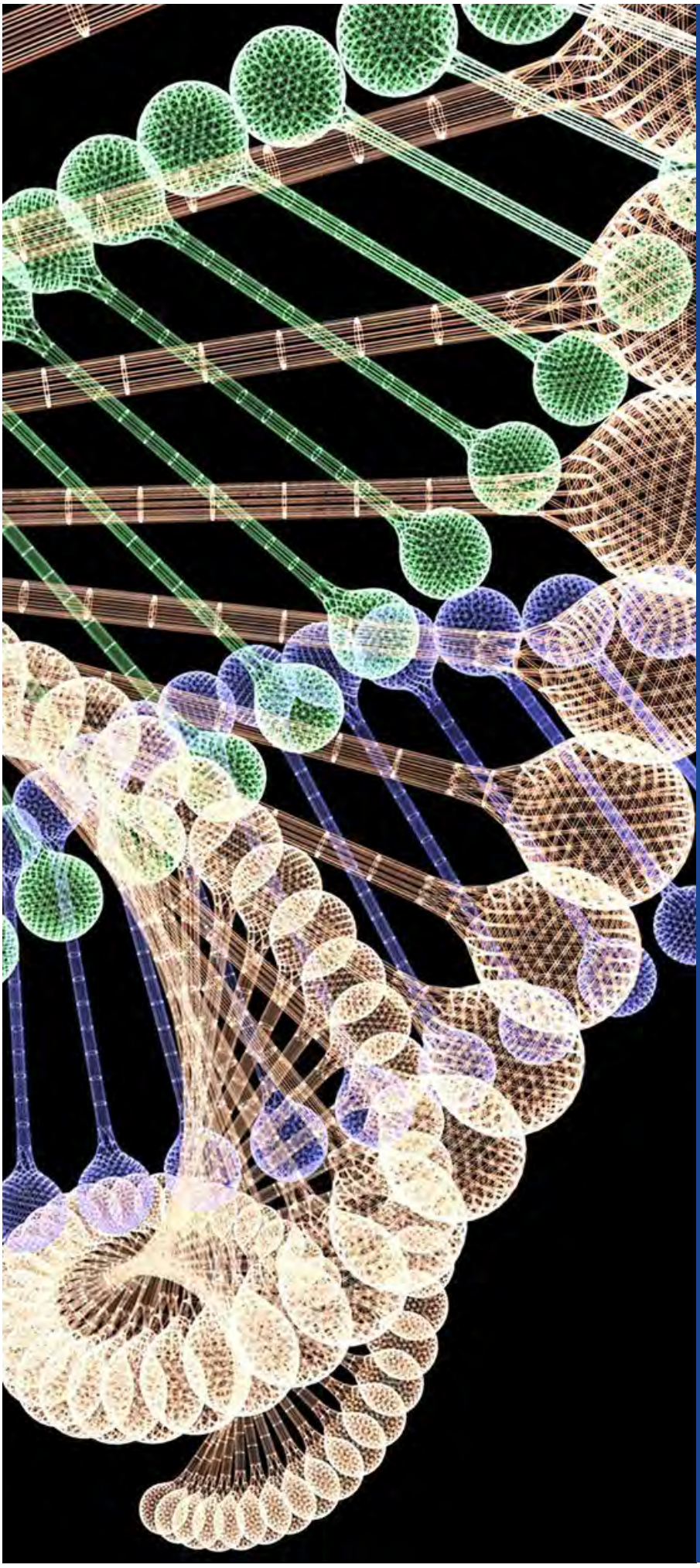
- Expansion of the definition of medical devices: The breadth of medical devices has been significantly expanded and includes certain products that previously did not fall under the definition of a medical device. For example, eye contact lens solution, liposuction equipment and laser equipment used for hair and tattoo removal.
- Enhanced vigilance and market surveillance: Once devices are available for use on the market, manufacturers will be obliged to collect data about their performance, and EU countries will coordinate more closely in the field of market surveillance. The new regulations will ensure vital information is easy to find through more stringent traceability measures. For instance, patients will receive an implant card with all the essential information, and a unique device identifier will be mandatory for every product.
- EUDAMED database: The Commission will establish a centralised EU database for the storage of information on medical devices (EUDAMED). This will facilitate the communication of both pre- and post-approval product information between economic operators, the Commission, member states and, in some cases, healthcare professionals and the public.
- Tighter regulatory controls: The new rules will impose tighter pre-market controls on high-risk devices, and apply a more rigid approach to the conduct of both clinical evaluation and the clinical investigation of clinical trials. The MDR will require device manufacturers to conduct clinical performance studies and provide evidence of safety and performance, proportionate with the risk associated with a given device. EU cross-border clinical trials will be subject to a single coordinated assessment. Stricter requirements on the use of hazardous substances will also be introduced, and device manufacturers will be required to collect and retain post-market clinical data, as part of the ongoing assessment of potential safety risks.
- Introduction of a risk-based classification system: A new system for risk classification, in line with international guidelines, will apply to in-vitro diagnostic medical devices, in addition to a wider medical device classifications definition for all products. While the classification system (Class III, Class IIa, Class IIb, and Class I) will be retained, some rules have been tightened. This may result in a significant number of product types – previously exempt from the regulations – now being included in the scope. Manufacturers will need to demonstrate that their medical device meets the requirements in the MDR and IVDR by carrying out a conformity assessment. The assessment route depends on the type and device classification.
- Post Market Surveillance System (PMSS): As part of their quality management system, manufacturers must also establish a PMSS, which should be proportionate to the risk class and the type of device in question. Manufacturers will have to report all incidents, injuries and deaths into an EU portal that will contain relevant data, so patients have access to safety-related information.
- Responsible Person (RP): Medical device manufacturers and authorised representatives will be required to designate at least one person with

responsibility for regulatory compliance; that person(s) must hold the prerequisite academic expertise and work experience in the field of medical devices.
- Financial compensation measures must be in place: The regulations require manufacturers to have measures in place to provide sufficient financial coverage in respect of their potential liability. Such financial coverage must be proportionate to the risk class, type of device and the size of the enterprise.



## Medical device manufacturers must prepare for regulatory changes

During the present transition period up to May 2020, manufacturers can choose to comply with either the existing MDD/AIMDD legislation requirements, or the new MDR.
However, as all medical device products marketed in the EU must eventually comply with the MDR, and any changes to pre-MDR products after full application of the MDR negate MDD compliance, companies are advised to define their strategies for regulatory transition.
Comparing and contrasting regulatory differences between the existing MDD and the new MDR is important. As it is highly likely for most legacy devices that a review of MDR requirements will identify regulatory issues that will need to be addressed for every device



Complying with the new MDR will prove a high task for most medical device manufacturers. Guidance and implementing measures under the current Directives will be reviewed by authorities over the next few years, in light of the new regulations.

At this present stage, the European Commission has a published list of legally non-binding guidance documents adopted by the Medical Device Coordination Group, to support the industry's efforts to apply relevant provisions of the MDR. These include consensus statements, informative documents and MEDDEVs for medical device manufacturers, authorised representatives, notified bodies, and competent authorities.

In addition, on October 30, 2018, the European Commission published the most recent version of the Borderline & Classification Manual. This document provides guidance on establishing the status of medical devices and IVDs, as well as their risk classifications. The current version, version 1.20, replaces version 1.19 released in April 2018.

The new medical device regulations in Europe present a huge challenge to manufacturers, but could also deliver improved confidence in the consistency and effectiveness of the EU regulatory process.

**Please note this article was first published in the Pharmatimes in February 2019**

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Science & Technology Article

# Risk & Science-Based Validation Of Cleanroom Garments

Authors: Milenko Pavičić and Thierry Wagner

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**ABSTRACT**

**Important quality attributes of cleanroom garments that will be worn during the manufacture of sterile medicines include: cleanliness; sterility; particle and microbial filtration efficiency; durability; usability; and comfort. Important risk factors related to cleanroom garment systems include gowning processes and related activities, such as laundering, packing, sterilization, repairs, storage, handling and logistics, as well as change management. Because many factors contribute to the overall quality, adequacy and suitability of cleanroom garment systems, a thorough and focused validation of cleanroom garments is critically important.**

**After providing a review of current and emerging regulations and standards, this article proposes a risk- and science-based quality-by-design approach for the development, implementation and validation of sterile cleanroom garment systems.**

**With this approach, more effort is spent at the front-end during the design phase as well as during design qualification. This will lead to designed-in risk reductions; enhanced scientific knowledge on selected technical solutions; and better awareness of limitations and residual risks. As a result, there should be fewer issues during cleanroom qualifications and process validations, leading to more effective routine operations as well as improved patient safety. The proposed approach, if implemented correctly, is not only the correct strategy to effectively control contamination risks related to people, but also an adequate response to the latest regulatory requirements.**

**Introduction**

Parenteral medicines are administered through injection, infusion or implantation, and must be sterile and pure to assure product safety. Therefore, the manufacture of parenteral medicines requires a controlled and validated clean production final packaging, resulting in a sterility assurance level (SAL) of at least $10^{-6}$. If terminal sterilization of the final product is not possible, aseptic manufacturing is the only alternative. In aseptic production, exposure of the sterile product to the using sterile ingredients to filling the product in its final container). In Europe, exposure of sterile products to the environment is only allowed in EU-GMP grade A zones placed in a grade B cleanroom or in an isolator[1].

Aseptic manufacturing of sterile products requires a high level of contamination control. Figure 1 shows the important elements that must be addressed in a contamination control strategy to assure purity of the products, sterility of sterile products and good microbiological quality of non-sterile products.

According to current Good Manufacturing Practices (GMP) guidelines, (EU[2], US[3], Japan[4], WHO[5,6]),processes, equipment, facilities and manufacturing activities should be managed in accordance with


Figure 1. Important elements of a contamination control strategy for sterile manufacturing.

Quality Risk Management (QRM) principles[7] that provide a proactive means of identifying, scientifically evaluating and controlling potential risks to quality. A quality-by-design approach[8], combined with effective risk management, should be applied to ensure the safety, quality and efficacy of sterile products. This comes from the belief that quality cannot be tested into the product, it can only be built into the design of products and the processes used to produce them. Risk

assessments must be performed to identify, assess, eliminate and control contamination risks from the design phase of an aseptic process; to monitor and detect contamination; and to establish process requirements and acceptance criteria for all elements of a sterile manufacturing process. Risk assessments should be documented as well as maintained and should include the rationale for decisions taken in relation to mitigating risks, discounting of potential risks and residual risk.

An important risk factor in sterile manufacturing is personnel. People can cause contamination of the production environment and products in many ways[9]. Contamination from people mainly consists of hair, skin cells, skin flakes, saliva, sebaceous matter, sweat, particles from clothing and many different exogenous particles and substances picked up in the environment. These contamination sources mostly contain different endogenous (i.e., commensal) and exogenous microorganisms present in numbers which vary from a few (e.g., skin cell) to thousands (e.g., sweat) or even millions e.g., saliva). Therefore, it is important that people working in an environment, where sterile products are manufactured, wear adequate cleanroom garments.

Contamination control related to people starts with good personal hygiene based on adequate hygienic procedures, such as hand washing and hand disinfection; aseptic behavior; aseptic skills; and a good working discipline. Adequate cleanroom garments, as well as undergarments[10] are critically important to minimize the risk of contaminating the environment or products with contamination generated by people. Cleanroom undergarments serve as a first barrier against contamination from people. The cleanroom garments must form a very robust barrier between the person and the environment. Additional protective measures, such as adequate cleanroom footwear, cleanroom socks, head coverings, face masks, eye coverings and (sterile) gloves, may be necessary or required from a regulatory point of view, to minimize the contamination risk.

Important quality attributes of cleanroom garments are cleanliness (free from chemicals, particles, pyrogens, fibers); sterility; particulate and microbial filtration efficiency; durability (tear, puncture, seam strength, abrasion); and comfort.

Personal protection against chemical or biological agents may also be a relevant quality attribute. A quality-by-design approach[8] combined with effective risk management[7], should also be applied to the design, selection and implementation of adequate cleanroom garments.

With the quality-by-design approach, more effort is spent at the front-end, in the design phase as well as in the design qualification, leading to designed-in risk reductions; better understanding of key aspects, limitations and residual risks; and fewer issues during final simulation runs and routine operations. This approach also creates the basis for proper root cause analysis (in case of issues) and adequate change management.

Other important risk factors related to cleanroom garments include gowning procedures and processes and activities related to cleanroom garments, such as laundering, packing, sterilization, repairs, storage, handling and logistics. Because many factors contribute to the overall quality, adequacy and suitability of cleanroom garment systems, a risk- and science-based validation of cleanroom garment systems is very important.

This article provides an overview of the various qualification, validation and monitoring aspects of cleanroom garments.

**Current Regulatory Guidance For Validation Of Cleanroom Garments**

Depending on the jurisdiction, aseptic production of sterile medicines must meet various regulatory requirements such as those set out in Annex 1 of the EU Guidelines to Good Manufacturing Practice[1] or in the U. S. Food and Drug Administration (FDA) Guidance for Industry on sterile drug production[3].

The FDA guidance outlines various recommendations for gowns, such as proper gown control; no unreasonable contamination risk to the gown; providing a barrier between the body and exposed sterilized materials; and preventing contamination from particles generated by, and microorganisms shed from the body. Gowns should be sterilized and non-shedding. Methods used to don each gown component in an aseptic manner should be detailed. Manufacturers should implement an aseptic gowning qualification program to assess the ability of a cleanroom operator to maintain the quality of the gown after performance of gowning procedures, including periodic requalification and microbiological monitoring of strategically selected locations on the gown.

The current EU-GMP guidelines require sterile (sterilized or adequately sanitized) garments to be provided for grade A/B areas and changing and washing to follow a written procedure designed to minimize contamination of clean area clothing or carry-through of contaminants to the clean areas. It further requires that clothing and its quality be “appropriate” without defining what would be considered appropriate. It also requires clothing to “be worn in such a way as to protect the product from contamination.” In terms of

attributes, “protective clothing should shed virtually no fibers or particulate matter and retain particles shed by the body”. Reusable garments are required to be cleaned and handled in such a way that the garment does not gather additional contaminants that can be shed later.
The current EU-GMP Annex 1 for the Manufacture of Sterile Medicinal Products[11] includes little guidance on cleanroom garment qualification except that it needs to be “appropriate”.

The new draft EU-GMP Annex 1 [12] published for consultation in December 2017 explicitly introduces the application of QRM principles and provides more details on gowning, including the requirement that gowning is part of a holistic contamination control strategy. It further requires that:

- Personnel are trained on gowning practices, which must be assessed
- Personnel are qualified through a successful aseptic process simulation test
- Microbial monitoring of personnel is performed

In addition, this new draft EU-GMP Annex 1 [12] requires that garments are visually checked for cleanliness and integrity. It also provides several new requirements regarding clothing of grade A/B; body parts that should be covered; the surfaces ofthe garment reduced to a minimum".

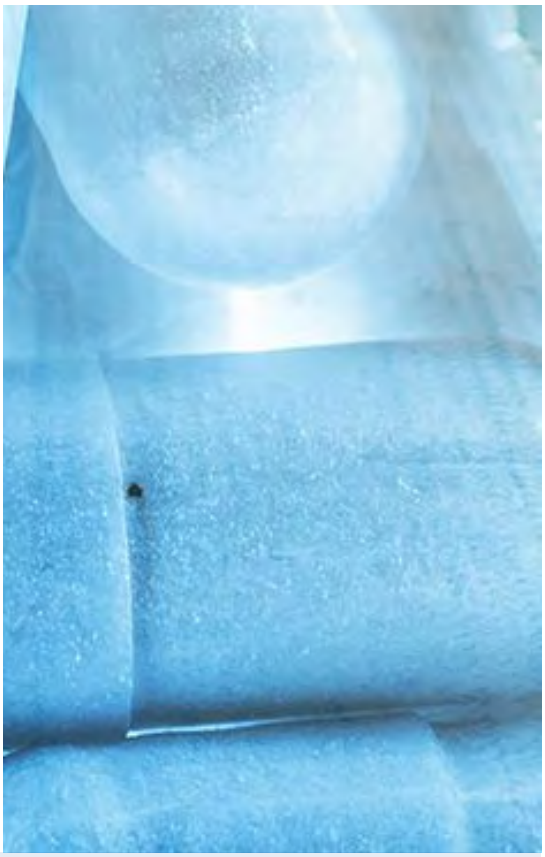
A key addition is the requirement that "reusable garments should be replaced based at a set frequency determined by qualification or if damage is identified". This requires manufacturers to produce data regarding the effect of However, there is little guidance on how to qualify those garments other than stating in subclause 4.11 that "clothing and its quality should be appropriate for the process and the grade of the working area. It should be worn in such a way The Japanese Guidance on the Manufacture of Sterile Pharmaceutical Products by Aseptic Processing[4], developed by a task force of Japanese experts, is an extensive document covering all areas of aseptic processing. It includes recommendations on gowning, operations, monitoring and controls that are comparable to other standards. It provides some interesting design recommendations on gowning and de-gowning areas and highlights the need to establish appropriate control procedures, including visual inspection. It also defines maximum allowable frequency of steam sterilization for reusable materials, such as aseptic gowns, to "ensure maintenance of specifications, safety, and intended functions after repeated exposure to steam at its maximum intensity".

ISO 14644-5[13] includes an informative annex B on cleanroom clothing requirements that provides some useful guidance on aspects to consider during qualification of such clothing. It provides guidance on barrier properties; evaluation of electrostatic properties; some guidance on fit and function; and helpful guidance on construction, finishing of seams and general design criteria that can be used to establish URS. It proposes use ofthe dispersal chamber or body box[14] as a simulation procedure to evaluate performance; recommends that shelf life of sterile packaging be determined; and includes considerations on thermal comfort and some guidance on cleaning, referring to IEST-RP-CC003.4[14] .

ISO 13408-1 [15] on general requirements of aseptic processing includes some general requirements on cleanroom garments but

does not provide much guidance on cleanroom garment system qualifications.
IEST-RP-CC003.4 "*Garment Systems Considerations for Cleanrooms and Other Controlled Environments*" [14] provides guidance on design, selection, specification, maintenance and testing of garment systems. It includes useful guidance on material qualification attributes, considerations on processing (cleaning, re-sterilization, etc.), gowning system specifications and quality management. Appendix B proposes various tests for assessments of particle penetration and garment cleanliness, which includes the well-known and very useful body box test[16,17] that allows simulation of the actual use of the garment, as well as the Helmke drum test[18] . Overall, IEST-RP-CC003.4[14] is the most useful document to support qualifications of cleanroom garment systems.

The EU general guidance on validation (GMP Annex 15[19]) provides the general framework that we will apply to the qualification of cleanroom garment systems.



## Validation Approach For Cleanroom Garments

The main stages of validation of equipment, facilities, utilities or systems are:
- Definition of User Requirements Specification (URS)
- Design Qualification (DQ)
- Installation Qualification (IQ)
- Operational Qualification (OQ)
- Performance Qualification (PQ)


*Figure 2. Overview of the different validation stages for cleanroom garments used in EU-GMP grade A/B cleanrooms.*

This approach is also appropriate for cleanroom garments. Some stages of the validation can focus mainly on the quality of the cleanroom garment itself, but in other stages, the other items of the cleanroom clothing system (i.e., cleanroom undergarments, footwear, socks, head coverings, face masks, eye coverings, gloves and other accessories) must be included. The packaging (sterile and non-sterile barriers) of the cleanroom garments should be part of the validation. An overview of the different validation stages and validation items for cleanroom garments is given in Figure 2. Each validation stage must be formally finalized before progressing to the next stage.

The GMP (EU, US, Japan) guidelines state that QRM[7] should be used for qualification and validation activities. If changes occur during the project phase or during commercial production (e.g., change of garment design, fabric, zipper type, packaging, laundering process or sterilization process), the risk assessments must be repeated to determine if additional validation must be performed. The way in which risk assessments are used to support qualification and validation activities should be clearly documented.

For critical goods and materials, such as cleanroom garments, it is also important to qualify the supplier. This qualification should provide an appropriate level of confidence that the supplier is able to supply cleanroom garments with consistent quality and acts in compliance with regulatory requirements. A supplier qualification should also include qualification of subcontractors, suppliers of base materials (e.g., fabric and garment accessories) and outsourced service providers (e.g., laundries and sterilization facilities).

**User Requirements Specification (URS)**

The specification for cleanroom garments should be defined in a User Requirements Specification (URS). The URS is a document that specifies requirements necessary and sufficient to create a feasible design, meeting the intended purpose of the material, equipment, utility or system. The URS may also include additional requirements, such as protection of people against chemical and/or biological agents. An example of a URS for cleanroom garments is given in Table I.

Cleanroom garments for use in EU-GMP grade A/B cleanrooms should adequately protect the environment and products against contamination from people. A trained and qualified operator, wearing a nonwoven polypropylene head cover, clean polyester two-piece undergarment, clean dedicated socks, double sterile gloves, a sterile face mask and sterile goggles, must be able to work at least three hours in the same set of cleanroom garments without causing unacceptable (cGMP) levels of contamination of the garments and the aseptic working environment.

| General | |
|---|---|
| 1. | The clothing must consist of a suit or coverall; a hood that can be firmly and reliably tucked into the neck of the suit; and boots. The hood may also be attached to the suit. It must be possible to tuck the trouser legs firmly and reliably into the boots. Boots shall have an anti-slip sole. |
| 2. | The garments may be reusable or single-use. |
| 3. | Unpacking and gowning must be conducted in an aseptic manner. |
| 4. | The garments must fit well and be available in different sizes (e.g. XS to XXL). |
| 5. | The clothing must be comfortable to wear (e.g., ease of movement, thermal, tactility). |
| 6. | Garments must be traceable, including number of laundering and sterilization cycles. |
| **Cleanliness and sterility** | |
| 1. | The garments should be clean (low levels of ions and organic extractables). |
| 2. | The garments shall not shed fibers, including filaments of the fabric and thread. |
| 3. | The garments shall not shed too many particles (≥ 0.5µm and ≥ 5µm). Maximum number of particles (≥0.5µm and ≥ 5µm) must be specified. |
| 4. | The garments shall be sterile (sterility assurance level ≤ $10^{-6}$). |
| 5. | The garments shall have a low pyrogenicity. |
| 2. | The sterile packaging must be robust and should endure common manipulations without jeopardizing the integrity of the packaging. |
| 3. | The sterile packaging shall be clean. |
| 4. | The sterile packaging shall not shed too many particles (≥ 0.5µm and ≥ 5µm) and shall not shed fibers. Maximum number of particles (≥ 0.5µm and ≥ 5µm) must be specified. |
| 5. | The space between the sterile packaging shall not contain too many particles (≥ 0.5µm and ≥ 5µm) and should not contain fibers. Maximum number of particles (≥ 0.5µm and ≥ 5µm) must be specified. |
| 6. | The sterile packed garments must be packed into a clean, low bioburden bag that is present in a firmly closed clean box. |
| 7. | The box and the last sterile packaging of the garments must be labeled with a clear description of the manufacturer, content, size, batch number, production date, expiry date, storage conditions and indicator that the sterilization was done. |
| **Identification** | |
| 1. | Each garment must have an unique identification number. |
| 2. | Labels must be firmly attached to the packaging or exist as an integral part of the packaging. |
| 3. | Labels must be clean and should not shed too many particles (≥ 0.5µm and ≥ 5µm) and should shed no fibers. Maximum number of particles (≥ 0.5µm and ≥ 5µm) must be specified. |
| 4. | Labels and printed text must be resistant to disinfectants that are commonly used (e.g., 70% alcohol, 6% hydrogen peroxide solution or a 600-ppm hydrogen peroxide vapor, 1000 ppm active chlorine). |

*Table I. Example of a URS for cleanroom garments used in EU-GMP grade A/B cleanrooms*

## Design Qualification (DQ)

During DQ, compliance of the cleanroom garment design with cGMP must be demonstrated and documented, and the requirements of the URS must be verified. The purpose of the DQ is to confirm that the selected cleanroom garment is qualified for the intended use. Therefore, it should also include tests to simulate the intended use. The DQ must be executed and authorized by suitably qualified persons who are knowledgeable enough to challenge the proposed design and its performance.

Following the model of design validations of sterile barrier systems described in ISO 11607-1[20] , it is recommended to split the DQ into four key areas: material qualification, performance testing, stability testing and usability evaluation. For reusable garments, reprocessing should be the subject of a separate DQ by the manufacturer and IQ-OQ-PQ by the supplier.

The **material qualification** includes the qualification of key characteristics and key properties of the materials and fabrics used, the cleanroom garments and the packaging.

**Performance testing** includes testing of the cleanroom garments and the packaging under simulated and standardized conditions using standardized test methods.

**Stability testing** must be performed to assure that key material characteristics and properties remain sufficiently stable during the life cycle. Characteristics and properties that change over time (e.g., deterioration of filtration efficiency of

garments due to repeated laundering and sterilization; wear of the garments due to multiple use; and changes to the integrity of the sterile packaging during storage due to long-term effects of gamma irradiation) should be validated under worst-case conditions. Information for material qualification, performance testing and stability testing normally should be provided by the supplier. It is important to verify that the data has been generated using validated and sound scientific methods.

The purpose of the **usability evaluation** is to assure that the cleanroom garments can be used with acceptable remaining contamination and safety risks due to the design of the garments and the established gowning, working and de-gowning procedures. The usability evaluation is typically done by the end-user however, suppliers can also evaluate their garments for the intended use and supply that data to users for verification and further mitigation of identified risks during gowning and operations. The concept of usability engineering and testing has developed into a well-accepted way to successfully reduce use-related risks by reviewing these risks during the design phase and systematically reviewing the design and use of the product against those identified risks (see also IEC 62366-1 on usability engineering of medical devices[21]).

Relevant items to be covered for each of the four areas are presented in Table II. A summary is given in Figure 2.

| Material qualification | Performance testing | Stability testing | Usability evaluation |
|---|---|---|---|
| *Cleanroom garments*<br>• Fiber and particle shedding<br>• Sterilization compatibility<br>• Sterility assurance level<br>• Pyrogenicity<br>• Particle filtration efficiency<br>• Bacterial filtration efficiency<br>• Porosity<br>• Surface resistivity<br>• Perforation resistance<br>• Mechanical strength<br>• Chemical resistance<br>• Protection against biological agents | *Cleanroom garments*<br>• Body box testing<br>• Helmke drum test | *Single-Use garments*<br>• Properties and characteristics at the end of shelf life<br>*Reusable garments*<br>• Properties and characteristics after maximum number of laundering and sterilization cycles | *Use scenarios*<br>• Transfer to classified storage area<br>• Readability of label<br>• Easy opening of packaging<br>• Aseptic unfolding of garments<br>• Gowning<br>• Donning additional accessories (e.g., sterile gloves, face mask, goggles)<br>• Work situations<br>• Safety, biosafety<br>• De-gowning |
| *Packaging*<br>• Fiber and particle shedding<br>• Bioburden<br>• Penetration of commonly used disinfectants<br>*Sterile packaging*<br>• ISO 11607-1 | *Sterile packaging*<br>• Influence of transport on integrity/sterility (ISO 11607-1) | *Sterile packaging*<br>• Packaging integrity/sterility at the end of shelf life (ISO 11607-1) | *Packaging*<br>• Aseptic presentation of garments (multiple layers) |

*Table II. The four key areas of the Design Qualification (DQ) for cleanroom garments used in EU-GMP grade A/B cleanrooms.*

**Reusable Versus Single-Use Cleanroom Garments.** The validation of reusable cleanroom garments is more complex and more extensive compared to single-use cleanroom garments. Repeated laundering, repeated sterilization, multiple use and repairs influence the quality of reusable cleanroom garments. This also means that the influence of these factors must be validated throughout the entire life cycle in the frame of the stability testing and the performance testing at the end of the life cycle. In addition, not only the supplier of the garments but also the cleanroom laundry, sterilization facilities and repair service must be qualified. Reprocessing should be the subject of a separate DQ by the manufacturer and IQ-OQ-PQ by the supplier.

### Installation Qualification (IQ)

The IQ for cleanroom garments is a formal check to verify if all required elements of the cleanroom gowning system are present. These include the gowning and de-gowning facilities; certificates of conformance and/or analysis; implementation of instructions from the supplier; standard operating procedures for gowning and de-gowning; logistical processes for the cleanroom garments and related accessories; and the operator training and qualification plan. Risk assessments that were executed as part of the DQ of the cleanroom garments should be finalized and risk controls should be

implemented.
In addition, it is important to verify that the correct materials have been received for performing the OQ and PQ (i.e., the correct cleanroom garments, correctly folded, in the correct packaging and correctly labeled). A summary of items to be included in the IQ is given in Figure 2.

**Operational Qualification (OQ)**
During the OQ, the objective is to qualify the gowning and de-gowning concept. For this purpose, all relevant steps of the gowning and de-gowning process, including logistics, should be qualified. In addition, the aseptic presentation of the garments should be qualified. To validate the aseptic gowning procedure, at least three independent, consecutive, successful visual and microbiological assessments for at least one person who is trained for aseptic gowning should be performed. The OQ should also include a formal assessment to verify that different work tasks can be executed properly from a practical point of view (e.g., moving, bending, stretching and lifting). It is recommended to perform this assessment with all available sizes of the cleanroom garments and with people of different body shapes. A summary of items to be included in the OQ is given in Figure 2.

**Performance Qualification (PQ)**
During PQ, the objective is to validate the performance of the cleanroom garment system when it is actually in use. The requirements specified in the URS must be complied with fully.

**The PQ of cleanroom garments consists of two stages**. In the **first stage** of the PQ, compliance with aseptic gowning procedures should be assessed and confirmed. This gowning qualification must involve both a visual and microbiological assessment. The visual assessment is to qualify that people don the cleanroom garments in a correct and aseptic manner, which shall be described in detail in a gowning procedure. After gowning, the microbiological quality shall be assessed by taking surface samples from several locations on the cleanroom garments, gloves, goggles and face mask. Locations must be determined based on a risk assessment.

Each person accessing an EU-GMP grade A/B environment must perform a gowning qualification. For the PQ, it is important to determine how many gowning qualifications are required to demonstrate compliance with the requirements. Typically, initial gowning qualification is performed three times for each person. It is important that adequately trained, qualified and experienced persons execute the PQ to exclude failures due to causes other than quality issues with the cleanroom garments.

The **second stage** of the PQ focuses on the validation of the microbiological quality of the gowned personnel with the garments and other accessories (e.g., gloves, face mask, goggles) during the actual work (e.g., aseptic compounding, aseptic filling, cleaning and disinfection, and other activities).

The second stage of the PQ also includes validation of the microbiological and particulate quality of the environment people are working in and the execution of aseptic process validations (i.e., media simulations or media fills). The number of runs for these

validations must be determined based on a risk assessment. Typically, these validations are performed three times. To exclude failures due to causes other than quality issues with the cleanroom garments, it is important that adequately trained, qualified and experienced personnel execute the PQ in areas with an excellent quality
history.

The PQ is typically done under worst-case conditions. These worst-case conditions must be determined based on a risk assessment. Also, the actions that should be taken if established criteria are not met during the PQ, must be defined before executing the PQ. Only after a successful PQ can the cleanroom clothing system be formally implemented. A summary of items to be included in the PQ is given in Figure 2.

**Revalidation And Change Management**

The cleanroom clothing system should be evaluated at an appropriate frequency (e.g., annually or biennially) to confirm that it remains in a state of control. Gowning qualifications shall be repeated at least annually and even more frequently in cases where there is doubt about the quality of the aseptic gowning process or aseptic gowning skills of specific persons. The cleanroom clothing system is included in validations which must be performed periodically (i.e., cleanroom qualifications under dynamic conditions and aseptic process validations).

Changes must be reviewed critically and may lead to revalidations that are more or less extensive, depending on the type of change. Properly and well documented DQs, as well as IQ-OQ-PQ, are the basis for successful change management.

**Monitoring**

Personnel monitoring must be part of the environmental monitoring program[1,3,22]. The microbiological quality of cleanroom garments for persons working in a grade A/B environment must comply with the EU-GMP[1] grade B limit for surface samples (i.e., the action limit is 5 CFU/contact plate). Alert limits are usually lower (e.g., 2 or 3 CFU/contact plate). Cleanroom garments, face masks, goggles and gloves are typically sampled at the conclusion of activities in a grade A/B area, but just before leaving the area. For this "exit monitoring," contact samples are taken from different locations. Sample locations must be determined based on a risk assessment. Commonly selected locations for exit monitoring are shown in Figure 3. After sampling, the person must leave the area to prevent spreading contamination due to medium residues present on the cleanroom garments.

Gloves should be sampled after performing activities in a grade A environment to verify the quality of the aseptic conditions and aseptic handling. Gloves of operators working in a grade B environment should also be monitored during each work shift. Gloves are typically sampled with a frequency ranging from once to multiple times per work shift.

In addition to personnel monitoring, samples from several locations in the cleanroom should be taken to determine if the production environment and processes are in control. Sampling is typically performed during (passive and active microbiological air sampling and particle counting) or at the conclusion (surface sampling) of operations but may also be performed under static conditions (i.e., in the at-rest state of an area), to verify cleanliness.

In the case of non-conformities, assessments must be done to determine root causes. The cleanroom clothing system as a potential root cause should be included in these assessments.

It is also recommended to assess if gowning procedures, cleanroom behavior guidelines and aseptic procedures are followed correctly. These visual assessments should be done on a regular basis.

**Conclusion**

A science- and risk-based quality-by-design approach for the development, implementation and validation of sterile garment systems for EU-GMP grade A/B aseptic processing areas is not only the correct approach to effectively control contamination risks related to people, but also an adequate response to the latest regulatory requirements. The new EUGMP Annex 1 draft is based on QRM principles and introduces the concept of a holistic contamination control strategy that considers all aspects of contamination control over the entire life cycle based on thorough technical knowledge and sound process know-how. Considerable efforts will be required by manufacturers to update their technical files, with cleanroom garments being just one of the many aspects.

With a risk-based quality-by-design approach applied to cleanroom garment systems, more effort is spent at the frontend, in the design phase, as well as in the design qualification. This will lead to designed-in risk reductions; better scientific knowledge of key aspects, attributes, limitations and residual risks of the selected technical solutions; and fewer issues during cleanroom qualifications, process validations and routine operations. In case of failures, it can be difficult to determine the root cause or the elements that have failed. That’s why a quality-by-design approach, with focused and extensive design qualifications for each element, is the only way to successfully and systematically reduce the risk of failure. This approach also creates the foundation for adequate and risk-based change management.

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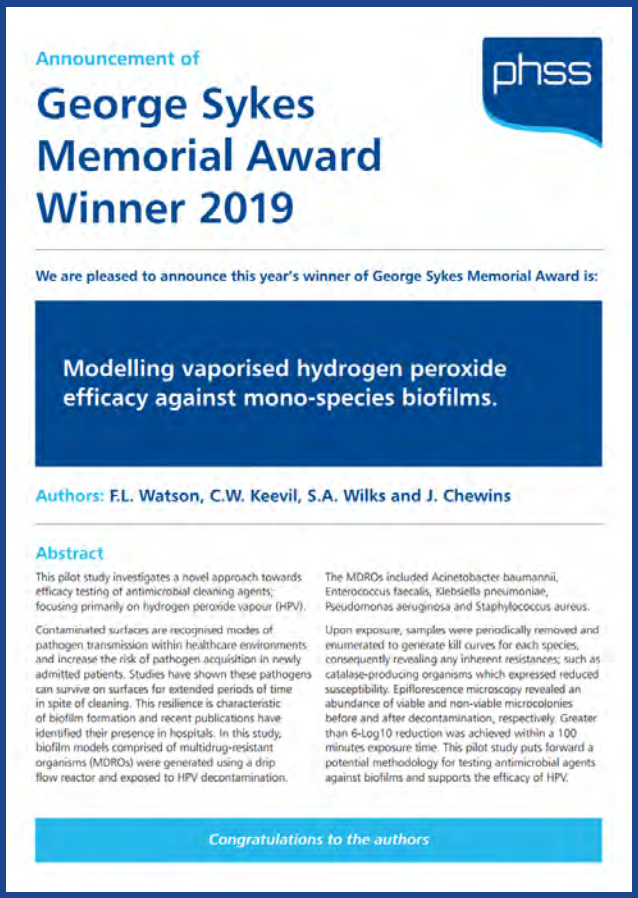
# George Sykes and John Sharp Memorial Awards 2019



Announcement of

George Sykes Memorial Award Winner 2019

phss

We are pleased to announce this year's winner of George Sykes Memorial Award is:

Modelling vaporised hydrogen peroxide efficacy against mono-species biofilms.

Authors: F.L. Watson, C.W. Keevil, S.A. Wilks and J. Chewins

Abstract

This pilot study investigates a novel approach towards efficacy testing of antimicrobial cleaning agents; focusing primarily on hydrogen peroxide vapour (HPV).

Contaminated surfaces are recognised modes of pathogen transmission within healthcare environments and increase the risk of pathogen acquisition in newly admitted patients. Studies have shown these pathogens can survive on surfaces for extended periods of time in spite of cleaning. This resilience is characteristic of biofilm formation and recent publications have identified their presence in hospitals. In this study, biofilm models comprised of multidrug-resistant organisms (MDROs) were generated using a drip flow reactor and exposed to HPV decontamination.

The MDROs included Acinetobacter baumannii, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus.

Upon exposure, samples were periodically removed and enumerated to generate kill curves for each species, consequently revealing any inherent resistances; such as catalase-producing organisms which expressed reduced susceptibility. Epifluorescence microscopy revealed an abundance of viable and non-viable microcolonies before and after decontamination, respectively. Greater than 6-Log10 reduction was achieved within a 100 minutes exposure time. This pilot study puts forward a potential methodology for testing antimicrobial agents against biofilms and supports the efficacy of HPV.

*Congratulations to the authors*

# 2019 Winner for George Sykes Memorial Award

**Authors: FL Watson, CW Keevil, SA Wilks and J Chewins**
We are very excited to announce that James Tucker has been voted our first John Sharp Memorial Award winner for the best Science & Technology Paper 2019.

**Title: Modelling vaporised hydrogen peroxide efficacy against mono-species biofilms.**

Please click on the link in order to download a copy of the winning paper.

**Click here to download**

# John Sharp Memorial Award 2019 Winner Announcement

**We are very excited to announce that James Tucker has been voted our first John Sharp Memorial Award winner for the best Science & Technology Paper 2019.**

Please click on the link in order to download a copy of the winning paper.

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Announcement of

John Sharp Memorial Award Winner 2019

We are pleased to announce this year's winner of John Sharp Memorial Award is:

The proposed changes to Annex 1: Considerations for the cleaning and disinfection processes.

Author: James Tucker

Abstract

The long-anticipated revision to Annex 1 (from the EudraLex Guidelines to Good Manufacturing Practices1 requirement for sterile drug manufacturing) is now closed for public consultation. Nearly 3 years after the initial announcement and more than 10 years since the previous revision was published, it is reasonable to suggest that an update is certainly due, and this new document delivers a considerable increase in the depth and breadth of Annex 1. Whilst many debates will continue around the timing of its publication, implications, perceived meaning and level of implementation expected from the final version post consultation; this article explores the author's view of the potential impacts specifically affecting cleaning and disinfection regimes. The following review will focus on a few key areas of the Annex understood to significantly affect the use of disinfectants within the sterile manufacturing areas once implemented. As a holistic, risk-based approach to contamination control is fundamental to the Annex, this is a critical aspect for consideration when reviewing the proposed updates.

*Congratulations to James*

# Regulatory update

Welcome to this quarter's Regulatory Update compiled by Malcolm Holmes.

**Each column has the monthly highlight for Europe in the first section and USA & International are accessed by scrolling further down the page**

Should you wish to receive a full copy supplied with hyperlinks then please go to the PHSS Website and join as a member.

## October 2019

**Europe**

- Ranitidine containing medicinal products
- Advice to companies on steps to take to avoid nitrosamines in human medicines
- Change of name of liposomal medicines at high risk of medication errors (update)
- Implementation of the new Veterinary Medicines Regulation
- MHRA Blog - Supply chain security: part 1 - introduction

October-2019-europe-regulatory-update-detail
Click for detailed Europe review

## November 2019

**Europe**

- New Guide to the quality and safety of tissues and cells for human application
- EMA encourages companies to submit type I variations for 2019 by end of November 2019
- European countries increase commitment to responsible antibiotic use in animals
- EMA Management Board: highlights of October 2019 meeting
- Dialogue with Chinese authorities on medicine regulation
- How to ensure that novel analytic methods are fit for decision-making
- MHRA Process Licensing: useful information
- MHRA - Digital Health and Pharma 4.0

November-2019-europe-regulatory-update-detail
Click for detailed Europe review

## December 2019

**Europe**

- New approach to extraneous agents testing of immunological veterinary medicinal products (IVMPs)
- Pharmeuropa 31.4 released
- Updated Q&A on “Information on nitrosamines for marketing authorisation holders”
- ‘Regulatory science to 2025’: live broadcast of post-consultation workshop
- Tripartite meeting held to discuss regulatory approaches for the evaluation of antibacterial agents
- Regulators' Advice Can Make a Difference: European Medicines Agency Approval of Zynteglo for Beta Thalassemia
- Dutch Authorities hand over final building to EMA in Amsterdam
- EMA Mid-year report 2019
- HMA/ EMA task force on Big Data

December-2019-europe-regulatory-update-detail
Click for detailed Europe review

Please scroll down for USA and International

# October 2019

**USA**

- NMDA impurity in certain ranitidine products
- Wholesale distributor verification requirement for saleable returned drug product
- Bringing remaining approved OTC medically important antimicrobial drugs used for animals under veterinary oversight
- Reorganization of the Office of New Drugs with Corresponding Changes to the Office of Translational Sciences and the Office of Pharmaceutical Quality
- TCPro simulates immune system response to biotherapeutic drugs
- Evaluation of Internal Standard Responses during Chromatographic Bioanalysis: Q&A

**International**

**Australia**

- Updated Guidelines for making an offer of enforceable undertaking to the TGA
- TGA business plan 2019-20

**PIC/s**

- Focused stakeholders consultation on revised draft PIC/S GMP Guide Annex 2A (Manufacture of Advanced Therapy Medicinal Products for Human Use) and Annex 2B (Manufacture of Biological Medicinal Substances and Products for Human Use)

**WHO**

- Proposal to discontinue the test for undue toxicity (chapter 3.7)

# November 2019

**USA**

- Clinical immunogenicity considerations for biosimilar and interchangeable insulin products
- Transdermal and topical delivery systems - product development and quality considerations

**International**

**Australia – Therapeutic Goods Administration (TGA)**

- Safety review of coumarin in topical listed medicines
- Consultation: Draft standards for faecal microbiota transplant (FMT) products

November-2019-row-regulatory-update-detail

Click for detailed USA & International review

# December 2019

**USA**

- Clinical immunogenicity considerations for biosimilar and interchangeable insulin products
- Transdermal and topical delivery systems - product development and quality considerations

**International**

**Australia – Therapeutic Goods Administration (TGA)**

- Safety review of coumarin in topical listed medicines
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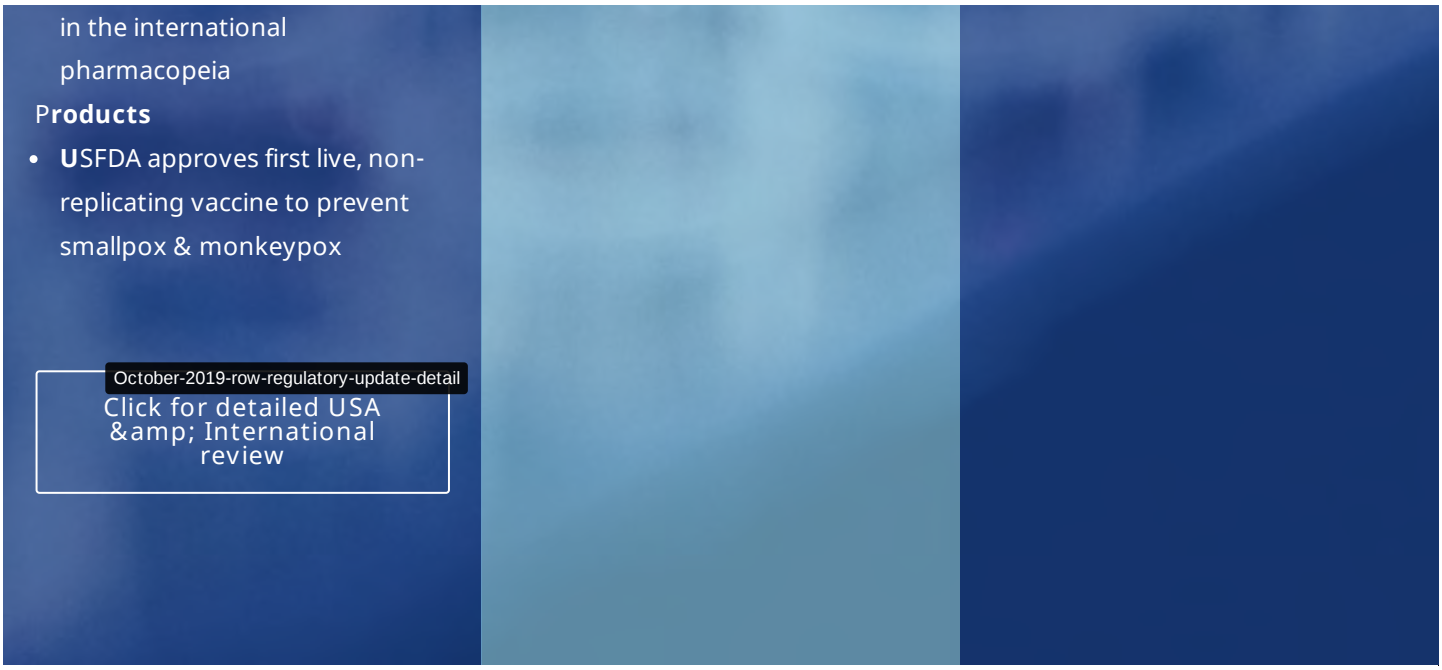
December-2019-row-regulatory-update-detail

Click for detailed USA & International review




Further information on these and other topics can be found in recent versions of the “Regulatory Update” on the PHSS website and on the websites of the relevant regulatory bodies and international organisations. We hope that our readers find our reviews to be both informative and helpful in keeping up to date with pharmaceutical legislation and regulatory guidance.

GMP Update is compiled by Malcolm Holmes an independent GMP consultant and member of the PHSS Management Committee

**Full regulatory update found on PHSS website**



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# Editorial and PHSS News

## Editorial (Guest)

By Guest Malcolm Holmes, PHSS Pharmaceutical Regulatory SME

**It's what we do**

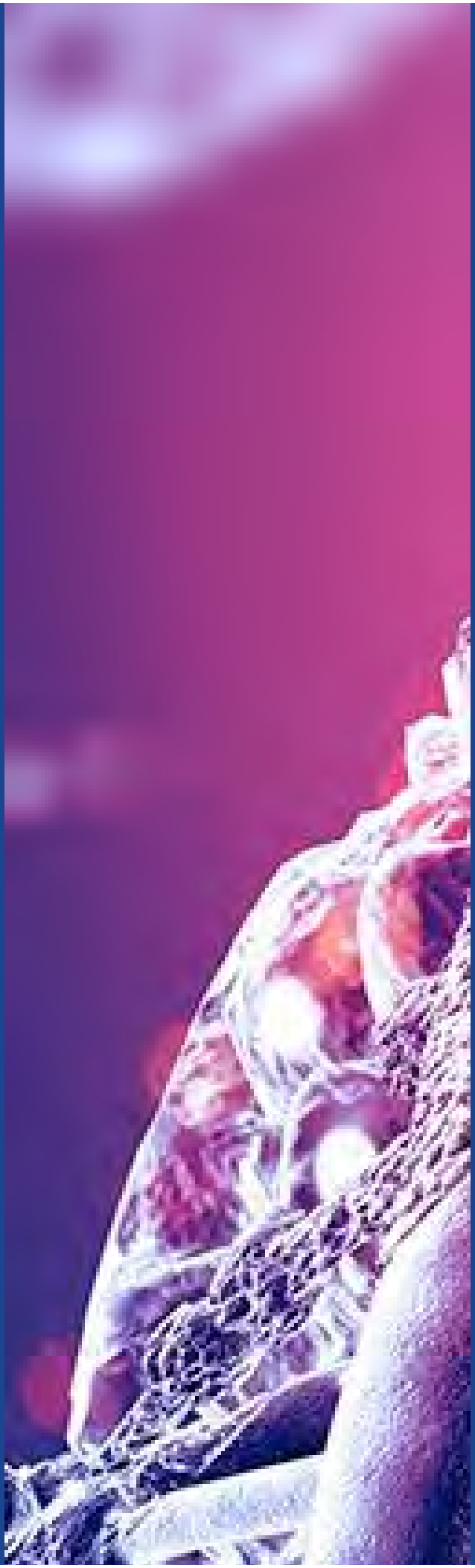
In the Pharmaceutical industry we discover new molecules with a potential to be used as medicines. Then we commence the long journey to turn that discovery into safe and effective medicines. All the time we should strive to gather knowledge which helps us understand and improve our products and processes, to ensure that the resultant medicines remain safe, effective and available to healthcare professional and patients.

**But there are hurdles to overcome**

**Manufacturing issues leading to shortages**

When a medicine becomes not available it is consequently of no use to the patient. From time to time firms experience manufacturing issues which can cause them to restrict or cease supply of a medicine until such issues are resolved. Regulators have in recent years placed a significant focus on need for reporting such shortages caused by such manufacturing issues and then steps being taken to alleviate the shortage as well as to prevent it happening in the future.

**Product recalls arising from previously unknown product and process issues**



Where a medicine is found to be sub-standard a common outcome following risk-based discussions between regulators and manufacturers is a product recall. Usually such events involve an individual batch or specific batches of a product which may be implicated. From time to time however the impact is much wider. For example, in 2007 it was noted that tablets of an antiviral drug VIRACEPT® (nelfinavir mesylate) exhibited a bad odour. This odour arose from ethyl mesylate – a genotoxic chemical. Product was recalled world-wide and the product authorisation in the EU was withdrawn by EMA and not reinstated until October that year. The investigation into the cause of the contamination of the product concluded that it had been caused by a reaction between ethanol, used in a cleaning process which was left in a manufacturing vessel subsequently used to hold methanesulphonic acid. An event such as this was very rare indeed.

More recently, in the summer of 2018 a U.S. drug manufacturer told the US FDA that it had discovered the impurity N-nitrosodimethylamine (NDMA) in a valsartan active pharmaceutical ingredient made its Chinese API supplier. Subsequently the FDA found NDMA and a similar chemical, NDEA, had contaminated certain valsartan APIs for years. The agency said the two contaminants, (both probable carcinogens) can form through specific and commonly used manufacturing processes.

Regulators worldwide were alerted and conducted their own investigations as well as jointly with the USFDA. Similar problems were identified with other Sartans and then in recent weeks with Ranitidine products. Multiple batch recalls have been undertaken as precautionary measures. These products are commonly used medicines and are often taken over

long time periods by patients thereby potentially increasing the risk of harm.

On 11 November 2019 EMA issued a Q&A document on "Information on nitrosamines for marketing authorisation holders". This 12-point Q&A indicates that all marketing authorisation holders (MAHs) of all human medicinal products containing chemically synthesised active pharmaceutical ingredients (APIs) should work with the manufacturers of their APIs and finished products in order to evaluate the risk of nitrosamines being present in their products, and take appropriate risk mitigating measures.

A risk-based prioritisation of products to be reviewed should be used and the outcome of the risk evaluation of all products should be concluded at the latest within 6 months of the publication of the Q&A i.e. by 11 April 2020.

Section 12 of the Q&A covers currently identified root causes for presence of nitrosamines. In this section the EMA has highlighted the involvement of agents such as sodium nitrite, and recovered solvents, but also reported that the use of certain packaging materials may contribute to contamination. Section 12, Point 8 notes that "Nitrosamine contamination has been observed by one MAH in a finished product stored in blister" and "the MAH has hypothesised that the lidding foil containing nitrocellulose printing primer may react with amines in the printing ink to generate nitrosamines, which would be transferred to the product under certain packaging process conditions".

Since that time Metformin tablets (used as a regular medicine for diabetes) have also been potentially implicated.

**Next steps**

Readers attention is drawn to the EMA Q&A document which is available on the EMA website and which should be urgently and carefully reviewed for scope, methodology and timelines. The timeline for compliance is short and the clock has been ticking since 11 Nov 2019.

Further information can be found in the Regulatory Updates in this and previous editions of EJPPS as well as on the PHSS and Regulators websites. PHSS has also issued two Impact Statements on this topic the first in May 2019 and most recently in Nov 2019.

**Contact Editor-in-Chief**

# PHSS News - Chair of The PHSS

Dear Readers

2019 has been an exciting year for PHSS, with many developments and changes, including a new chair!We have been able to facilitate two outstanding conferences in June and September, together with the Aseptic Processing Workshop and QP Forum. Our focus remains on bringing the opportunity for industry discussion and engagement, by sharing knowledge, regulatory guidance and GMP practical guidance.

We have continued to focus on delivering practical guidance through our special interest groups, this will be maintained under James Drinkwater's leadership as we go into 2020.

In September we launched the new look EJPPS. I am very proud that we are now able to bring the digital version to the industry. To view our latest edition please visit website.

I would like to thank Tamsin Marshall, PHSS Operations Manager and Kay O'Hagan, EJPPS Editor in Chief for all the hard work, drive and dedication to deliver this new platform for the journal.

In 2019, we have been honoured to welcome some new faces joining our management committee as co-opted members, this has brought experience and diversity to enhance the PHSS team.

It is an honour to be able to step into the chair position, taking over from James Drinkwater after his 10 years in the post. James will, of course, continue to be very involved with the PHSS, staying on the management committee as well as leading the Aseptic Processing Special Interest Group (SIG). For more details on our 2019 Event and SIG summary please see the full update from James.

On behalf of the PHSS, I would like to wish you all the best wishes for the festive season.

**Jenni Tranter, PHSS Chair**
**November 2019**

# PHSS Activities and initiatives EJPPS report December 2019

**By James Drinkwater - Head of PHSS Aseptic processing and Containment Special interest group and Annex 1 Focus group.**

**PHSS QP Forum in Wales November 2019**

This year the PHSS QP Forum was supported by the MHRA with a Key note presentation from Phillip Rose a senior GDMP Inspector. The program of presentations were high quality leading to a sold out event with the best ever attendance. To build on this success and transition from a discussion forum for QPs to a full conference the PHSS will introduce a new venue for 2020 will enhanced conferencing facilities.

**PHSS initiative in preparation of Clarity on GMP Guidance notes**

Focus groups are now formed for each of the Clarity on Guidance note topics with supporting scope and content for each topic prepared. Significant progress is expected in 2020 with a target to complete as many as possible by the annual Aseptic processing & Bio-contamination special interest group meeting the day before the Annual PHSS Challenges in Sterile product manufacturing conference in June, Manchester UK.
Current topics to be covered are:

1.Assurance of Sterility for Indirect product contact parts: Vial/ Syringe filling Stopper/ plug pathway. Published but update in progress to cover points to consider for existing


filling lines. Second publication to be in collaboration with Biophorum: BPOG.

2.Environmental Classification, Qualification, Monitoring of GMP controlled areas. Connects all three stages.

3.Localized Uni-Directional Airflow: L-UDAF definition and alignment with ISO UDAF definitions and Annex 1 Grade A air supply definition: includes case study examples.

4.Barrier Leak rates and Leak integrity applied to Pharmaceutical Isolators at Filling line scale.

5.Continuous particle monitoring: Compliance and event monitoring with trend predictor. Facilitates Aseptic process filling from start of monitoring before a one cubic metre compliance sample volume is reached. Trending facilitates incidence rates analysis as KPI.

6.Risk assessment in setting EM sample locations in Isolator filling lines at design stage to facilitate sample system technical integration. Aligns with BPOG EM risk assessment guidance for full cleanroom/ facilities.

7.Airflow visualisation in controlled areas: CFD: Computational Fluid Dynamics (DQ) Smoke studies visualisation of airflow patterns (OQ) and LR Method contamination transfer challenge study (PQ).

8.Moist heat sterilisation key points to consider including avoiding Wet loads in porous load sterilisation.

9.Glove Management strategy for Barrier systems: Isolators and RABS Gloves based on Life cycle approach: Selection, Visual and physical integrity testing, Risk assessment for post batch production leak integrity test failure with consideration of impact.

10.VHP/vH202 bio-decontamination of loads:
A) Loads in: Isolators and Material transfers

B) Bio-compatibility of H202 residuals, impact on biological products and associated impact studies.

11.Definitions of ‘Open and Closed’ applied to aseptic processing.

12.NTT: No-touch-transfer of pre-sterilised ready to use (RTU) product containers into Grade A filling environments following GMP and QRM principles. Guidance covers requirements for pre-sterilised container suitability for NTT (Container supplier and supply chain qualification) and qualification of application in a filling line that applies NTT for Sterile product Aseptic process filling.

13.Contamination Control Strategy: CCS. Annex 1 compliance requirements. Guidance on preparation of a CCS based on three steps: Definition of application and principle contamination control measures for the facility and process by design. Identification of contamination vectors applied to the process/ facility design with associated risk and contamination control strategy based on technical and procedural control measures for each vector of transient and intrinsic contamination.

14.Aseptic-Containment strategy: ACS for processing sterile toxic and biologically hazardous products. Aseptic containment also applies where cross contamination control is required and prepared with an alignment to the CCS. A three step approach is considered: Define hazards based on Health based exposure limits, Set control measures based on two levels of containment; Primary and Secondary containment, Qualification of containment with challenge testing commensurate with level of hazard; Toxic or Bio-Hazard.

15.Media fill – Process simulation design in Aseptic processing. Points to consider for different product and process types with different process durations; batch and campaign.

16.Cleanroom garments selection, gowning qualification, Change-rooms for gowning and behaviour after entering cleanroom. Includes examples of MHRA regulatory observations on Cleanroom behaviour.

17.Disinfectant Rotation: Required? Why, what, when? Considers spectrum of efficacy, material compatibility and justification for rotation or no rotation, as required.

18.PUPSIT and Fluid pathway leak integrity and Sterilising filter configurations in filling lines.

19.Visual inspection of sterile products in final containers and alignment of acceptance criteria between stopper manufacturers and pharmaceutical manufacturers.

20.Definitions of Hold times: Aseptic holds, sample hold, sterile hold, stability holds.

**Planning is now finalised for 2020 Aseptic processing workshop syndicates** and Annual Challenges in Sterile product manufacturing conference in Manchester UK April and June respectively. These are typically sold out events so those interested should contact the PHSS as soon as information is released. Details of events and content will be released early 2020 to facilitate reservation of places.

**The Annex 1 revision status.** There is information that in Q1 of 2020 a targeted consultation will be completed by the EMA/IWG; Inspectors Working Group on the next draft of Annex 1 revision. Following review the final draft is expected to be published Q3/Q4 2020 with a period before implementation (without further changes).

The PHSS have sent a letter to the EMA to register interest as an 'Interested party' to facilitate joining the targeted consultation alongside other interested parties; including ISPE and PDA.

The **Annual PHSS Conference** on challenges in sterile product manufacture, June 2020, will include an update of current status of Annex 1 revision with a PHSS 'Hot Topic' Annex 1 conference expected once the final draft is published to start to understand content: GMP requirements, QRM application and regulatory expectations.