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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

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 cm^2 are aseptically filled with 2 ml of product solution in batches of 4 000, which takes approximately 4 hours.

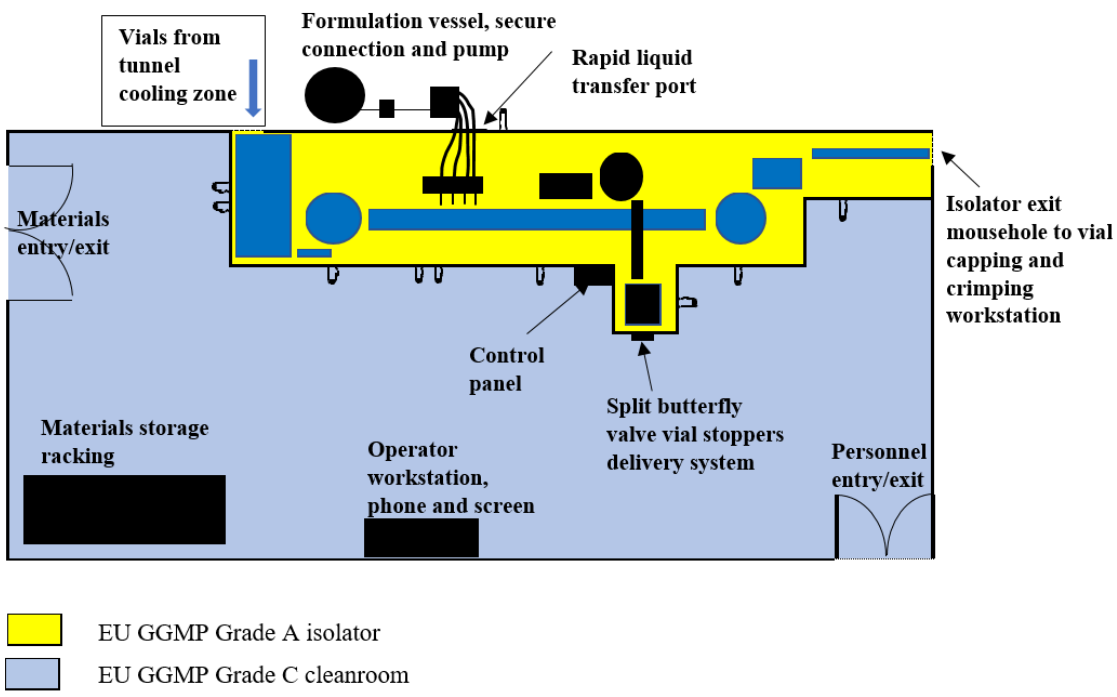


Figure 1 Cleanroom and isolator schematic

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 is 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.

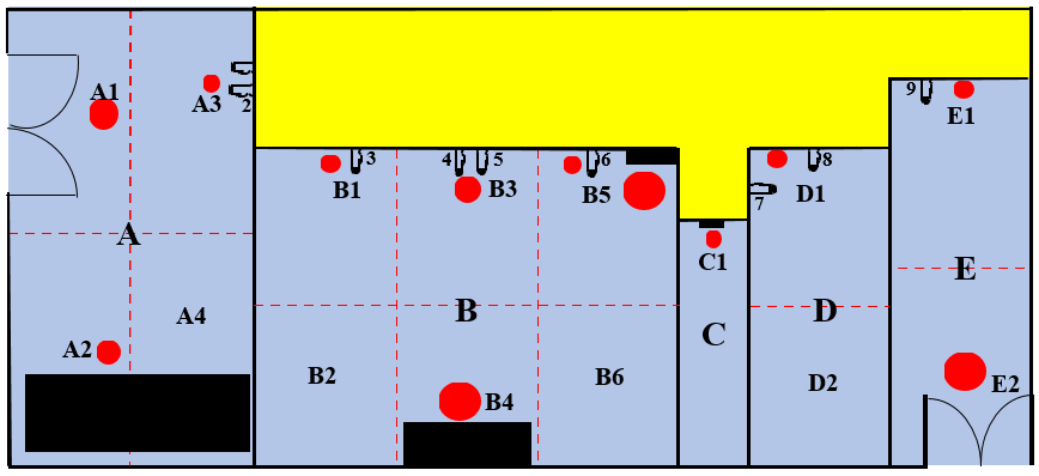
Table 1 In operation classification considerations for the cleanroom containing the isolator

Classification Parameter	Cleanroom 'in operation' classification considerations
1. Facility installation status	**1.1 Installation testing** All cleanroom installation and equipment testing to be satisfactorily completed.
	1.2 Air conditioning system 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 The in-situ integrity testing of all cleanroom terminal air supply filters to be satisfactorily completed.
	1.4 'At rest' testing 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** The worst-case maximum number of 6 people to be present and performing the established cleanroom manufacturing activities.
	2.2 Personnel cleanroom garments 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** 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. 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** The 'in operation' limit for ≥0.5 µm 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 ≥5 µm 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 ≥5 µm particle size of 29 000 /m^3 is applied.
5. Sampling volumes and sampling times	**5.1 Sample volume** 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 /m^3, the minimum sample volume (V_S), in litres, is calculated from the formula to be; $V_S = (20/ C_{n,m}) \times 1000 = (20/29000) \times 1000 = 0.69$ l 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 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** 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** 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. **7.2 Airflow considerations** 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. **7.3 Sampling locations** The final sampling locations are shown in Figure 2 and detailed in Table 2.
8. Sampling probe and tubing	**8.1 Air sampler tubing** 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. **8.2 Sampling height** 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** 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. 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** 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** 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.



● Areas of activity
—— Solid grid lines indicate suitable sizes of rectangular sub-areas
---- Dotted grid lines indicate the resultant sections to be sampled
A1 etc. Locations to be sampled
1 etc. Barrier gauntlet number

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 exception at location A2, all the samples had air concentrations below the EU GGMP Grade C limits set for particles ≥0.5 µm and ≥5 µm.

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 **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 **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 **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 **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 **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 **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 **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 **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 **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 **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 **Activity;** Operator adding the closures split butterfly delivery system to isolator	**≥0.5 µm** 4998 4251	99 960*	Pass	1.0
		≥5 µm 455 472	9 440*	Pass	0.7
D1	**Location;** Between barrier gauntlets 6 and 7 **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 **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 **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 **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 (5).

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

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 (6) and explained elsewhere (5). 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

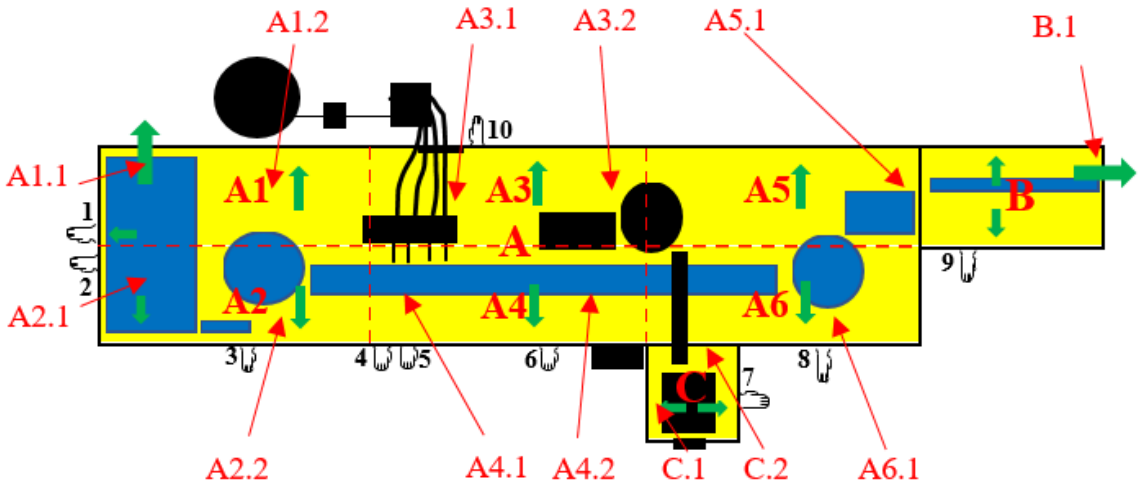
Classification Parameter	Isolator 'in operation' classification considerations
1. Facility installation status	**1.1 Installation testing** All isolator installation and equipment testing to be satisfactorily completed.
	1.2 Air conditioning system The isolator ventilation system to be operating in the established manner to provide the required level of airborne contamination control.
	1.3 Isolator terminal HEPA filters The in-situ integrity testing of all isolator terminal air supply filters to be satisfactorily completed.
	1.4 'At rest' testing 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** 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 All cleanroom personnel were required to wear the designated cleanroom garments.
3. Particle size	**3.1 EU GGMP Annex 1 particle sizes** 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. 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** 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 3520 /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** 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; $V_S = (20/ C_{n,m}) \times 1000 = (20/20) \times 1000 = 1000$ l 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 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** 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** 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. **7.2 Airflow and other considerations** 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. **7.3 Overall number of sampling locations** 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** 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** 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** 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.

Table 3 In operation classification considerations for the isolator

Classification Parameter	Isolator 'in operation' classification considerations
11. Out-of-Specification result	**11.1 Investigation and remedial actions** 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.



—— Solid grid lines indicate suitable sizes of rectangular sub-areas

---- Dotted grid lines indicate the sections to be sampled

A1.1 Locations within sections to be sampled

➡ Airflow direction

1 etc. Barrier gauntlet number

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 ≥0.5 µm and ≥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 corrections	1.05	**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** 20	Pass
			≥5 µm 0	Pass
A5.1 Vial exit and correction	0	**Location;** Rear of filled vial exit accumulation zone **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 **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 **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 **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 **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 ≥5 µm 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 m^3 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 m^3, a more appropriate limit would be 88 000 per m^3. 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.

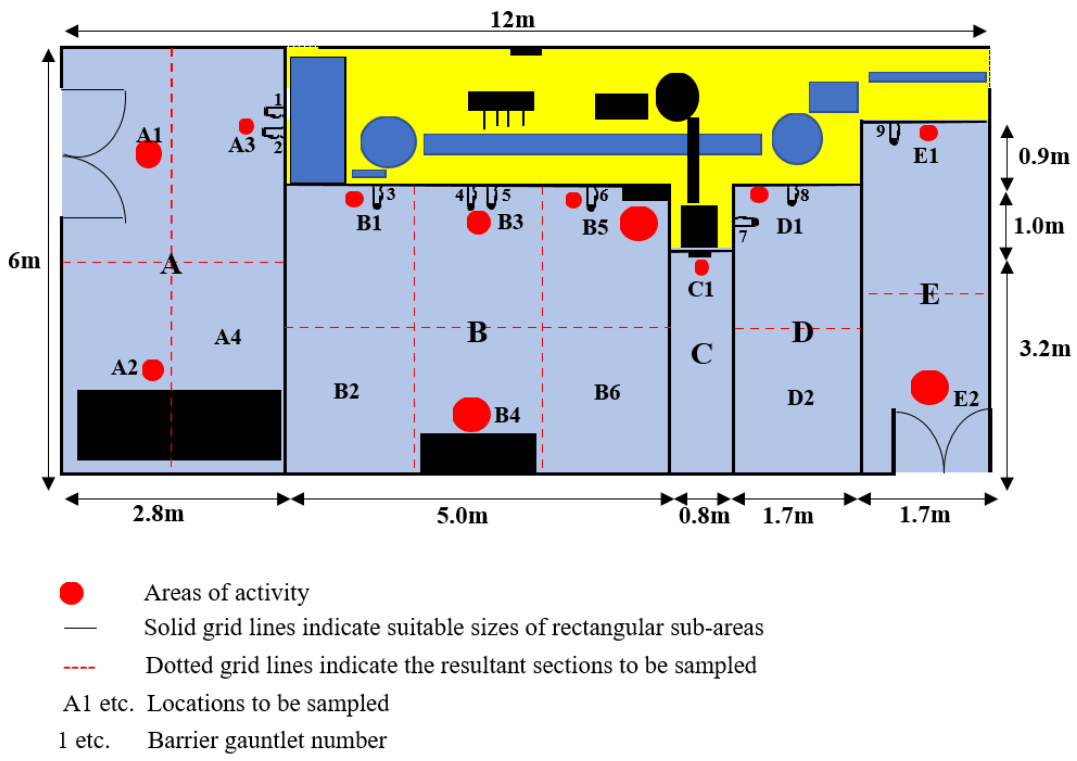

Figure A1 Cleanroom activity areas, sub-areas and sampling locations

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 m^2) is divided into 3 rectangular sub-areas, A (13.5 m^2), B (1.53 m^2) and C (0.8 m^2) 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.

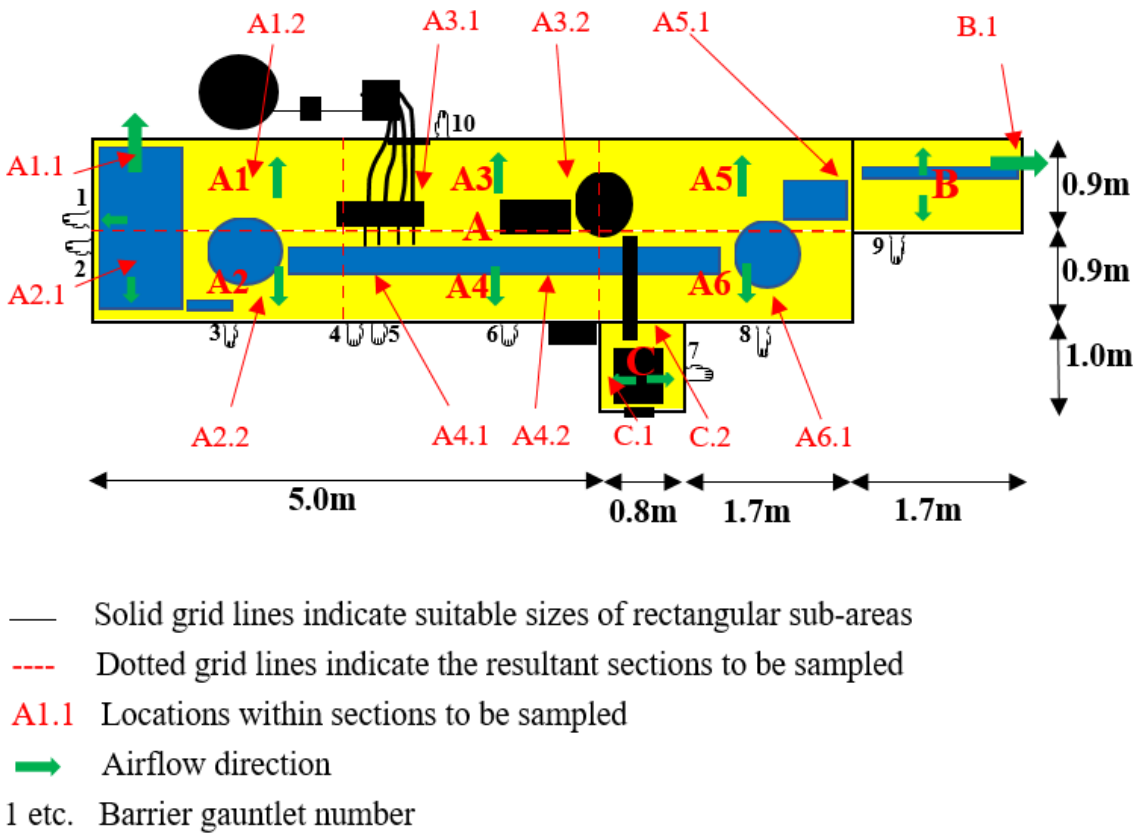

Figure B1 Isolator sampling sections and sampling locations

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.