Particle Counter Routine Monitoring Best Practices

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INTRODUCTION

Particulate counts as well as microbial counts within a cleanroom vary with the sampling location and the activities being conducted during sampling. Monitoring the environment for nonviable particulates and microorganisms is an important control function in achieving product compendial requirements for: 1) Foreign and Particulate Matter; and 2) Sterility.[1]

In the life science industry and especially regulated organizations engaged in the industrial manufacture of parenteral, enteral, or topical drugs are involved in substantial risk mitigation strategies and practices on a daily basis.

The manufacture of either Drug Products[2] or Drug Substances[3] in particular poses a high risk to the general public.[4] Subsequently, organizations involved in the industrial manufacture of

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[1] USP <1116>. "Importance of Microbiological Evaluation Program For Controlled Environments."

[2] **Drug Product**: *Drug product* means a finished dosage form, for example, tablet, capsule, or solution, that contains a drug substance, generally, but not necessarily, in association with one or more other ingredients (Defined per CFR Regulation, Title 21, Part 314, Subpart A, Section 314.3). A Drug product is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of humans or other animals.

[3] **Drug Substance**. *Drug substance* means an active ingredient that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or any function of the human body, but does not include intermediates use in the synthesis of such ingredient (Defined per CFR Regulation, Title 21, Part 314, Subpart A, Section 314.3). Drug Substance and Bulk Pharmaceutical Chemical (BPC) are commonly used terms descriptive of active ingredients, may be considered equivalent to the term used herein as API.

An **active pharmaceutical ingredient (API)** is defined in ICH Q7 as "Any substance or mixture of substances intended to be used in the manufacture of a drug product and that, when used in the production of a drug, becomes an active ingredient in the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease or to affect the structure and function of the body." Currently, other terms are also used by FDA and industry to mean an API. "Drug substance" and "bulk pharmaceutical chemical" (BPC) are terms commonly used to mean API and, for

biopharmaceutical[5] and other medical products are routinely audited by: (i) the Food and Drug Administration (FDA), (ii) the European Medicines Agency (EMA), and/or (iii) other regulatory authorities. These agencies audit the manufacture of products and substances used for medical (human) and veterinary (animal) treatments.

Biopharmaceutical Quality Managers, in many cases, create a conservative risk assessment using a combination of elements from a variety of applicable standards.

For non-viable ***monitoring*** of aerosol particles, the standards that are most referred to are ISO 14644-1/2:2015; ISO 21501-4:2018; EU GMP, Annex 1; PIC/S, GMP Annex 1 (2017); FDA Aseptic processing cGMP:2004.

For viable ***monitoring***, <USP> 1116[6] , FDA cGMP:2004[7]; ISO 14698-1:2003; WHO[8] [9]; and EU GMP, Annex 1[10] are frequently referred.

BPC, inactive ingredients. The use of these terms to describe active ingredients may be considered equivalent to the term used here, API.

[4] FDA cGMP: 2004, ii(B).

[5] A **biopharmaceutical**, also known as a **biologic(al) medical product**, **biological**, or **biologic**, is any pharmaceutical drug product manufactured in, extracted from, or semisynthesized from biological sources. These include, but are not limited to vaccines, blood, blood components, allergenics, somatic cells, gene therapies, tissues, recombinant therapeutic proteins, and living cells used in cell therapy. **Biologics** can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances, or may be living cells or tissues.

[6] http://www.ccv.com.ve/SalasLimpias/USP_1116_USP_36_NF31S1.pdf
(USP <1116>)

[7] http://www.fda.gov/downloads/Drugs/.../Guidances/ucm070342.pdf
(Aseptic Processing)

[8] http://www.who.int/immunization_standards/vaccine_quality/env_monitoring_cleanrooms_final.pdf
(Monitoring in Vaccine Manufacturing Facilities)

Other standards may also be applicable.

This Application Note is intended to provide a non-comprehensive general overview of applicable standards, and will provide a number of industry best practices used in biopharmaceutical industrial manufacturing and research. It is intended to provide a very basic and fundamental instructional guide. Actual practices at a facility may differ from the information presented herein due to managerial decisions and prerogatives, and/or individual risk assessments.

Applicability of each practice may vary depending on product or substance being manufactured, the documented risk assessment plan, and past validation studies conducted by the manufacturer.

[9] http://apps.who.int/prequal/info_general/documents/TRS961/TRS961_Annex6.pdf
(Annex 6, Sterile Pharmaceutical Products)

[10] http://ec.europa.eu/health/files/eudralex/vol-4/2008_11_25_gmp-an1_en.pdf

RISK ASSESSMENT – ENVIRONMENTAL MONITORING

The validation (or certification) of a cleanroom is conducted generally on an annual basis, or semi-annual for critical areas, using different standards and methods when compared to routine monitoring.

Cleanroom Classification: ISO 14644-1:2015 defines certification as a method of assessing level of cleanliness against a specification for [an entire] a cleanroom or clean zone. Levels should be expressed in terms of an ISO Class, which represents maximum allowable concentrations of particles in a unit volume of air.[11] In Europe, zone classifications are defined as Grade A, B, C, or D for sterile medicinal products[12], which will be discussed shortly. Qualification results should be no older than 12 months to be valid. [13]

Cleanroom Monitoring ISO 14644-2:2015 defines monitoring as observations made by measurement in accordance with a defined method and plan to provide evidence of the performance of an installation. Monitoring may be continuous, sequential, or periodic; and if periodic the frequency shall be specified. This information may also be used to detect trends in operational state and to provide process support. [14]

Monitoring plans take into account a documented **risk assessment**, which is a written plan made to account for levels of air cleanliness required, as well as critical locations and performance attributes of

[11] ISO 14644-1: 2015, 3.1.4

[12] EU GMP, Annex 1.

[13] [13] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities.", 3.2.4, page 19

[14] ISO 14644-2: 2015, 3.2

the clean area. As with any business practice, this plan should undertake periodic evaluation and review of the monitoring plan, and improvements should be implemented where appropriate.[15]

Risk assessment plans for environmental monitoring are generally created using a team approach (Microbiologist, Quality Assurance, Quality Control, Manufacturing, Facilities, and Engineering), and this team often includes a consultant familiar with FDA/GMP regulations.

Sources:

- International Conference Harmonization (ICH) Q9 – Quality Risk Management

- European Medicines Agency (EMA)
    - Eudralex – Volume 4, Good Manufacturing Practices (GMP) Guidelines

  Guide Presented in 3 parts :
    - Part I covers GMP Principles for the manufacture of medicinal products
    - Part II covers GMP for active substances used as starting materials
    - Part III is intended to host a collection of GMP related documents, which are not detailed guidelines on the principles of GMP laid down in the directives (EU Commission Directive 2003/94/EC and 91/412/EC)

- FDA: Guidance For Industry – Q9 Quality Risk Management: https://www.fda.gov/downloads/Drugs/Guidances/ucm073511.pdf

- WHO Guidelines for Quality Risk Management: http://www.who.int/medicines/areas/quality_safety/quality_assurance/Annex2TRS-981.pdf

[15] ISO 14644-2: 2015, 4.1.

SOURCES OF CONTAMINATION

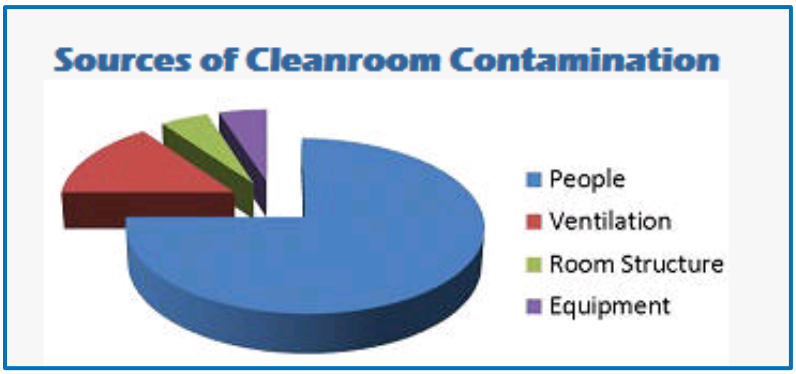
Processes that generate submicron particles include:

People: Contamination generally comes from skin flakes and oil, cosmetics and perfume, spittle, clothing debris (lint, fibers, etc.), and hair. People are the #1 cause of bio and particle burden.

Process and machinery. Basically, anything that creates friction is creating particles.

Fluids: Particles floating in air, bacteria, organics and moisture, floor finishes or coatings, cleaning chemicals, plasticizers (outgasses), and deionized water.

Simply, any kind of process, machinery, and especially people add to the particle burden of the room.



Particle counters or microbial samplers have an exhaust mechanism. These components obviously have moving parts, which create friction. This friction increases the particle burden of the cleanroom by generating inert particles. It is industry best practice to ensure all environmental monitoring equipment has a HEPA filtered exhaust, or that the exhaust emissions are externally removed from the clean zone.

ISO 14698-1:2008, Section A.3.2 states, "The exhaust air from the sampling apparatus should not contaminate the environment being sampled or be reaspirated by the sampling device."

It is therefore of paramount importance that all environmental monitoring equipment used in a clean area, especially aseptic operations, incorporate a HEPA filtered exhaust.

CLEAN AREA CLASSIFICATIONS

Cleanroom classification is provided by two primary sources, the first is ISO 14644-1:2015.

Table 1 — ISO Classes of air cleanliness by particle concentration

ISO Class number (*N*)	Maximum allowable concentrations (particles/m³) for particles equal to and greater than the considered sizes, shown below[a]					
	0,1 µm	0,2 µm	0,3 µm	0,5 µm	1 µm	5 µm
1	*10*[b]	d	d	d	d	e
2	100	*24*[b]	*10*[b]	d	d	e
3	1 000	237	102	*35*[b]	d	e
4	10 000	2 370	1 020	352	*83*[b]	e
5	100 000	23 700	10 200	3 520	832	d, e, f
6	1 000 000	237 000	102 000	35 200	8 320	293
7	c	c	c	352 000	83 200	2 930
8	c	c	c	3 520 000	832 000	29 300
9g	c	c	c	35 200 000	8 320 000	293 000

[a] 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.

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

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

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

[e] 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.

[f] 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.)

[g] This class is only applicable for the in-operation state.

Air cleanliness class by particle concentration shall be designated by an ISO Class number, N. The maximum permitted concentration of particles for each considered particle size is determined by the above table.

The other classification in the Life Science industry is the EU GMP, Annex 1, Sterile Medicinal Products, which is mirrored by the PIC/S, and other biopharmaceutical standards.

	Maximum permitted number of particles per m^3 equal to or greater than the tabulated size			
	At rest		In operation	
Grade	0.5 µm	5.0µm	0.5 µm	5.0µm
A	3 520	20	3 520	20
B	3 520	29	352 000	2 900
C	352 000	2 900	3 520 000	29 000
D	3 520 000	29 000	Not defined	Not defined

The GMP Grade A is equivalent to an ISO Class 4.8 as dictated by the > 5µm particle limit.

Grade A / ISO Class 4.8 – High Risk Operations

High Risk operations (e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections) are conducted in ISO Class 5 or EU GMP Grade A/B zones. Normally, such processes are conducted in a laminar flow work station, with flow range between 0.36 – 0.54 m/s (guidance). A laminar air flow and lower velocities may be used in closed isolators and glove boxes.[16]

For these high risk zones, non-viable particle counts > 5 µm may be indicative of a biocontamination problem.

Aseptic Support Areas:

Grade B / ISO Class 5

These areas can be monitored either continuously with a low flow (1 CFM) fix particle counter, or sequentially with a portable particle counter.

[16] EU GMP: 2008, Annex 1, Clause 3

Grade C and D

Clean areas for carrying out less critical stages in the manufacture of sterile products.[17] These areas are monitored sequentially with a portable particle counter.

ISO Class 6-8

The nature of the activities conducted in a supporting clean area determines its classification. FDA recommends that the area immediately adjacent to the aseptic processing line meet, at a minimum, Class 10,000 (ISO 7) standards under dynamic conditions. Manufacturers can also classify this area as Class 1,000 (ISO 6) or maintain the entire aseptic filling room at Class 100 (ISO 5). An area classified at a Class 100,000 (ISO 8) air cleanliness level is appropriate for less critical activities (e.g., equipment cleaning).[18] These areas are monitored sequentially with a portable particle counter.

The standards provide guidance for the various clean areas, but much of the monitoring practices are directly a result of the risk management assessment.

As stated previously, it is good business practice to evaluate the monitoring plan on a regular basis, and Climet also recommends that Standard Operating Procedures (SOP's) and the User Requirement Specification (URS) also be evaluated and revised as necessary on a periodic basis. Business Best Practices is to evaluate annually.

There has been quite a bit of technological progress in the past five to ten years, and a periodic review and modification: 1) may eliminate unneeded requirements, 2) may incorporate new technology, processes, and procedures; and 3) should incorporate Cost of Poor Quality (CoPQ) requirements for any laboratory equipment.

[17] EU GMP, Annex 1, clause 3.

[18] FDA cGMP: 2004, IV.(b).

STANDARDS

The manufacture of sterile products is subject to special requirements in order to minimize risks of microbiological contamination, and of particulate and pyrogen contamination. [19]

Cleanroom classifications as specified by different standards are shown below in Table 1.

TABLE 1. Particle Concentration Limits, Non-Viable Monitoring

Obsolete Fed Std 209E	ISO 14644-1	EU GMP, Annex 1	Max. Concentration Limits (particles/m^3 of air) for particles equal to and larger than the sizes listed below			
			0.3µm	0.5µm(1)	1µm	5µm (2)
	ISO Class 4.8 (3)	Grade A	not defined	3,520	not defined	20
100	ISO Class 5	Grade B	10,200	3,520	832	29
1,000	ISO Class 6	- - -	102,000	35,200	8,320	290
10,000	ISO Class 7	Grade C	not defined	352,000	83,200	2,900
100,000	ISO Class 8	Grade D	not defined	3,520,000	832,000	29,000

(1) EU GMP Annex 1 and ISO 14644-1:2015 limits are identical on the 0.5 µm particle size "at rest".

(2) EU GMP, Annex 1 requirements for 5 µm. Virtually every pharmaceutical monitors 5µm particles in Class 5 areas as a key indicator of biocontamination and risk assessment.

(3) For Grade A the airborne particle classification is ISO 4.8 dictated by the limit for particles ≥5.0 µm

[19] EU GMP, Annex 1, Principal. As of **2 October 2020**, standard is currently in DIS (Daft), with 5 µm Grade A particle limit increased to 29 .

Non-viable monitoring is conducted with the use of particle counters, and is the metaphorical canary in a coal mine. It provides an early warning of a possible contamination problem.

Particles are significant because they can enter a product as an extraneous contaminant, and can also contaminate it biologically by acting as a vehicle for microorganisms.[20]

The table above is a conflation of the various standards, and where applicable the most conservative value is shown.

In aseptic monitoring, the particle sizes of interest are 0.5 µm and 5 µm.[21]

IMPORTANCE OF MACROPARTICLES

In biopharmaceutical industrial manufacturing, the >5 µm particle size (defined as a Macroparticle in ISO 14644-1:2015) is an important size of interest. Viable microorganisms generally aggregate in chains, clusters or pairs (i.e., in colony forming units or CFUs) greater than 5 µm in size. This measurement is therefore an important early indicator of a contamination problem, and is confirmed in both EU GMP, Annex 1, clause 3; as well as ISO 14644-1:2015:

ISO 14644-1:2015, 4.3, Table 1, foot note (f) states, "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)." Annex C is dedicated to macroparticles, and was added to the ISO 14644-1 DIS prior to being ratified in 2015. This was due to concerns expressed by the life science industry as the 5 µm particle size limitation was removed from the classification for ISO 5 zones. In this light, a joint study by AstraZeneca and Glasgow University (March 2014) confirms that the 5 µm channel on a particle counter was more accurate in detecting airborne microorganisms than fluorescence-based real time microbial air samplers, which have a tendency to grossly overcount (i.e.,

[20] FDA cGMP. Aseptic Processing, iv(a), page 5

[21] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities.", 12(c), page 17.

false-positives).[22] Contrary to marketing claims, not only do viable microorganisms fluoresce, but virtually all carbon-based life, including but not limited to, dead microorganisms, sterile skin flakes, pollen, and vegetative matter. Additionally, many minerals fluoresce. Per the AstraZeneca and Glasgow University study, IPA, as well as particles from gowning, gloves, and other cleanroom materials fluoresce. Also, the manufacturers of fluorescence counters fail to share that many APIs fluoresce. Subsequently, when validating alternative methods, its critical to test for false positives that might result in unnecessary batch rejections.

EU GMP, Annex 1, clause 3 most eloquently states, "The monitoring of the ≥ 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."

It is therefore recommended that those organizations involved in the regulated life science industry follow the >5 µm size limits for the maximum number of particles per cubic meter sample as given in EU GMP, Annex 1.

FREQUENCY OF MONITORING

Environmental monitoring should be conducted based on a scheduled frequency as determined by a documented risk assessment conducted by the manufacturer. [23]

Operations where products are likely to be contaminated and affect the health of the vaccine require more frequent Environmental Monitoring sampling. Areas where values exceeding the regulatory limit

[22] https://journal.pda.org/content/68/2/172; and http://eprints.gla.ac.uk/84187/1/84187.pdf

[23] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." Section, "Routing Monitoring for Particulates." Section 3.2.6, no. 23, page 22 (2012).

have been detected require increased EM sites and frequency compared to areas where monitoring results consistently fall within set specifications over time.

PDA's Fundamentals of Environmental Monitoring offers the following Guidance: [24]

Monitoring Guidance	USP <1116>	EU Annex 1, PIC/S and WHO Annex 4	Japan (Aseptic Processing Guidance)
FREQUENCY Airborne total particulate and viable count.	**ISO 5**: Each production shift **ISO 7**: Each operating shift **ISO 8**: Twice per week	**A**: In Operation, continuous with frequent viable sampling. **B**: In operation, frequent particle monitoring required. **C, D:** Monitoring on risk basis.	**A, B:** Each operating shift for airborne viable, and continuous for airborne particulate monitoring. **C, D:** Airborne viable twice per week; airborne particulate once per month.

According to the EU GMP, Annex 1, 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.[25]

This is echoed by the FDA, which states in regards to ISO Class 5 areas, that regular monitoring should be performed during each production shift. They recommend conducting non-viable particle monitoring with a remote counting system as these systems are generally less invasive than portable particle counters.[26]

Grade B or ISO Class 5 locations that are background for a Critical Area:

Cubic meter sampling is required in the at rest state.[27] If using a portable particle counter, the source implies that 100 LPM should be used with a 10 minute sample (one cubic meter sampling). Frequency of monitoring is made in accordance with the principles of risk management.

[24] Parenteral Drug Association, Inc. "Fundamentals of an Environmental Monitoring Program: Technical Report No. 13 (Revised)." Table 3.0-2, Page 9. ISBN: 978-0-939459-67-4 (2014).

[25] EU GMP, Annex 1, no. 9, pages 3-4

[26] FDA cGMP:2004. Aseptic Processing. Section iv(a), Page 6

[27] EU GMP, Annex 1, no. 5; and FDA cGMP, Section A Page 5-6. Cubic meter sampling is required for ISO Class 5.

Grade B-D zones, or Non-Critical ISO Class 5-8 Areas, should be monitored sequentially, with portable particle counters. Due to longer tubing lengths and the risk of particle loss, sequential monitoring with a portable particle counter in these areas would yield more accurate results.

Grade B corridors and Grade B areas should be monitored at least once during each shift using a portable particle counter.[28]

Grade C process areas should be monitored at more frequent intervals than less critical areas such as corridors, changing rooms, and Grade D areas. [29]

The monitoring of Grade C and D areas in operation should be performed in accordance with the principles of quality risk management.[30]

Enhanced monitoring should be provided in certain Grade C and D areas, for example, in facilities processing biological products where low grade areas can potentially contribute a significant bioburden (to the point of sterility failure).

SAMPLE LOCATIONS

The objective of **particle monitoring** in a cleanroom or clean zone is to provide evidence that the required level of cleanliness is achieved at critical control points. Risk assessment and evaluation of data from formal cleanroom or clean zone classification in accordance with ISO 14644-1 should be used to determine the monitoring locations (critical control points).[31]

The number of sample locations when certifying or validating a cleanroom is determined by a chart provided in ISO 14644-1, A.4.1, and shown below:

[28] Best Practices for Particle Monitoring in Pharmaceutical Cleanrooms, Technical Monograph No. 16. Page 23.

[29] Best Practices for Particle Monitoring in Pharmaceutical Cleanrooms, Technical Monograph No. 16. Page 24.,

[30] EU GMP, Annex 1, no 15, page 4

[31] ISO 14644-2: 2015, B.3.1.1, page 11.

Table A.1 — Sampling locations related to cleanroom area

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
1 000	27
> 1 000	See Formula (A.1)

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.

Clean rooms 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.[32]

With regards to **clean zone monitoring**, "Locations should be representative of all areas in the clean room, but locations where product is put at high risk of contamination should be included during **routine monitoring**. As an example, in rooms where open operations are carried out in a unidirectional airflow hood, the hood should be sampled routinely; the surrounding area may be sampled at a lower frequency, or in multiple sites sampled on a rotating basis. **Areas of low risk** (such as those distant from product, materials, or air flows) should be sampled occasionally to provide confidence that low levels of contamination are maintained in such areas. Sampling plans where a central point in a room is chosen and samples exclusively taken at this point are not an optimal use of Environmental Monitoring."[33]

Clean rooms and clean air devices should be routinely monitored in operation and the monitoring locations based on a formal risk analysis study (EU GMP, Annex 1, #8). Air and surface samples should be taken at the locations where significant activity or product exposure occurs during production.[34]

If you are not sure about what sample points to use for monitoring purposes, please contact Climet, and we would be happy to have one of our local validation partners contact you directly to provide any necessary consultation or advice.

[32] EU GMP, Annex 1, no.8, page 3

[33] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." Section, "Routing Monitoring for Particulates." Section 3.2.4, no. 23, page 21 (2012).

[34] EU GMP, Annex 1, section x(a)(1), page 32.

SAMPLE VOLUMES & TIMES

ISO 14644-1:2015 states that at each sample location a volume of air sufficient to detect a minimum of 20 particles for the largest selected particle size when at the class limit for the designated ISO Class.

ISO 14644-1:2015 has no class limit for the 5 µm particles in an ISO Class 5 area. Therefore, we must now generally assume that $C_{n,m}$ = 20 if the 5µm particle is a size of interest.

The following equation in the ISO 14644-1:2015 standard confirms what is already an industry best practice. In critical areas such as ISO Class 5 or GMP Grade A/B, a full cubic meter **sample volume** is required.

> The single sample volume, V_s, per sampling location is determined by using Formula (A.2):
>
> $$V_s = \left(\frac{20}{C_{n,m}}\right) \times 1000 \qquad \text{(A.2)}$$
>
> where
>
> Vs is the minimum single sample volume per location, expressed in litres (except see Annex D);
>
> $C_{n,m}$ is the class limit (number of particles per cubic metre) for the largest considered particle size specified for the relevant class;
>
> 20 is the number of particles that could be counted if the particle concentration were at the class limit.

Sample times are a function of the particle counter's nominal flow rate, largest particle size of interest, and the ISO or GMP classification of the clean area. The following are recommended sample times in minutes:

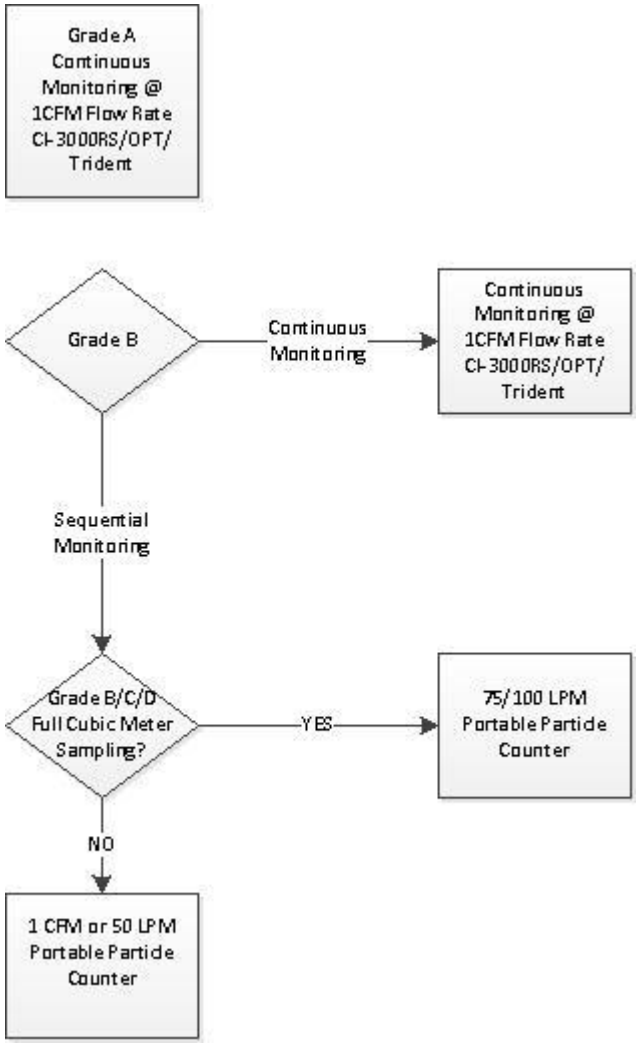
			Flow Rates & Times (minutes)				
ISO	GMP	5 µm	0.1 CFM	1 CFM	50 LPM	75 LPM	100 LPM
4.8	A	20	353.4	35.3	20.0	13.3	10.0
5	B	29	243.7	24.4	13.8	9.2	6.9
7	C	2930	2.4	1 minute	1 minute	1 minute	1 minute
8	D	29300	1 minute	1 minute	1 minute	1 minute	1 minute

** Fill-Stations or other continuous monitoring applications (aka "Critical Areas" or Grade A) must use a 1 CFM particle counter (Climet model no. CI-3100 OPT or CI-3100 RS).

For aseptic preparation and filling, this is a background area for the Grade A zone. A one cubic meter sample must be taken when certifying a Class A (ISO 5) zone, and this rule is also required for monitoring purposes. [35]

Additionally, for certification of a cleanroom, "The volume sampled at each location shall be at least two liters, with a minimum sampling time of one minute for each sample at each location." [36] It is industry best practice when monitoring to also sample a minimum volume of 2 liters, with a minimum sampling time of one minute.

As you can see, if you wish to perform full cubic meter sampling for your sequential monitoring, labor efficiencies can be attained by using higher flow rate particle counters when 5 µm is a size of interest (e.g., life science industry). The additional cost for a higher flow rate instrument can generally be recovered in labor hour savings required to perform sampling/monitoring. If doing sequential monitoring, you can use the same high flow rate particle counter in clean areas with high particle limits, aka higher ISO/GMP classifications.



[35] EU GMP, Annex 1, clause 3.

[36] ISO 14644-1:2015, A.4.4

Flow Rate when Measuring 5µm

The particle counter should have a sample flow rate of at least 28.3 liters per minute (1 CFM) and should be fitted with an isokinetic probe for sampling in unidirectional flow zones. [37]

In areas where non-unidirectional flow exists, the LSAPC should be located with the sample [probe] inlet facing vertically [upwards].[38] Please, refer to the "Isokinetic Sampling" section of this document when the >5 µm particle is a size of interest.

Enclosed Areas – BSC's

The rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and compromise the partial containment barrier provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce this risk. Other personnel activities in the room (e.g., rapid movements near the face of the cabinet, walking traffic, room fans, open/closing room doors) may also disrupt the cabinet air barrier. BCSs should be located away from doors, hallways and traffic areas. [39]

[37] ISO 14644-1:2015, C.4.1.2

[38] ISO 14644-1:2015, C.4.1.2

[39] Center for Disease Control (CDC). Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, Appendix A, page 300.

Enclosed work spaces (isolators and Class III biosafety cabinets) should be monitored by means of a remotely located isokinetic probe(s). The connection between the sampling probe and the particle counter should be kept short enough so that loss of particles does not occur.[40]

In BSC's, the air curtain is very fragile, and it is ***not*** recommended the particle counter be located in front (or inside) of the BSC as exhaust emissions may disrupt the laminar flow increasing the risk of contamination.[41] Best practice is to locate the instrument on the side, and at least 12 inches away (with an internal HEPA filter), or alternatively users may externally exhaust the air from the environment.

In small areas such as within isolators or cabinets where only one sampling site is possible, three replicates must be taken. Results of these tests should not be averaged. [42]

Laboratory Equipment Enclosures

The preferred material is stainless steel for numerous reasons:

1) Stainless steel is one of the few static neutral materials:
   Aluminum is a good second choice. However, plastics can carry a very high negative static charge, which attracts particles of all sizes, including Microbe-Carrying-Particles.[43] Plastics can also be a food source for some bacteria (*Ideonella sakainesis*). Thus, plastic enclosures increase the risk of a cleanroom's bioburden.

[40] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." No. 25, page 20.

[41] Center for Disease Control (CDC). Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, Appendix A, page 300.

[42] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." No. 20, page 19.

[43] USP <1116>

2) Stainless steel does not biodegrade like plastic:
   Plastic biodegradation occurs from bacteria, enzymes, UV Light, and exposure to moisture and harsh chemicals, which cause cracking and micro-fractures of the plastic. Where bacteria and fungi are allowed to grow in recesses or when cleaning and sanitation procedures are ineffective, continuous or even resistant environmental strains can be developed. [44]

3) Is not easily damaged.
   Scuffs, cracks, and innocuous damage on plastics will create perfect hiding places for bacteria, and other viable microorganisms.

4) Easily cleaned and sanitized

Given the aforementioned, it is industry best practice that all cleanroom laboratory equipment is constructed of Stainless steel, or alternatively the second best choice is aluminum.

Probe Locations

The location of the sample probe depends on the criticality of the clean zone.

Sample probes used in sterile and aseptic environments should be located normally not more than 1 foot (30 cm) away from the work site, within the airflow, and during filling/closing operations."[45]

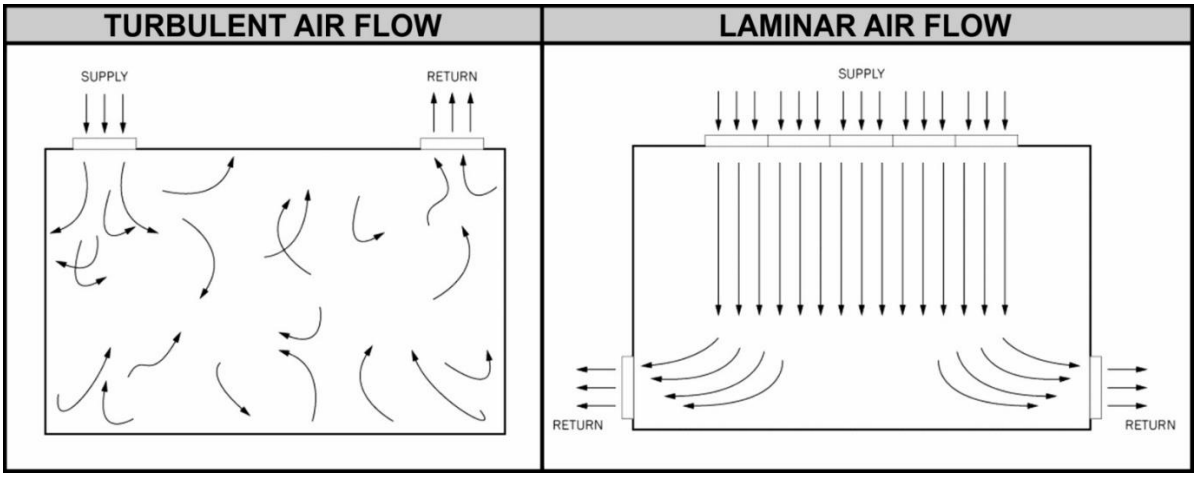
However, both EU GMP and the FDA cGMP recognize that, "Some operations can generate high levels of product (e.g., powder) particles that, by their nature, do not pose a risk of product contamination. It may not, in these cases, be feasible to measure air quality within the one-foot distance and still differentiate background levels of particles from air contaminants. In these instances, air can be

[44] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." Page 4.
[45] FDA cGMP: 2004. "Sterile Drug Products Produced by Aseptic Processing." Section iv(a), page 5-6; and WHO, no. 24, page 20.

sampled in a manner that, to the extent possible, characterizes the true level of extrinsic particle contamination to which the product is exposed."[46] And, "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."[47]

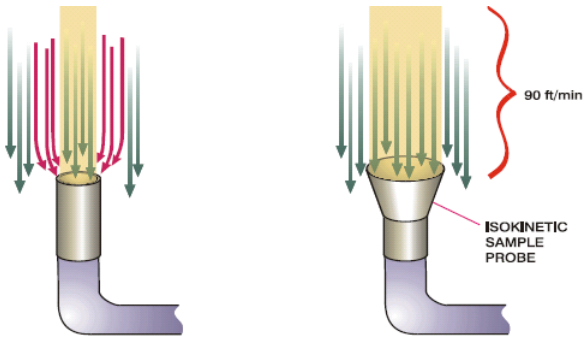
In these situations, it is recommended the probe be mounted on a moveable bracket to allow the variable adjustment of the isokinetic probe.

ISOKINETIC SAMPLING



Isokinetic sampling is required in laminar flow (aka unidirectional flow) areas.

During routine monitoring in critical areas, "Sample probes should be positioned at work height and pointed in a direction such that the probability of detecting particles is maximized. In unidirectional airflow environments, where possible, probes should point into the airflow that has just passed the product. Where this is not possible, probes should be directed



[46] FDA cGMP: 2004. " Sterile Drug Products Produced by Aseptic Processing." Section iv(a), page 5-6.

[47] EU GMP, Annex 1, no.9, page 4.

towards the area surrounding the product and **not towards clean air flowing directly out of the HEPA filter**." [48] [49]

In areas where non-unidirectional flow exists, the LSAPC should be located with the sample inlet [probe] facing vertically [upwards].[50] If 5 µm particles are a size of interest, Climet recommends the use of an isokinetic probe in non-unidirectional or turbulent air flow zones. Research conducted by Climet has demonstrated that an inlet without an isokinetic probe will have a circular turbulence donut-like pattern around the particle counter inlet. Heavier 5 µm particles are not entrained in the airflow as easily as smaller particles, and are more influenced by gravity. Subsequently, these macroparticles will frequently be bounced away from the inlet due to turbulence. The isokinetic probe can be used as a tool to entrain and capture heavier macroparticles in turbulent air flow environments.

The term Isokinetic is defined as an equal or uniform sampling of particles in motion within the air. Isokinetic sampling means that the velocity at the tip of the probe is equal to the inlet velocity. The true particulate concentration will therefore be measured.

To ensure isokinetic sampling, each probe is designed for the airflow of the particle counter. For example, never use a 1 CFM isokinetic probe with a 50 LPM particle counter. This will result in turbulence at the probe's inlet and thus sampling errors.

For example, when the inlet velocity is less than the calculated probe velocity, the sample results will be biased high (i.e., oversampled) due to inertia of large macroparticles < 5 µm.

When the inlet velocity is greater than the calculated probe velocity, the sample results will be biased low, (i.e., undersampled) as larger macroparticles break through the airstream and bypass the inlet.

[48] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." Section 3.2.4, no. 23, page 20 (2012).

[49] ISO 14644-2:2015, Section A.4.5

[50] ISO 14644-1:2015, C.4.1.2

ZERO COUNT TEST (THE PURGE TEST):

Before you begin your daily round of sampling, it is good practice to perform a False Count Test (also known as a Zero Count Test). The **purpose** is to verify there is no internal contamination of the particle counter, or electronic problems that would produce false-high counts. Important to note this Purge Test alone will not tell you if the instrument is grossly undercounting.

1) Establish Company Limits: Counts for the Purge Test for a one minute sample should be no more than 10% of the counts typically obtained in the cleanest area to be monitored. You can calculate limits using the following equation:

   Limits = (flow rate LPM ÷ 1000 L) x Class Limit per m3 x 10%

   Important to note, this is a logical test, not an absolute test. If you slightly exceed the limit established above, you are probably okay.

2) First do a one minute test **without** the filter installed to verify the instrument counts at a believable level in each channel. Here you are looking for abnormally low counts. If counts in the instrument seems abnormally low, do another side-by-side test with another Climet particle counter to determine if they are reasonably close. If okay, clear the counts, and go to the next step.

3) Install the zero-count (or Purge) filter on the inlet of the particle counter. Do another one minute sample. If the instrument produces zero counts or meets the company specifications for the test (Per step 1 above), the particle counter is ready for use.

Performing a Zero Count Test for a longer period of time serves no additional benefit. To the contrary, the cumulative hours spent performing excessive Zero Count Testing will result in the laser diode being operated unnecessarily, thus reducing the monitoring life of the instrument not to mention labor costs.

Again, the purpose of the test is to ensure you do not have a **catastrophic** failure of the instrument. A small number of counts is likely a result of what we call "Dark Counts." Solar flares and other cosmic events may result in spikes of Gama Radiation, X-Rays, etc. These are not uncommon events. The radiation spike will strike the particle counter's photo detector and registers as energy that's indiscernible from light scatter. Subsequently, it's normal to get some false counts, and it's the reason

ISO 14644-1:2015 de-emphasized the 5 um channel in an ISO Class 5 environment to optional macroparticle counts - - the limit of 29 was simply too low.

If you are failing the Purge Test, you likely have internal contamination. Top the inlet to knock free particles that may be sloughing off of the inlet during the test. Continue to top more forcefully until there are no more count bursts.

The filter may also be the reason for stray counts. Try testing with another filter, if available, or test another particle counter. This may help to determine whether the particle counter or the filter is the problem.

If you are a Climet customer, and would like a boilerplate procedure, please contact service@climet.com

SAMPLE AVERAGING:

It is common to take three samples of the same volume for *each sample location*, and then average the counts.[51] If you begin monitoring immediately, and you take (for example) three one minute samples to average, you will likely see that the 5 micron count on each sample decreases while the 0.5 micron count does not change significantly. This is because 5 micron particles fall out more quickly than 0.5 micron particles (walking into or out of the areas being monitored by the particle counter will stir up > 5 µm particles from the floor).

For all forms of environmental monitoring, the assumption should be made that contaminants are introduced into the clean room from finite points, and their subsequent distribution may be limited or

[51] ISO 14644-1:2015, 5.3 (page 6), and A.6.2.1 (page 12)

sporadic. For this reason, averaging of values across sampling points is not appropriate for *in-operation* monitoring and for *at rest* monitoring. [52]

According to the World Health Organization, "If room is small and only one location needs to be probed, at least three replicates should be made and values may be averaged."[53]

Also, "In small areas such as within isolators or cabinets where only one sampling site is possible, three replicates must be taken. Results of these tests should not be averaged.[54]

AIR SETTLEMENT DELAY:

When monitoring a cleanroom it is good practice to let the air settle prior to taking a sample. Many of our customers program a minute or two delay into their particle counter and microbial air sampler so that the sampling begins after the air in the sample area is allowed to settle. Again, this allows the person taking the sample an opportunity to at least step back 6-10 feet to allow the air to settle (remember, people are a source of particles). You are also monitoring a process *in operation*, and therefore evacuating an entire cleanroom to do monitoring is not recommended.

SAMPLE HEIGHT:

It is good practice to take the sample from *at work height* and about 1 meter above the floor, and pointed in a direction such that the probability of detecting particles is maximized.[55] This would be from a counter top or often from a cart when doing sequential monitoring.

[52] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." no. 19, page 19.

[53] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." no. 20, page 19.

[54] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." no. 20, page 19.

We must remember that most particle and bio burden in a clean area is a result of people and equipment. Sampling at a height well above these factors will provide a false sense of comfort with regards to the compliance of your cleanroom.

A poor practice is placing the particle counter on an elevated stand or tri-pod. The only logical explanation for sampling in this fashion is to perform a HEPA filter scan. In such cases, one would normally be performing an overlapping sweeping scan of the entire filter. Simply, mounting a particle counter in an elevated position is a wasted effort. Monitoring from an elevated height also puts the instrument at risk if knocked over. The World Health Organization states in a section pertaining to unilateral or laminar airflow zones, "[..] probes should be directed towards the area surrounding the product and not towards clean air flowing directly out of the HEPA filter." [56]

Additionally, "It may not be appropriate to locate a sample probe directly under a HEPA filter in a non-unidirectional area because such a location may not be representative of the cleanroom or clean zone, and may prevent detection of contamination events in operation."[57]

TREND ANALYSIS:

Particle counts should be uploaded into a database to allow for trend analysis. The whole idea of trend analysis is to identify worsening trends before it becomes a deviation, which requires a considerable amount of paperwork and cost. By identifying an early trend, you can start your investigation and take early corrective actions before a problem occurs. Increasing counts may occur slowly over the course of months, and those performing the monitoring should know the normal particle burden of the cleanroom as well as any trends. There are certainly LIMS solutions that large

[55] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." no. 23, page 20.

[56] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." no. 23, page 20.

[57] ISO 14644-2:2015, A.4.5, NOTE.

pharmaceuticals utilize. For the smaller biotech or pharmaceutical firms, Climet offers a proprietary software solution that's a fraction the cost of a LIMS system.

HEPA FILTERED EXHAUST:

A HEPA filtered exhaust is required, and is an industry best practice for all particle counters and microbial air samplers.

Exhaust from laboratory equipment may contain either inert or viable particles, and these viable particles are frequently in the form of microbe-carrying-particles (MCPs). This is true for even microbial samplers as studies from decades ago have confirmed that as physical collection efficiency nears 100%, there is a substantial decline in biological efficiencies. Therefore, even the most effective microbial sampler will not collect all viable microorganisms. [58] [59]

Biocontainment technology incorporates unidirectional (or laminar) air flow with the use of HEPA filters to capture and remove airborne contaminants from the air stream. These combinations of technologies help to both protect the worker from potentially infectious aerosols and provide necessary product protection.[60] The use of a HEPA filter that is only 90% efficient, when tested against sub-micron particles used in standard classification methods, was greater than 99.99% efficient in removing microbe-carrying-particles (MCPs) in occupied rooms, such as cleanrooms. [61]

In addition to infectious biohazard concerns, all laboratory equipment that creates air movement (instruments that incorporate centrifuges, fans, vacuum pumps, etc.) have internal components that create mechanical friction. When mechanical friction occurs, inert particles are generated and expelled through the exhaust. These particles on new equipment are generally, at a minimum, in the thousands

[58] Whyte, Green and Albisu. "Collection efficiency and design of microbial air samplers." Department of Mechanical Engineering, University of Glasgow, Scotland.

[59] Stewart, Grinshpun, Willeke, Terzieva. "Effect of Impact Stress on Microbial Recovery on an Agar Surface." Applied and Environmental Microbiology, April 1995, p. 1232-1239.

[60] Center for Disease Control (CDC). Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, Appendix A. Page 293.

[61] Whyte, Green and Whyte. "Removal of Microbe-Carrying-Particles by HEPA Filters in Cleanrooms." International Jounal of Ventilation, Vol. 10, 21012, issue 4.

at 0.5 µm and in the tens at 5.0 µm and above. These aerosol sized particles spread widely through production areas.[62]

As a result of the aforementioned, virtually all standards[63] require a HEPA filtered exhaust in both particle counters and microbial samplers as they will contribute to the bioburden and particle burden of a cleanroom by the production of inert and potentially infectious aerosols through exhaust emissions that expelled widely throughout the cleanroom.

The CDC states that best practices are to insist the device's exhaust air is HEPA filtered or be removed from the laboratory.[64] This is further confirmed twice in ISO 14698:

"The sampling plan shall take into account the cleanliness level of the risk zone and the degree of biocontamination control required for the activity being conducted, to protect individuals, the environment, the process and the product. Elements to be considered include, but is not limited to the impact of operations, personnel and equipment in risk zones which contribute to biocontamination, such as monitoring/measuring devices".[65]

The exhaust air from the sampling apparatus should not contaminate the environment being sampled or be reaspirated by the sampling device. [66]

[62] World Health Organization (WHO). "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities.", no2, page 4

[63] Center for Disease Control (CDC). "Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition", Appendix A.; and ISO 14698-1:2003(E), Section 5.3.2.4(h)(4); and ISO 14698-1:2003(E), Section A.3.2, last paragraph.

[64] Center for Disease Control (CDC). Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, Appendix A. https://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_appendixa.pdf

[65] ISO 14698-1: 2003, Section 5.3.2.4(h)(4)

[66] ISO 14698-1: 2003, Section A.3.2

According to the World Health Organization, "When a process generates particles or microorganisms, it may be difficult or even impossible to demonstrate compliance with Environmental Monitoring requirements. In such cases a detailed validation study should be conducted that demonstrates that the nature of the product alone is responsible for these results. This may take the form of repetitive simulation studies (e.g., using an innocuous replacement of product such as growth media) where all Environmental Monitoring results are found to be acceptable." [67]

ALERT AND ACTION ALARMS

Alert and Action Level information is covered in detail in Application Note 170712, which can be downloaded by CLICKING HERE

Alternatively, you can login to Climet's Tech Library at http://www.climet.com/library/

VHP SANITATION

It is an excellent practice to VHP all microbial samplers and particle counters at least every six months to ensure no biocontamination growth occurs within the equipment. Climet recommends monthly VHP.

DATE AND TIME PERMISSIONS:

It is industry best practice that date and time programmed into any environmental monitoring equipment be set by Administrator security privileges (Username and Password Protected).

[67] World Health Organization (WHO). "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." No. 73, page 34.

TRANSPORT BETWEEN AREAS

When portable counters are transported between areas, companies must demonstrate the effectiveness of measures taken to avoid cross-contamination. Specially segregated areas (such as for spore-forming microorganisms or microorganisms handled in biosafety facilities) must have dedicated particle counters. [68]

BATTERY PREVENTATIVE MAINTENANCE

The battery in a portable particle counter will not last forever. Climet recommends a preventative maintenance program to replace the battery every 3-5 years, depending on use, and criticality/expense of the product being monitored. The 3 year replacement is for both Nickel Metal Hydride or Lithium Ion batteries. Additionally, whenever the battery icon display is on it last cell before the daily sampling rounds are completed, a new battery should be installed.

REVIEW OF RISK ASSESSMENT PLAN, SOPS, AND URS'S

A **Risk Assessment Plan** is a written document made to account for levels of air cleanliness required, and includes critical locations and performance attributes of the clean area. As with any business practice, this plan should undertake **periodic evaluation and review of the monitoring plan**, and improvements should be implemented where appropriate.[69]

[68] World Health Organization (WHO). "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." Section 3.2.3, no. 18, page 18 (2012).

[69] ISO 14644-2: 2015, 4.1.

CALIBRATION REQUIREMENTS

The calibration of particle counters is highly regulated, and is subject to FDA and GMP regulatory compliance. Regarding particle counters, **ISO 14644-2, Section 4.4** confirms that all particle counter calibrations must be ISO 21501-4:2018 compliant. **ISO 21501-4:2018, Section 6.10 (NOTES),** confirms that calibrations must be made by a laboratory that is **ISO 17025 accredited**. Failure to follow these standards will, with a high probability, result in regulatory action.[70] [71]

SAMPLE TUBING

Several factors may affect the efficacy of a particle counter including: (1) air velocity or flow rate of the particle counter; (2) tubing length; (3) number of tubing bends; (4) the radius of these bends, (5) tubing diameter; and (6) tubing material.

In pharmaceutical industrial manufacturing, particle sizes of interest are generally >0.5 µm and >5 µm. The latter (>5 µm) particle size is very important as it is a leading indicator of a biocontamination problem as viable organisms usual aggregate in chains clusters or pairs – colony forming units (cfu's) of >5µm in size.

Today, a Climet particle counter (even 100 LPM) has strong efficiency in being able to count and size small particles less than 1 µm. These small particles remain entrained in the air flow, and are not susceptible to what the industry has termed "**Tubing Loss**."

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.[72]

[70] ISO 14644-1:2015, A.2.2, page 8.

[71] ISO 14644-2:2015, 4.4, page 3.

[72] EU GMP, Annex 1, clause 11

Manifold systems were at one time recommended in critical areas (and are still mentioned in EU GMP, Annex 1, no. 11). These are now defined as, "**Inappropriate for monitoring particles >5 µm due to losses in long transport tubing lengths required by these systems**." [73]

Today, it is a well-established fact that particle sizes greater than 1 µm are susceptible to Tubing Loss. Tubing Loss is caused by: 1) impaction of particles onto the walls of the transport tubing; and 2) sedimentation or gravity.

The now retired Fed. Std. 209E recommended transport tubing be of a specified Reynolds number, and due to particle loss in transport tubing established the maximum length at 3 meters when a particle size of interest is between 2-10 µm.[74] The maximum flow rate of a particle counter during this period was only 1 CFM (28.3 LPM), and the Fed. Std. 209E explicitly made this recommendation for this specific flow rate.

However, the Federal Standard 209E (ratified September 11, 1992) was cancelled November 29, 2001, and superseded by ISO 14644-1 and 14644-2. These ISO standards mention in several places: 1) the negative effect of >5µm tubing loss; and 2) that **transport tubing length should be kept as short as possible**. ISO 14644 makes no tubing length recommendations or requirements.[75]

Quality Managers must remember that in 2005, Climet was the first to introduced the 50 LPM particle counter. In 2006, Climet again pioneered the 75 LPM particle counter, and in 2007 Climet again brought to market the world's first useable and reliable 100 LPM particle counter. These all occurred ***after*** Fed. Std. 209E was cancelled.

As part of Climet's new product validation procedures, our engineers at that time conducted a **Tubing Loss Study** to determine particle collection efficiency for our new high flow rate aerosol counters. The

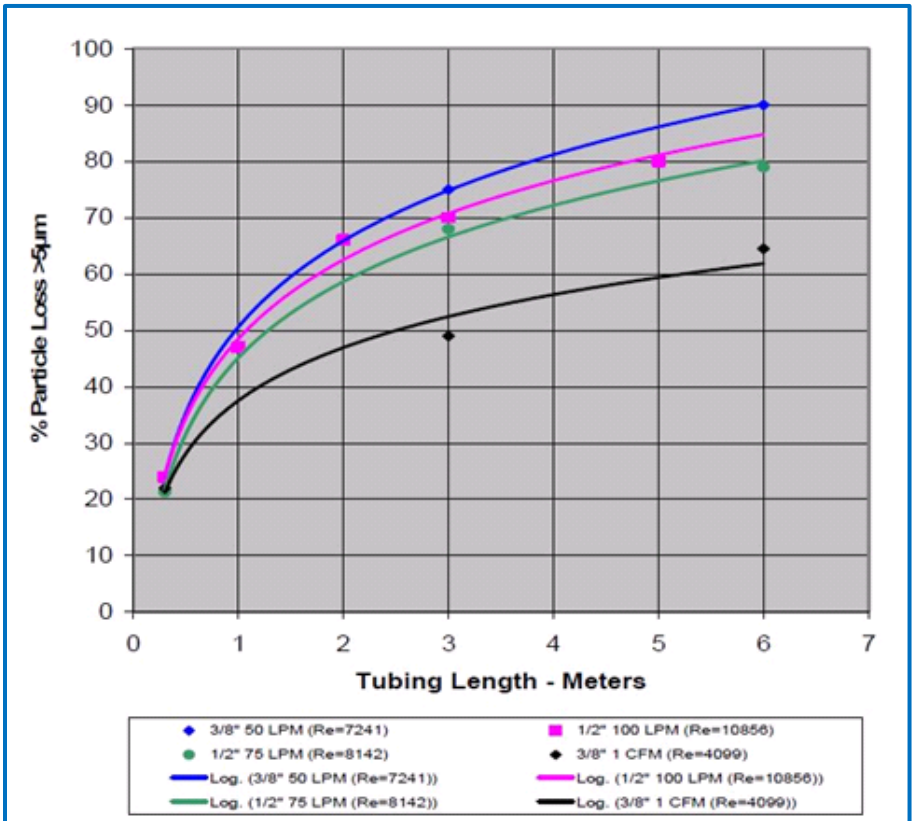
[73] ISO 14644-2:2015(E), Section A.4.2, NOTE 2.

[74] Federal Standard 209E:1992, Section B40.2.1, page 27.

[75] Institute of Environmental Sciences and Technology. IEST Work Group CC100 , "NOTICE OF CANCELLATION FED-STD-209 NOTICE 1", November 29, 2001. And, ISO 14644-1 and ISO 14644-2.

study confirmed that losses occur due to: 1) the length of the transport tubing, and 2) previously unknown, turbulence inside the tubing (i.e., intra-tubular turbulence) is present in all flow rates, and varies by the inside diameter of the tubing. This, we learned, was responsible for the initial >35% loss in the first 1 meter of tubing. In the first 1 meter of tubing: 1) 50 LPM at 3/8" tubing is ~50%; 2) 75 LPM with larger ½" tubing is ~45%; and 3) 100 LPM at ½" tubing is ~ 48%.

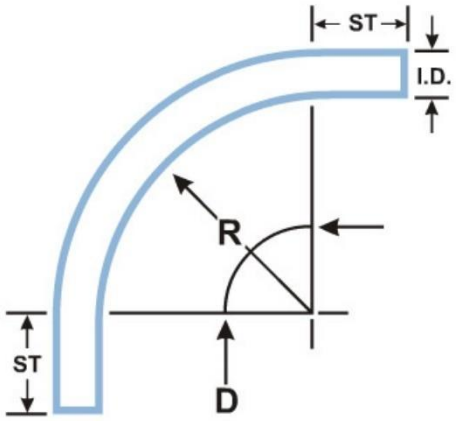
Particle Counter Flow Rate	Recommended Max. Tubing Length
1 CFM	3 meters (10 feet)
50 LPM	3 meters (10 feet)
75 LPM	3 meters (10 feet)
100 LPM	2 meters (6 feet)



PARTICLE LOSSES >5µm IN URBAN AIR FOR VARIOUS FLOW RATES AND TUBING LENGTHS

As a consequence of Climet validation procedures, Climet recommended a shorter transport tubing length for a 100 LPM particle counter of 2 meters (~6 feet) maximum.

Since introducing the higher flow rate particle counters, the Climet high airflow design has been reversed engineered by all our major competitors. This includes past patent violation issues, which forced one of our competitors to re-design their particle counter. Regardless, Climet innovations a decade later are currently an industry standard. Unfortunately, many of those imitators are still recommending or referring to the obsolete Federal Standard 209E for maximum tubing length.



D = Degrees Bend Angle
ST = Straight Tangent
I.D. = Inside Dimension
R = Radius (15 cm or 6 inches min.)

Tubing Bends

In order to mitigate tubing loss of >1 µm particle sizes, Climet recommends that bends be eliminated. If this is not feasible, that bends be minimized, and that the bends be no less than a radius of 15 cm (6").

Tubing Preventative Maintenance & Replacement

How you maintain tubing should be part of a written plan or SOP (Standard Operating Procedure).

As stated previously, >5 µm particles may stick to the inner surface of transport tubing due to sedimentation and impaction. This will eventually result in a sudden release of particles and unusually high 5 µm counts that have nothing to do with the actual conditions inside the clean area.

Subsequently, Climet recommends a preventative maintenance program be implemented to both clean and eventually replace transport tubing at regular intervals. This can potentially save significant expenses as a single deviation investigation has an average cost between $8,000 to $12,000 among pharmaceutical manufacturers, which far exceeds the cost of simply replacing transport tubing.

CLIMET RECOMMENDATIONS:

Type: Climet recommends (and sells) BEV-A XX transport tubing.

Sterilize: BEV-A XX can be sterilized with Ethylene oxide, and the BEV-A XX transport tubing sold by Climet can also be autoclaved.

Frequency of Sanitization and Replacement: Climet recommends replacing or sanitizing transport tubing every quarter, and we are aware of some companies that replace or sanitize transport tubing on a monthly basis. Obviously, the frequency would depend on the amount of sampling performed, and the classification of the cleanroom. If properly maintained, we recommend replacing the tubing every 3-5 years. ***HOWEVER, if the sample tubing is exposed to VHP****, the inner lining is made of Hydrel, which is not compatible with VHP. In these cases, we would highly recommend replacing the transport tubing at least annually.*

Tubing Type

With regards to tubing type, the following is optional.

1. Stainless Steel
2. Bev-A-Line XX
3. Polyester (polyurethane)
4. Polyester lined vinyl
5. Copper
6. High Density polyethylene
7. Glass
8. Teflon

Climet recommends autoclavable Bev-A-Line XX tubing be installed within Stainless Steel tubing. The use of Bev-A-Line makes cleaning and replacement of the tubing itself a simple, inexpensive, and a quick process, while the rigidity of the Stainless Steel provides great support and prevention against accidental crimping, pinching or damage to the Bev-A-Line XX tubing by external forces.

Moreover, Stainless Steel is one of the few static neutral materials and provides an excellent barrier for biocontamination. Plastics should never be exposed in critical areas, and should even be eliminated or

mitigated in non-aseptic areas when possible. Plastics represent a substantial increase in biocontamination risk, as plastics carry a high negative static charge. Positive and negative static charges will subsequently attract particles of every size. This can present an increased risk in biocontamination due to microbe-carrying-particles, or particle-carrying-microbes.

Finally, it is important to use the correct diameter of tubing recommended by the manufacturer of the particle counter. Using a small inside diameter of tubing will prevent the particle counter's blower to pull the proper flow through the tubing, and will result in under-sampling.

COMPRESSED AIR GASES

Microbial monitoring of manufactured clean rooms, RABS, and isolators should include compressed gases, surfaces, room or enclosure air, and any other materials and equipment that might produce a risk of contamination.[76]

When microbial monitoring is not conducted on compressed gases, it is industry best practice to conduct particle monitoring on compressed gases. The logic behind particle monitoring is the concern regarding the sustained viability of microorganisms after having been compressed, decompressed, run through a high pressure diffuser, and finally having been impacted onto agar.

Depending on the manufacturer, typically you see monitoring of microbial, particle, and in many instances, both. This is largely dependent on the risk management report, validation studies, and product or substance being manufactured.

In either case, a *high pressure diffuser* would be required to ensure the measurement instrument (particle counter or microbial sampler) is not damaged by excessive pressure, and should always be used.

Also refer to the following:

Application Note:
http://www.climet.com/library/app_notes/hpd/HPD_Application_Note_r1-0.pdf

Configuration & Best Practices
http://www.climet.com/products/ci302.html

[76] USP <1116> page 787