Learning Objectives

Learn how contamination control standards and guidance sets requirements for Critical Parameters

- ISO-14644-1 – Classification of air cleanliness
- PIC/S Annex 1, Manufacture of Sterile Medicinal Products
- Some remarks about FDA Guideline on Sterile Drug Products Produced by Aseptic Processing – Sept 2004
ISO Cleanroom Standards

ISO 14644-1:2015 Classification of air cleanliness

Classification of air cleanliness in cleanrooms and associated controlled environments.

• Defines the concentration of all airborne particles (does not differentiate bio-contamination)

• Limited to a designated range of considered particle sizes from ≥0.1 to 5.0 micron particles for determination of particle concentration limits.

• Generic standard, not application specific.
## ISO 14644-1:2015 – Classification Table

### Table 1 — ISO Classes of air cleanliness by particle concentration

<table>
<thead>
<tr>
<th>ISO Class number (N)</th>
<th>Maximum allowable concentrations (particles/m³) for particles equal to and greater than the considered sizes, shown below&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0,1 µm</td>
</tr>
<tr>
<td>1</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
</tr>
<tr>
<td>4</td>
<td>10 000</td>
</tr>
<tr>
<td>5</td>
<td>100 000</td>
</tr>
<tr>
<td>6</td>
<td>1 000 000</td>
</tr>
<tr>
<td>7</td>
<td>c</td>
</tr>
<tr>
<td>8</td>
<td>c</td>
</tr>
<tr>
<td>9g</td>
<td>c</td>
</tr>
</tbody>
</table>
All concentrations in the table are cumulative, e.g. for ISO Class 5, the 10 200 particles shown at 0.3 μm include all particles equal to and greater than this size.

These concentrations will lead to large air sample volumes for classification. Sequential sampling procedure may be applied; see Annex D.

Concentration limits are not applicable in this region of the table due to very high particle concentration.

Sampling and statistical limitations for particles in low concentrations make classification inappropriate.

Sample collection limitations for both particles in low concentrations and sizes greater than 1 μm make classification at this particle size inappropriate, due to potential particle losses in the sampling system.

In order to specify this particle size in association with ISO Class 5, the macroparticle descriptor M may be adapted and used in conjunction with at least one other particle size. (See C.7)

This class is only applicable for the in-operation state.
ISO 14644-1:2015 Cleanroom Standard

Defines classification by

- ISO Class
- Occupancy State
- Designated particle sizes

3 Occupancy States

- As Built
- At Rest
- Operational

Specifies

- Number of sample locations for classification
- The sample size required at each location
- How to evaluate the date to determine the class
Number Sample Locations – ISO 14644-1:2015

- Now a Look-up table
- Vertical plane for horizontal UDAF
- Horizontal plane for vertical UDAF

<table>
<thead>
<tr>
<th>Area of cleanroom (m²) less than or equal to</th>
<th>Minimum number of sampling locations to be tested (N&lt;sub&gt;L&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>36</td>
<td>9</td>
</tr>
<tr>
<td>52</td>
<td>10</td>
</tr>
<tr>
<td>56</td>
<td>11</td>
</tr>
<tr>
<td>64</td>
<td>12</td>
</tr>
<tr>
<td>68</td>
<td>13</td>
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<tr>
<td>72</td>
<td>14</td>
</tr>
<tr>
<td>76</td>
<td>15</td>
</tr>
<tr>
<td>104</td>
<td>16</td>
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<tr>
<td>108</td>
<td>17</td>
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<td>116</td>
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<td>156</td>
<td>20</td>
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<tr>
<td>192</td>
<td>21</td>
</tr>
<tr>
<td>232</td>
<td>22</td>
</tr>
<tr>
<td>276</td>
<td>23</td>
</tr>
<tr>
<td>352</td>
<td>24</td>
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<tr>
<td>436</td>
<td>25</td>
</tr>
<tr>
<td>636</td>
<td>26</td>
</tr>
<tr>
<td>1000</td>
<td>27</td>
</tr>
</tbody>
</table>
A.4.2 Positioning the sampling locations

In order to position the sampling locations

a) use the minimum number of sampling locations $N_L$ derived from Table A.1,

b) then divide the whole cleanroom or clean zone into $N_L$ sections of equal area,

c) select within each section a sampling location considered to be representative of the characteristics of the section, and
The equation (below) from Annex B of ISO 14644-1 can be used to calculate the single sample volume per location.

\[ V_s = \frac{20}{C_{n,m}} \times 1000 \]

- \( V_s \) = minimum single sample volume per location expressed in liters
- \( C_{n,m} \) = the class limit (number of particles per cubic meter) for the largest considered particle size specified for the relevant class.
- 20 = the defined number of particles that could be counted if the particle concentration were at the class limit.
• Average counts at each location.
• Standardise to counts/m\(^3\).
• Each location must comply with the class limit, for the class of cleanliness of the complete zone/space/room to be claimed.

• Real-time particle monitoring is very different - here we focus on the cleanliness of a critical control point over a short period of time.
Time to Look at the GMP Environmental Requirements

Remember.....ISO Standards are the Toolbox for Classification – to be used in conjunction with the levels defined in the GMP Guidance.
The requirements considered

Environmental cleanliness

- Airborne particles
- Microbiological contamination
  - Airborne
  - Surfaces

Pressure differentials

Air-change rates and recovery time

Uni-directional airflow velocities

Air filtration (final filters) requirements
Cleanliness Requirements
## Annex 1 - Area Cleanliness

<table>
<thead>
<tr>
<th>Grade</th>
<th>At Rest</th>
<th>In Operation</th>
<th>Microbiological</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max Particles ≥ stated sizes</td>
<td></td>
<td>Air sample cfu/m³</td>
</tr>
<tr>
<td></td>
<td>0.5µ</td>
<td>5.0µ</td>
<td>0.5µ</td>
</tr>
<tr>
<td>A</td>
<td>3 520</td>
<td>20</td>
<td>3 520</td>
</tr>
<tr>
<td>B</td>
<td>3 520</td>
<td>29</td>
<td>352 000</td>
</tr>
<tr>
<td>C</td>
<td>352 000</td>
<td>2 900</td>
<td>3 520 000</td>
</tr>
<tr>
<td>D</td>
<td>3 520 000</td>
<td>29 000</td>
<td>Not defined</td>
</tr>
</tbody>
</table>
### US FDA Area Cleanliness (2004)

<table>
<thead>
<tr>
<th>Clean Area Classification (0.5 um particles/ft³)</th>
<th>ISO Designation b</th>
<th>≥ 0.5 μm particles/m³</th>
<th>Microbiological Active Air Action Levels (cfu/m³) c</th>
<th>Microbiological Settling Plates Action Levels (diam. 90mm; cfu/4 hours) c, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>5</td>
<td>3,520</td>
<td>1e</td>
<td>1e</td>
</tr>
<tr>
<td>1000</td>
<td>6</td>
<td>35,200</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>10,000</td>
<td>7</td>
<td>352,000</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>100,000</td>
<td>8</td>
<td>3,520,000</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

a. All classifications based on data measured in the vicinity of exposed materials/articles during periods of activity.

b. ISO 14644-1 designations provide uniform particle concentration values for cleanrooms in multiple industries. An ISO 5 particle concentration is equal to Class 100 and approximately equals EU Grade A.

c. Values represent recommended levels of environmental quality. You may find it appropriate to establish alternate microbiological action levels due to the nature of the operation or method of analysis.

d. The additional use of settling plates is optional.

e. Samples from Class 100 (ISO 5) environments should normally yield no microbiological contaminants.

**Colony Forming Unit (CFU)**
The Value – 20 Particles

Comes from ISO 14644-1:1999 & 2015

• For classification, the air sample size shall be sufficient such that if you were at the class limit for the largest considered particle size, you would count at least 20 particles.

• Rule of thumb to give some confidence that real particles are > noise in the system.
Annex 1 – Occupancy State

“At-rest”
• The “at-rest” state is the condition where the installation is installed and operating, complete with production equipment but with no operating personnel present.

“In-operation”
• The “in-operation” state is the condition where the installation is functioning in the defined operating mode with the specified number of personnel working.
Annex 1 – Grade A

Grade A
The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Conditions normally provided by a laminar airflow work station.

- Particles 0.5 micron and larger < 3500/m³
- Particles 5 micron and larger ≤ 20
- Counts apply “at rest” & “in operation”
- Microbial Limits < 1 CFU/m³
Sample Size Calculation – ISO 14644-1 & Annex 1 Grade A ≥5.0m

\[ V_s = \frac{20}{C_{n,m}} \times 1000 \]

\( V_s = \) minimum single sample volume per location expressed in liters

ISO standard for ≥5µ particles is 29/m³

\[ V_s = \frac{20}{29} \times 1000 \]

\[ V_s = 689 \text{litres} \]

689 liters = 0.689 m³ = 24.33 ft³
Annex 1 – Sample size
Basis for Classification

For classification purposes EN/ISO 14644-1 methodology defines both the minimum number of sample locations and the sample size based on the class limit of the largest considered particle size and the method of evaluation of the data collected.

Clearly states ISO 14644-1 to be used for classification. Will change sampling for classification.

• Grade A (rest & op) → $1m^3$ sample each location [35 mins]
• Grade B (at rest) → 690 litres [25 mins]
• Grade B (operation) → 28.3 litre [1 min]
• Grade C & D → 28.3 litre [1 min]
Annex 1 – Vial Capping

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- The container closure system for aseptically filled vials is not fully integral until the aluminium cap has been crimped into place on the stoppered vial. Crimping of the cap should therefore be performed as soon as possible after stopper insertion.

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- As the equipment used to crimp vial caps can generate large quantities of non-viable particulates, the equipment should be located at a separate station equipped with adequate air extraction.
- Reinforces established practice.
Annex 1 – Vial Capping

Vial capping can be undertaken as an aseptic process using sterilised caps or as a clean process outside the aseptic core.

- Where this latter approach is adopted, vials should be protected by Grade A conditions up to the point of leaving the aseptic processing area, and thereafter stoppered vials should be protected with a Grade A air supply until the cap has been crimped.

A clear requirement quoting 2 basic options

1. Aseptic process.
2. Clean process with local clean air protection.

There isn’t any defined requirement about the environment surround local protection for option (2)

Grade A air supply is quite different from designating a Grade A zone
Annex 1 – Vial Capping

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- Vials with missing or displaced stoppers should be rejected prior to capping. Where human intervention is required at the capping station, appropriate technology should be used to prevent direct contact with the vials and to minimise microbial contamination.

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- Restricted access barriers and isolators may be beneficial in assuring the required conditions and minimising direct human interventions into the capping operation.
  - Then we have some much more explicit requirements about detection of stopper faults and process intervention.
Capping Options

Clean Protected

Aseptic
Industrial Integrated Vial Line

- Wash
- Depyrogenisation & cool
- Fill & stopper
- Cap Overseal
ISO 5 (Grade A Air Supply) Protected Capper in Grade D Surrounding Room
Annex 1 – “Operational” Monitoring

Clean room and clean air device monitoring

8. Clean room and clean air devices should be routinely monitored in operation and the monitoring locations based on a formal risk analysis study and the results obtained during the classification of rooms and/or clean air devices.

➢ Provides a clear basis for developing the monitoring system
Annex 1 – “Operational” Monitoring – Grade A

9. For Grade A zones, particle monitoring should be undertaken for the full duration of critical processing, including equipment assembly, except where justified by contaminants in the process that would damage the particle counter or present a hazard, e.g., live organisms and radiological hazards.

- In such cases monitoring during routine equipment set up operations should be undertaken prior to exposure to the risk.
- Monitoring during simulated operations should also be performed.
- The Grade A zone should be monitored at such a frequency.
  - The practical issues of airborne particle monitoring during set-up yet to be challenged.
  - Still retains the option of monitoring a simulated operation if the product is inherently hazardous to the particle counting system.
The Grade A zone should be monitored at such a frequency and with suitable sample size that all interventions, transient events, and any system deterioration would be captured and alarms triggered if alert limits are exceeded.

It is accepted that it may not always be possible to demonstrate low levels of >5.0 µm particles at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself.

• Suggests the principle of a practical basis for developing a monitoring system.
• Recognises that the process can generate aerosols. Not only restricted to ≥5.0 micron, will affect ≥ 0.5 also!
It is recommended that a similar system be used for Grade B zones although the sample frequency may be decreased. The importance of the particle monitoring system should be determined by the effectiveness of the segregation between the adjacent Grade A and B zones. The Grade B zone should be monitored at such a frequency and with suitable sample size that changes in levels of contamination and any system deterioration would be captured and alarms triggered if alert limits are exceeded.

- Suggests a similar principle of a practical basis for developing a monitoring system.
- Recognises that RABS are present a lesser risk than poorer separative devices.
Airborne particle monitoring systems may consist of independent particle counters; a network of sequentially accessed sampling points connected by manifold to a single particle counter; or a combination of the two.

The system selected must be appropriate for the particle size considered. Where remote sampling systems are used, the length of tubing and the radii of any bends in the tubing must be considered in the context of particle losses in the tubing.
The selection of the monitoring system should take account of any risk presented by the materials used in the manufacturing operation, for example those involving live organisms or radiopharmaceuticals.

- Sets out basic requirements.
- Recovery of 5.0 micron particles will dictate the nature and capability of the system.
- Recognises some of the contamination risks that can affect particle monitoring systems.
-Revision of ISO 14644-2 and UK PHSS monograph will address the configuration and use of such systems.
12. The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of clean rooms and clean air devices.

- Recognises that sample size and frequency of sampling need to be developed on a risk basis.
- Need also to refer back to clause 9 as this is more helpful.
In Grade A and B zones, the monitoring of >5.0 µm particle concentration count takes on a particular significance as it is an important diagnostic tool for early detection of failure. The occasional indication of >5.0 µm particle counts may be false counts due to electronic noise, stray light, coincidence, etc. However consecutive or regular counting of low levels is an indicator of a possible contamination event and should be investigated. Such events may indicate early failure of the HVAC system, filling equipment failure or may also be diagnostic of poor practices during machine set-up and routine operation.

- This is contentious for some people.
- US FDA guidance doesn’t recognise this occurrence.
- Research by Whyte & Eaton, and Lujunqvist & Reinmuller suggests that ≥ 0.5 micron and ≥ 5.0 micron rise together. Remember the ≥ 0.5micron includes all the ≥ 5.0 micron particles.
Annex 1 – “Operational” Monitoring – Systems

15. The monitoring of Grade C and D areas in operation should be performed in accordance with the principles of quality risk management. The requirements and alert/action limits will depend on the nature of the operations carried out, but the recommended “clean up period” should be attained.

- Gives us more flexibility for Grades C and D.
- Be careful not to develop over onerous compliance rather than risk based monitoring.
- “Clean-up” time means recovery test.
- Doesn’t automatically demand recovery testing of all Grade C and D areas!
Filling Machine Particle Monitoring
Filling Machine Particle Monitoring
Differential Pressure $\Delta P$

Positive?  Negative?
Differential Pressure Requirements

GMPs require Cascading Air Flows using differential pressure from cleanest to least clean. Pressure difference between areas of different class:

- 0.05” wg = 12.5 Pascals
- US FDA: at least 10 – 15 Pa
- Annex 1: 10 – 15 Pa (guidance value)
Air Change Rate & Recovery Time
Non-unidirectional Airflow (Non-UDAF)
US FDA Guidance – Air Changes per Hour

- For Class 100,000 (ISO 8) supporting rooms, airflow sufficient to achieve at least 20 air changes per hour is typically acceptable.
- Significantly higher air change rates are normally needed for Class 10,000 and Class 100 areas.
Air Changes per Hour

- Air changes not applicable for Grade A UDAF – here velocity and uniformity of airflow applies.
- Air changes aren’t specified for Non-UDAF zones.
- Clean-up or recovery time is defined:
  - “The particle limits given in the table for the “at rest” state should be achieved after a short “clean up” period of 15-20 minutes (guidance value) in an unmanned state after completion of operations.”
  - This will generally require 20-35 ac/hr depending on the effectiveness of the mixing and dilution.
- A recovery time test is required to qualify this performance.
Unidirectional Airflow (UDAF)

UDAF replaces terminology LAF
Annex 1 – UDAF Requirements

Grade A: The local zone for high risk operations, e.g., filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally such conditions are provided by a laminar air flow work station. Laminar air flow systems should provide a homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance value) at the work position in open cleanroom applications. The maintenance of laminarity should be demonstrated and validated.

A uni-directional air flow and lower velocities may be used in closed isolators and glove boxes.

Should say...
“Non-unidirectional airflow and lower unidirectional airflow velocities may be used in closed isolators and glove-boxes.”
Airflow patterns should be evaluated for turbulence or eddy currents that can act as a channel or reservoir for air contaminants (e.g., from an adjoining lower classified area).

In situ air pattern analysis should be conducted at the critical area to demonstrate

- **Unidirectional airflow**
- **Sweeping action over and away from the product under dynamic conditions.**
Guidelines – Air Flow Patterns

The studies should be well documented

- Written conclusions
- Evaluation of the impact of aseptic manipulations (e.g., Interventions) and equipment design.

Video record

- Aides in assessing airflow initially
- Facilitating evaluation of subsequent equipment configuration changes.
Air Flow Patterns

Terminal HEPA Filtered Air Supply
Airflow Visualization Studies – Guidelines

- Have written objectives for each visualisation study.
- Even if studies are conducted during a shutdown, try to operate in a production mode with personnel in full garb observing good aseptic technique during a simulation.
- Begin video taping the study by showing the equipment below the ceiling level that is being washed by airflow and then pan up to show the smoke at the ceiling.
- Use a smoke source that is adequate to show good airflow.
- Do not get too close to equipment with the smoke source. This can cause smoke to display a turbulent action as it strikes flat surfaces.
- Record all data at the time smoke studies are conducted. (time, date, personnel present, room location, etc.)
Temperature and Humidity
Temperature and (Relative) Humidity

Activities in clean areas - The ambient temperature and humidity should not be uncomfortably high because of the nature of the garments worn. – Annex 1

- [US GMPs require temperature and humidity controls]

Identify criticality

- Product requirements – (GMP)
- Comfort of operators – (Good cleanroom practice)
- Contamination dissemination of operators – (Good cleanroom practice)
Relative Humidity Is...

The ratio of the amount of water vapor in the air at a specific temperature to the maximum amount that the air could hold at that temperature, expressed as a percentage.
Potential Problems with Low Relative Humidity

- Static electricity (problem with plastic vials)
- Human discomfort due to dryness
- Product impact due to low humidity (sterile powder filling)
Potential Problems with High Relative Humidity

- Encouraging environment for mold and bacteria growth
- Human discomfort due to high humidity
- Product impact during sterile powder filling
- Product degradation post lyophilization and prior to sealing.
What are the Correct Temperature and Relative Humidity Ranges?

GMPs are not specific.

If there is no product requirement, then...

- Temperature: 17-23 degC
- RH: 25 – 55%
Final Filters (HEPA)
Annex 1 – Filtered Air Supply

“53. A filtered air supply should maintain a positive pressure and an air flow relative to surrounding areas of a lower grade under all operational conditions and should flush the area effectively.”

• There is no grade specified.
• Current good practice would employ an H14 (to EN 1822) as a terminal filter for Grades A, B, and C.
“HEPA-filtered air should be supplied in critical areas at a velocity sufficient to sweep particles away from the filling/closing area and maintain unidirectional airflow during operations.”

“...leak tests should be performed at suitable time intervals for HEPA filters in the aseptic processing facility. For example, such testing should be performed twice a year for the aseptic processing room.”
“Any aerosol used for challenging a HEPA filter should meet specifications for critical physicochemical attributes such as viscosity. Dioctylphthalate (DOP) and poly-alpha-olefin (PAO) are examples of appropriate leak testing aerosols. Some aerosols are problematic because they pose the risk of microbial contamination of the environment being tested. Accordingly, the evaluation of any alternative aerosol involves ensuring it does not promote microbial growth.”

“There is a major difference between filter leak testing and efficiency testing. An efficiency test is a general test used to determine the rating of the filter.8 An intact HEPA filter should be capable of retaining at least 99.97 percent of particulates greater than 0.3 μm in diameter.”
Conclusion

FDA and PIC/S guidelines set important requirements

ISO-14644-1 – Classification of air cleanliness - gives important information

Clarity about in operation and at rest, in US and PIC/S

Understand and put in place air quality design, flow, cascades, filtration etc
Thank you for your time. Questions?

Gordon Farquharson

gordon.farquharson@pharmout.net
Executive Consultant

www.pharmout.net