FAQs ON PARTICLE COUNTING

1. Minimum Sampling Flow Rates & Volumes?
2. Maximum Permissible Sampling Tube Length
3. How to conduct Recovery Test?

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FAQs ON PARTICLE COUNTING

What is the Minimum / Maximum / Recommended Flow Rate specified in Cleanroom Standards?

FLOW RATES

- Airborne particle counters are available with flow rates of 0.1 cfm, 1.0 cfm, 50 lpm or 100 lpm.
- Most standards do not specify using a particular flow rate. The new ISO14644 standard only specifies a flow rate of 1 cfm for counting particles >=5 microns. So what flow rate should you use?
- The following would be the problems using a low flow rate 0.1 cfm counter while validating pharmaceutical clean rooms:
Flow rates (contd.)

- We would have to multiply the 1 minute measurement result by 10 to get counts per cft. And again by 35.2 to get counts/cu metre. This is a valid approach provided that the measured value has statistical significance i.e. it is 20 counts per minute or greater to provide a reliable basis.
- If the measured value was only 3 particles in 1 minute measured with a 0.1 cfm counter, this would convert into 30 particles/cu ft. but if count changed to 4 particles it would jump to 40/cu ft.

FLOW RATES (Contd.)

- The other method would be to let the 0.1 cfm counter run for 10 minutes to get a sample volume of 1 cubic foot. The drawback of this approach is that, if any short term particle burst occurs during the relatively long 10 minutes count cycle, this burst would be integrated and would not show up as significant.
- The best way to count large particles is to sample a large quantity of air, thus maximising the chances of capturing any 5 micron particles that may be present. Therefore 1 cfm flow rate is better than 0.1 cfm as you get 10 times the sample quantity.
Ultra High Flow Considerations (100 lpm)

- Impact on immediate environment and airflow patterns
  - Isokinetic probe must be matched to particle counter flow rate
  - High exhaust rate of sampled air may disturb room airflow

- Disturbance of airflow patterns in restricted areas due to high rate of sampled air
  - Modest air supply of most LAF cabinets may not be sufficient to support high flow rates without substantial disturbance

Smoke Studies
(Impact of exhausted air)

- Particle Counter Off
  - Photo shows horizontal laminar flow across table

- Particle Counter On
  - Photo shows disruption of laminar flow near the particle counter exhaust

The greater the flow rate of sampled air, the greater the amount of exhaust air.

DON'T SACRIFICE SENSITIVITY FOR SPEED!!

- If you study the specifications of most particle counters with 100 lpm flow rate, one fact clearly stands out. The smallest particle size they can measure is only 0.5 microns, not 0.3 microns.

- And the counting efficiency for the most sensitive threshold of 0.5 microns is 50%. In other words, compared to a theoretically perfect counter, they are only counting half the particles of 0.5 micron size!!

- Counting efficiency is an expression of the probability that a Particle Counter will sense and count a particle passing through its sample volume. This probability is a function of size up to a certain critical size above which all particles are normally sensed and counted.
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What is the Minimum Sample Volume to be collected?

**Sample Volumes per ISO 14644-1:2015**

No change in minimum sampling volume and time in 2015 version

Clause A.4.4: “At each sample location, sample a sufficient volume of air that a minimum of 20 particles would be detected if the particle concentration for the largest considered particle were at the class limit for the designated ISO Class”

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

*V_s* is the minimum Sample Volume Per Location (in Litres)

*C_{n,m}* is the class limit (particles / m^3) for the largest considered particle size specified for the relevant class

20 is the defined number of particles that could be counted if the particle concentration were at the class limit

“The Volume Sampled at Each Location Must be at Least 2 Litres, With a Minimum Sample Time at Each Location of 1 Minute”

What volume do I need to collect?

ISO 14644 requires sample with 20 theoretical particles

ISO 14644 allows 3520 particles (0.5um) in 1m^3 (1000 litres)

for Class 5

\[ \text{Volume} = \frac{20 \text{ particles}}{3520 \text{ particles}} \times 1000 \text{ litres} \]

Volume = 5.7 litres

At sampling rate of 1cfm (28.3 lpm) this would take less than one minute

Greater than 2.0 litres so minimum sample volume is satisfied
Minimum Sample Volume

Regulations and Guidance for the Manufacture of Sterile Medicinal Products ISO 14644 and EU GMP Annex 1

EU GMP Annex 1 2009 states:

“For classification purposes in Grade A zones, a minimum sample volume of 1m³ should be taken per sample location”

This is sometimes mistaken to imply that 1 cu metre sample per location is required even during routine monitoring of the Grade A zone.

EU GMP Annex 1 2009

EU GMP Clearly differentiates classification and monitoring

Section 4.1 Clean room / clean air device classification

General interpretation: The GMP Annex 1 Revision distinguishes very clearly between clean room / clean air device classification which is described in sections 4 to 7, and clean room monitoring, which is described in sections 8 to 20.

In general, clean room/clean air device classification is required to be performed according to ISO 14644-1 with the applicable limits for particle counts defined in the table in section 4 of GMP Annex 1. Monitoring, on the other hand, does not need to be performed according to ISO 14644-1. It can be performed for a reduced number of sampling points and sampling volumes.

EU Annex 1 : Monitoring

Section 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.”

• It is not necessary to sample 1m³ during verification or monitoring

• Particle counters used for monitoring may have the same or different flow rate from those used for classification.
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What is the Maximum Permissible Sampling Tube Length?

SAMPLE TUBING LENGTH

• Particle loss in the sample tubing applies more to larger particles of 5 microns and larger, and less to smaller particles of 0.3 and 0.5 microns. The inertia of larger particles makes them bump into the wall of the tubing and they may stick to the tubing wall.

To lessen this problem, two methods are recommended:

• Flow rate should be fairly high i.e. 1 cfm not 0.1 cfm. The higher the flow rate, the lesser the number of larger particles that will be "lost"
• Sample tubing length should be minimised, although tubing cannot be totally eliminated.
• In the case of portable particle counters, the tubing length should not be more than 3 metres i.e. 10 ft

Sample Tubing Length

EU GMP Annex 1 states:

"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."

and

"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."

You will notice that nowhere is the "short" length specified.
Sample Tubing Length

ISO 14644-3 Annex B Test methods Clause B1.2.2 states:
"The transit tube from the sample probe inlet to the Discrete Particle Counter sensor should be as short as possible. For sampling of particles larger than or equal to 1 µm, the transit tube length should not exceed the manufacturer’s recommended length and diameter."

Most particle counter manufacturers recommend a maximum tubing length of 3 metres.

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Particle Sampling Tubing

Fed Std 209E: Obsolete Since 2001: But Helpful:

► B40.2.1 Particle transit considerations. The probe and transit tube should be configured so that the Reynolds number is between 5,000 and 25,000.

► For particles in the range of 0.1 to 1.0 µm and a flow rate of 0.028 M³/min (1.0 ft³/min), a transit tube up to 30 m long may be used.

► For particles in the range of 2 to 10 µm the transit tube should be no longer than 3 m.

ASTM F50: Standard Practice for Continuous Sizing and Counting of Airborne Particles in Dust-Controlled Areas and Clean Rooms Using Instruments Capable of Detecting Single Sub-Micrometre and Larger Particles (Not ISO but Helps and is Current)

► For particles in the size range 0.1 µm to 2 µm in diameter and a Single Particle Counting Device flow rate of 0.028 m³/min (1 ft³/min), a transit tube up to 30 m long can be used.

► For particles size range 2 µm to 10 µm, a maximum transit tube length of 3 m can be used.
**Particle Sampling Tubing**

**ASTM F50: (Not ISO but Helps)**

► If a flexible transit tube is to be used, then no radius of curvature below 15 cm shall be used.

**PARTICLE SAMPLING TUBING**

*New ISO 16441:2015 Annex C (Informative not Mandatory)* – Counting and sizing of airborne macroparticles states:

Clause C.4.1.2 “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 metre in length.”

**FAQs ON PARTICLE COUNTING**

How Often Should we Classify an ISO Class 5 cleanroom?
FREQUENCY OF CLASSIFICATION OF CLEANROOMS

• At-rest and operational classification should be performed periodically based upon risk assessment of the cleanroom and clean zone operations, typically on an annual basis.

• Where the cleanroom and clean zone is equipped with instrumentation for continuous or frequent monitoring of air cleanliness (airborne particles, room pressure differentials), the time intervals between classification may be extended provided that the results of the monitoring remain within the specified limits. However, in the pharmaceutical and related industries formal classification / re-qualification must be undertaken at least annually.

• Frequency of re-testing cleanroom no longer dictated by Class, to be done annually. Previously it was 6 months for ISO Class 5.

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How Should we conduct the Recovery Test?

Revision to Room Recovery Rates

ISO/DIS 14644-3 Section B.4:

For non-unidirectional cleanroom only

This test now incorporates 100:1 and 10:1 tests.

Test should be performed when the clean room is in the 'As Built' or 'At Rest' states, or after major modification to the cleanroom, or its operation.

No requirement to retest every 2 years.

It is not recommended that the 100:1 test be used for ISO 8 & 9.
RECOVERY TEST according to new ISO/DIS 14644-3:2016
Clause B.4 Recovery Test (previously Clause B.12)
This test is performed to determine the ability of the installation to reduce the concentration of airborne particles by dilution. (Previously clause said This test is performed to determine the ability of the installation to eliminate airborne particles.)
Recovery performance is evaluated by using the 100:1 or 10:1 recovery time and/or the cleanliness recovery rate. The 100:1 or 10:1 recovery time is defined as the time required for decreasing the initial concentration by a factor of 100 times (and 10 times). *Previously only 100:1 test was mentioned, now 10:1 also.*

RECOVERY TEST
• Where to test:
  • Working level
  • At places where particle concentration indicates further investigation
  • At product/process level

Do not test at:
• Places where there is Direct influence of air supplies
• Near air returns
When an artificial aerosol is used, the risk of residue contamination of the installation should be considered.

RECOVERY TEST- PROCEDURE
• Care should be taken to avoid coincidence error and potential contamination of the Particle Counter optics. Before testing, calculate the concentration required to carry out the recovery test. If the concentration exceeds the maximum capability of the Particle Counter such that coincidence loss occurs: 1) use the dilution system; or 2) reduce the concentration to avoid coincidence losses.
• The particle size used in this test should be not greater than 0.5 μm. The cleanroom area to be examined should be contaminated with an aerosol while the air-handling units are in operation.
• Raise the initial particle concentration to more than 10 or 100 times depending on the target cleanliness level
• Commence measurements at not more than 1 min intervals and record time and concentration.
Evaluation by 10:1 or 100:1 recovery time

Evaluation procedure:
a) note the time when the particle concentration reaches the 10 or 100 × target concentration threshold (t_{10n} or t_{100n})
b) note the time and concentration when the particle concentration reaches the target cleanliness level, (t_n)
c) the 10:1 recovery time is represented by t_{0.1} = (t_n - t_{10n});
d) the 100:1 recovery time is represented by t_{0.01} = (t_n - t_{100n}).

• Any Questions about the Questions?
• Any Questions about the Answers?