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 non-viable particulates and microorganisms is an important control function in achieving product compendial requirements for 1) Foreign and Particulate Matter; and 2) Sterility.[1]

The manufacture of either Drug Products or Drug Substances, in particular, poses a high risk to the general public. In the life science industry, 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. [2] Subsequently, organizations involved in the industrial manufacture of biopharmaceuticals 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.

> **Drug Product** (Definition): *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.
>
> **Drug Substance** (Definition): A d*rug 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.
>
> A **biopharmaceutical** (Definition), also known as a **biologic(al) medical product**, **biological**, or **biologic**, is any pharmaceutical drug product manufactured in, extracted from, or

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

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

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.

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[3] , FDA cGMP:2004[4]; ISO 14698-1:2003; WHO[5] [6]; and EU GMP, Annex 1[7] are frequently referred.

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.

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

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

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

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

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

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.

RISK ASSESSMENT – ENVIRONMENTAL MONITORING

The validation (or certification) of a cleanroom is conducted annually, or semi-annually for critical areas, using different standards and methods 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.[8] In Europe, zone classifications are defined as Grade A, B, C, or D for sterile medicinal products[9], which will be discussed shortly. Qualification results should be no older than 12 months to be valid. [10]
>
> **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. [11]

The *Risk Assessment* is performed to establish the *Monitoring Program*, i.e., sample locations, including identification of critical monitoring locations, frequency of monitoring each of these locations, monitoring methods (continuous or sequential), etc. [12]

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

[8] ISO 14644-1: 2015, 3.1.4

[9] EU GMP, Annex 1.

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

[11] ISO 14644-2: 2015, 3.2

[12] EU GMP: 2022, Annex 1, §9.4

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***.[13]

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

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

- Eudralex, EU Legislation
  The body of **European Union legislation** in the pharmaceutical sector is compiled in Volume 1 and Volume 5 of the publication "The rules governing medicinal products in the European Union":

  - EudraLex, Volume 1 - Pharmaceutical legislation for medicinal products for human use
  - EudraLex - Volume 5 - Pharmaceutical legislation for medicinal products for veterinary use

  The basic legislation is supported by a series of **guidelines** that are also published in the following volumes of "The rules governing medicinal products in the European Union":

  - 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

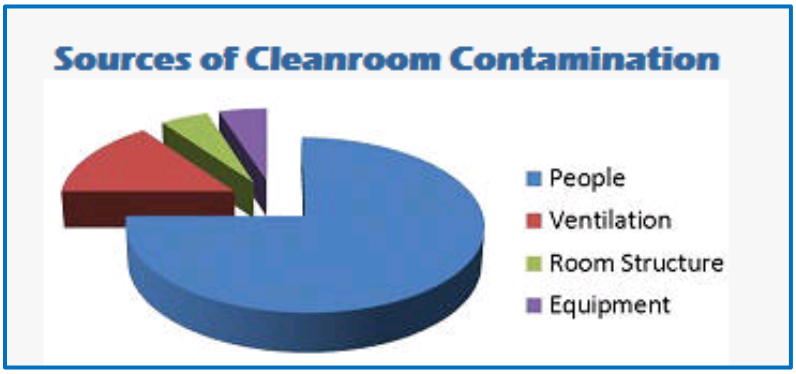
[13] ISO 14644-2: 2015, 4.1.

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 viable and inert particle burden. Estimates suggest up to 80% of contamination is associated with personnel.
>
> **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.

STANDARDS & 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.

Another classification in the Life Science industry is the EU GMP, Annex 1, Sterile Medicinal Products,

Table 5: Maximum permitted total particle concentration for monitoring.

Grade	Maximum limits for total particle ≥ 0.5 µm/m³		Maximum limits for total particle ≥ 5 µm/m³	
	at rest	in operation	at rest	in operation
A	3 520	3 520	29	29
B	3 520	352 000	29	2 930
C	352 000	3 520 000	2 930	29 300
D	3 520 000	Not predetermined [a]	29 300	Not predetermined [a]

PIC/S (Annex 1, revised 1 Feb 2022: pp 2) has more stringent guidelines for 'at rest' and the 5 µm 'in operation' limits:

Clean rooms and clean air devices should be classified in accordance with EN ISO 14644-1. Classification should be clearly differentiated from operational process environmental monitoring. The maximum permitted airborne particle concentration for each grade is given in the following table:

Grade	Maximum permitted number of particles/m³ equal to or greater than the tabulated size			
	At rest		In operation	
	0.5µm	5.0µm	0.5µm	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

Routine monitoring, according to PIC/S (2022: §8), should be done "in operation." This is also confirmed in EU GMP: 2022, Annex 1, §9.5.

PIC/S: 2022 states the particle limits given in the table (on page 2) 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.[14] EU GMP: 2022, Annex 1 mirrors this requirement, albeit the maximum concentration limits are slightly different. [15]

Grade A / ISO Class 5 – 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 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 airflow and lower velocities may be used in closed isolators and glove boxes.[16]

For grade A, particle monitoring should be undertaken for the full duration of critical processing, including equipment assembly. [17] The grade A area should be monitored continuously (for particles ≥0.5 and ≥5 µm) and with a suitable sample flow rate (at least 28 litres (1ft3) per minute) so that all interventions, transient events and any system deterioration is captured [18]

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

[14] PIC/S: 2022, Annex 1, §14

[15] EU GMP: 2022, Annex 1, §9.15, Net 1

[16] EU GMP:2022, Annex 1, §4.30

[17] EU GMP:2022, Annex 1, §9.16

[18] EU GMP:2022, Annex 1, §9.17

EU GMP: 2022, Annex 1, the Grade A zone (both "at rest" and "in operation") is equivalent to an ISO Class 5.

PIC/S: 2022, Annex 1, the Grade A zone (both "at rest" and "in operation") is equivalent to an ISO Class 4.8.

Aseptic Support Areas:

Grade B / ISO Class 7

In EU GMP:2022, Annex 1, while "at rest", the Grade B area is equivalent to ISO Class 5. However, "in operation", the Grade B area has the limits of an ISO Class 7.

PIC/S: 2022, Annex 1, the Grade B zone is equivalent to an ISO Class 5 while "at rest" and ISO Class 7 while "in operation."

It is recommended that a similar system be used for the grade B area although the sample frequency may be decreased. The grade B area should be monitored at such a frequency and with a suitable sample size that the programme captures any increase in levels of contamination and system deterioration. If alert levels are exceeded, alarms should be triggered. [19]

In practice, these areas can be monitored either continuously with a low flow (1 CFM) fix particle counter, or sequentially with a portable particle counter, and the appropriate method would be identified on the end-user risk assessment.

Grade C / ISO Class 8

Routine monitoring while "at rest," the particle limits are equivalent to an ISO Class 7 clean area.

Routine monitoring while "in operation" have limits of an ISO Class 8 clean area.

[19] EU GMP, Annex 1, §9.18

Grade D

Routine monitoring while "at rest," the particle limits are equivalent to an ISO Class 8 clean area.

For Grade D, "in operation" limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and on routine data, where applicable.

These areas are monitored sequentially with a portable particle counter.

General Guidance

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 Practice 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.

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 ISO 14644-1:2015 and PIC/S:

> **ISO 14644-1:2015, 4.3, Table 1, footnote (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., false-positives).[20] 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.
>
> **PIC/S, Annex 1, §13 (1 FEB 2022)** most eloquently states, "In Grade A and B zones, 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

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

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."

EU GMP: 2022, Annex 1, §9.15, Note 2: "The occasional indication of macro particle counts, especially ≥ 5 µm, within grade A may be considered to be false counts due to electronic noise, stray light, coincidence loss etc. However, consecutive or regular counting of low levels may be indicative of a possible contamination event and should be investigated. Such events may indicate early failure of the room air supply filtration system, 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.

FREQUENCY OF MONITORING

Environmental monitoring should be conducted based on a scheduled frequency as determined by a documented risk assessment conducted by the manufacturer. [21]

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 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: [22]

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.

Grade A or ISO Class 5

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.[23] 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

[21] 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).

[22] 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).

[23] EU GMP, Annex 1, no. 9.16

remote counting system as these systems are generally less invasive than portable particle counters.[24]

PIC/S: 2022 states the particle limits given in the table (on page 2) 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.[25] EU GMP: 2022, Annex 1 mirrors this requirement, albeit the maximum concentration limits are slightly different. [26]

In Grade A and B zones, 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.[27]

Grade B or ISO Class 7 locations that are background for a Critical Area:
A similar system of sampling (i.e., Grade A) is recommended for Grade B zones. This implies full cubic meter sampling, although the sampling frequency may be decreased. [28] The frequency of monitoring is made in accordance with the principles of risk management.

PIC/S: 2022 states the particle limits given in the table (on page 2) 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.[29] EU GMP: 2022, Annex 1 mirrors this requirement, albeit the maximum concentration limits are slightly different. [30]

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

[25] PIC/S: 2022, Annex 1, §14

[26] EU GMP: 2022, Annex 1, §9.15, Net 1

[27] PIC/S: 2022, Annex 1, §13

[28] PIC/S:2022, §10; and EU GMP: 2022, Annex 1, §9.18)

[29] PIC/S: 2022, Annex 1, §14

[30] EU GMP: 2022, Annex 1, §9.15, Net 1

In Grade A and B zones, 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.[31]

Grade B-D zones, or Non-Critical ISO Class 7-8 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.[32]

Grade C process areas should be monitored at more frequent intervals than less critical areas such as corridors, changing rooms, and Grade D areas. [33]

The monitoring of Grade C and D areas in operation should be performed in accordance with the principles of quality risk management.[34]

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).

[31] PIC/S: 2022, Annex 1, §13

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

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

[34] EU GMP: 2022, Annex 1, no 15

SAMPLE LOCATIONS

Online Calculator: https://www.climet.com/toolbox/index.html

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).[35]

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:

[Intentionally Blank – Please see chart on next page]

[35] 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.[36]

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 (i.e., critical locations) 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."[37]

[36] EU GMP: 2022, Annex 1, §9.5

[37] 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).

SAMPLE VOLUMES & TIMES

Online Calculator: https://www.climet.com/toolbox/index.html

This information will be found in your organization's **Monitoring Plan**, which itself is based on a **Risk Assessment**. The following is provided as general guidance.

ISO 14644-1:2015, §A.4.4 (Annex A is Normative) provides an equation to determine the **minimum single sample volume size** per location (Vs). This standard 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. Note, ISO 14644-1:2015, Table 1, has no class limit for the 5 µm particles in an ISO Class 5 area. Therefore, ***if the 5µm particle is a size of interest*** C*n,m* = 20.

ISO 14644-1:2015, A.4.4, the volume sampled at each location shall be **at least 2 liters, with a minimum sample time of 1 minute** for each sample at each location. When not taking full cubic meter samples, it is industry best practice, where applicable, to take three samples and average.

ISO 14644-1:2015, §5.3 in summary, states that *if a full cubic meter (1000 L) sample is not taken, multiple samples should be taken (i.e., three) and averaged. These smaller sample times are taken in Grade C and D areas (ISO Class 8+)*

A similar system of sampling used in Grade A monitoring is recommended for Grade B zones, although the sample frequency may be decreased.[38] This implies full cubic meter sampling for Grade B areas.

[38] PIC/S:2022, §10; and EU GMP: 2022, Annex 1, §9.18)

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 / GMP classification of the clean area. The following are recommended sample times in minutes:

				Minimum Time in Minutes				
ISO	**PIC/S**	**5µm**	**VOL (L)**	**0.1 CFM**	**1 CFM**	**50 LPM**	**75 LPM**	**100LPM**
5	A	20	1000L	353.1	35.3	20.0	13.3	10.0
7	B	2900	1000L	353.1	353.4	20.0	13.3	10.0
8	C	29000	1 min	1	1	1	1	1
	D			1	1	1	1	1

** 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).

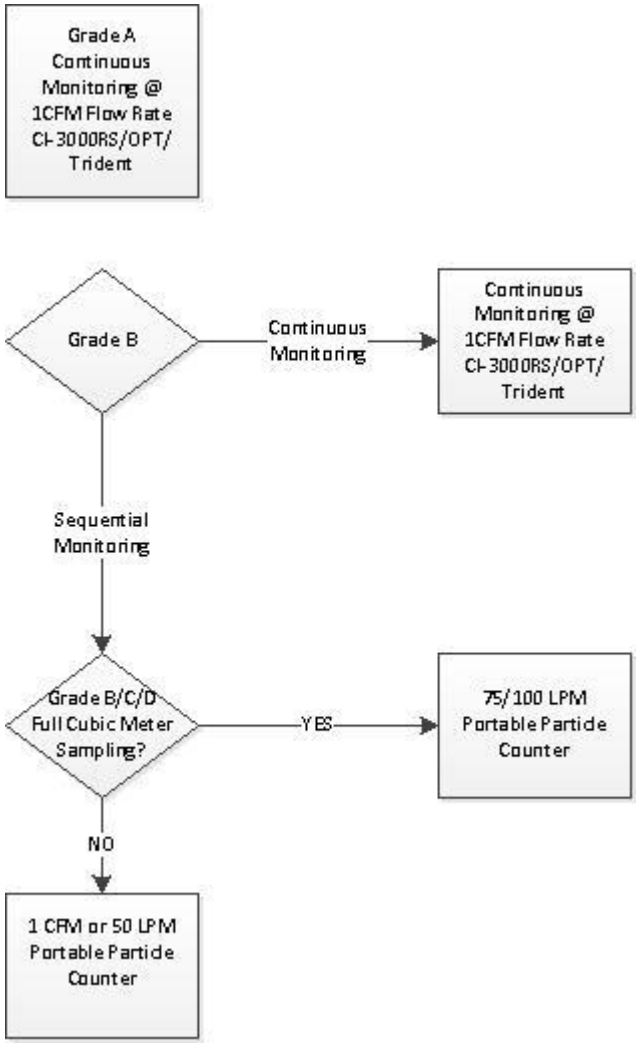
For aseptic preparation and filling, this is a background area for the Grade A (where it is not an isolator), air

e. 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. [39]

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

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." [40] 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.



[40] 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**.[41]

In areas where non-unidirectional flow exists, the LSAPC should be located with the sample [probe] inlet facing vertically [upwards].[42] Climet particularly recommends this when ≥ 5 µm particles are a size of interest. Please, refer to the "Isokinetic Sampling" section of this document when the >5 µm particle is a size of interest.

If conducting full cubic meter *sequential* sampling, and if 5 µm is a particle size of interest, Climet recommends using a 75 LPM or 100 LPM portable particle counter. The higher flow rates will allow you to complete a sample in significantly less time.

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

[42] ISO 14644-1:2015, C.4.1.2, first paragraph, last sentence.

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. [43]

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.[44]

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.[45] 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. [46]

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

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

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

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

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.[47] Plastics can also be a food source for some bacteria (*Ideonella sakainesis*). Thus, plastic enclosures increase the risk of a cleanroom's bioburden.

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. [48]

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.

[47] USP <1116>

[48] World Health Organization. "Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities." Page 4.

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."[49]

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 sampled in a manner that, to the extent possible, characterizes the true level of extrinsic particle contamination to which the product is exposed."[50] 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."[51]

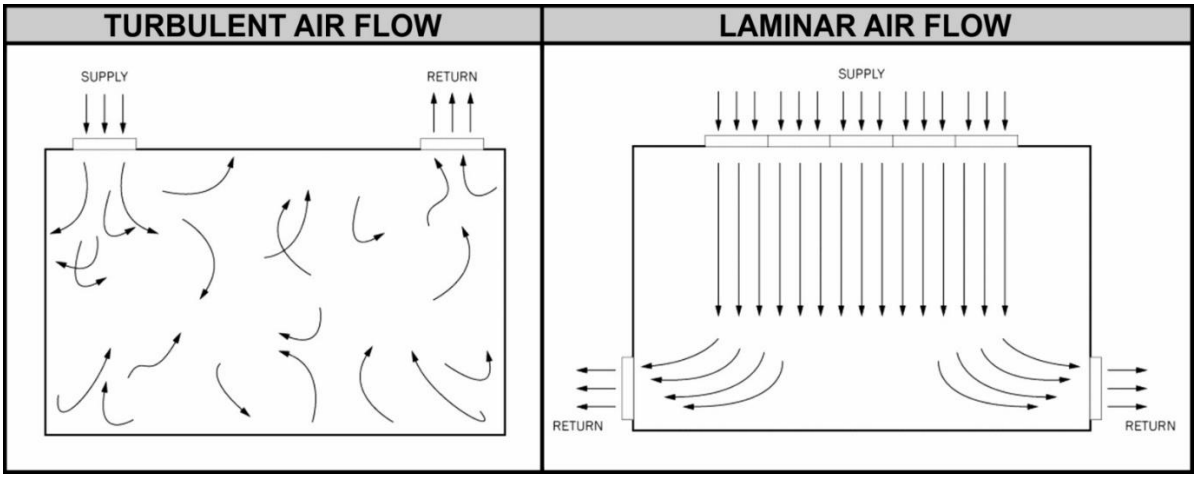
In these situations, it is recommended the probe be mounted on a moveable bracket to allow the variable adjustment of the isokinetic probe.

[49] FDA cGMP: 2004. "Sterile Drug Products Produced by Aseptic Processing." Section iv(a), page 5-6; and WHO, no. 24, page 20.

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

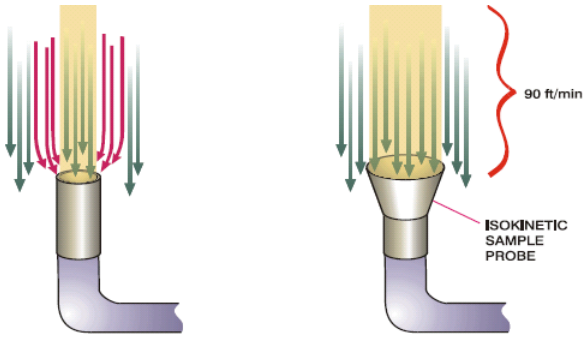
[51] EU GMP, Annex 1, no.9, page 4.

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 towards the area surrounding the product and **not towards clean air flowing directly out of the HEPA filter**." [52] [53]



In areas where non-unidirectional flow exists, the LSAPC should be located with the sample inlet [probe] facing vertically [upwards].[54] If 5 µm particles are a size of interest, Climet recommends the

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

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

[54] ISO 14644-1:2015, C.4.1.2, first paragraph, last sentence. This is particularly important when 5 µm is a particle size of interest.

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.

PURGE TEST / FALSE COUNT TEST / ZERO COUNT TEST

Before you begin your daily round of sampling, it is good practice to perform a False Count Test (better known as a Purge Test). The **purpose** is to: (1) verify there is no internal contamination of the particle counter, and (2) ensure no electronic noise problems exist 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**:

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

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:

Equation 1:

$$Plim = \left(\frac{F}{1000} \text{x M}\right) x\ CL\ x\ 10\%$$

Where, *F* = Particle Counter Flow Rate in LPM (i.e, 28.3 LPM, 50 LPM, etc.)
M = Time of Purge Test in minutes
CL = Class Limit for the particle size of interest (*cleanest area*)
Plim = Purge Test Limits

For example, the Class Limit for a Grade A area *in operation* for the > 0.5 µm channel is 3,520 particles according to the 2022 EU GMP, Annex 1 standard (Table 5). These limits are also mirrored in PIC/S (2022). If we are sampling with a 1 CFM (28.3 LPM) particle counter for 3 minutes:

$$Purge\ Test\ Limits = \left(\frac{28.3}{1000} \text{x } 3\right) x\ 3{,}520\ x\ 10\%$$

$$Purge\ Test\ Limits = 29.88, \text{rounded equals } \mathbf{30\ count\ limit}$$

Settings - - be sure you are measuring in "**Total Counts**" and not "Counts/Meter."

If measuring in Counts/Meter, you will need to also use Equation 2 below:

Equation 2

$$Adjusted\ Plim = \left(\frac{1000}{F}\right) xPlim$$

Where, F = Particle Counter Flow Rate in LPM (i.e, 28.3 LPM, 50 LPM, etc.)
Plim = See Equation 1 above

Particle counter calibrations, particle counting and sizing is not an exact science. For example, ISO 21501-4:2018, §6.2, the **50% Count Efficiency Test** allows for a ± 20% variance. Climet finds this way too loose, and has established our own tolerance of 10%. Additionally, the **100% Count Efficiency Test** also allows for a ± 10% variance.

Also, referring to the EU GMP, Annex 1 (2022), §19.15 (Note 2); and PIC/S (2022) §13:

> *The occasional indication of macro particle counts, especially ≥ 5 µm, within grade A may be considered to be false counts due to electronic noise, stray light, coincidence loss etc. However, consecutive or regular counting of low levels may be indicative of a possible contamination event and should be investigated.*

So, when conducting a 1 minute purge test, it is probably okay if the > 5 µm channel has a count of 1-2, so long as its not consequtive or routine.

2) **Test for Expected Range – Low Side**
Do a one minute test **without** the filter installed to verify the instrument counts at the expected range in each channel. Here you are looking for abnormally low counts. If counts seem abnormally low, do a side-by-side test with another Climet particle counter to determine if they are reasonably close (±20% in the 0.5 micron channel). If okay, clear the counts, and go to the next step. NOTE: for comparison testing on the 5 µm channel, you need to condut a minimum of a 10 minute sample, and please, see Technical Note TN-0022 in our online ***Tech Library*** at www.climet.com.

3) **Conduct Purge Test**
Install the zero-count (or Purge) filter on the inlet of the particle counter. Do a 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 will register 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.

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

Contamination may also come from a damaged or old Purge Filter. If the filter is dropped, discard it immediately and replace with a new filter. Climet recommends Purge Filters be replaced annually.

If you are still having problems, speak to a **Climet Applications Engineer** by calling 1-909-793-2788, or email engsupport@climet.com.

SAMPLE AVERAGING:

It is common to take three samples of the same volume for *each sample location*, and then average the counts.[55] 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 sporadic. For this reason, averaging of values across sampling points is not appropriate for *in-operation* monitoring and for *at rest* monitoring. [56]

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."[57]

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.[58]

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

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

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

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

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.[59] This would be from a counter top or often from a cart when doing sequential monitoring.

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." [60]

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."[61]

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

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

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

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 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. [62] [63]

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.[64] 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. [65]

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

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

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

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

[65] 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.[66]

As a result of the aforementioned, virtually all standards[67] 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.[68] 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".[69]

The exhaust air from the sampling apparatus should not contaminate the environment being sampled or be reaspirated by the sampling device. [70]

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

[67] 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.

[68] 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

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

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

If using a particle counter or microbial air sampler WITHOUT an internal HEPA filtered exhaust, the best advice is provided by 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*." [71] There was an FDA 483 that cited a pharmaceutical drug producer for using a particle counter without a HEPA filter and who failed to conduct a validation study.

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

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, and at least every six months.

VHP Testing by Independent Lab

1) Application Note – Curis Whitepaper
2) Earlier Climet Testing

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).

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. [72]

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

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.[73]

[73] 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.[74] [75]

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

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

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.[76]

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**." [77]

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

[76] EU GMP, Annex 1, clause 11

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

3 meters when a particle size of interest is between 2-10 µm.[78] 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.[79]

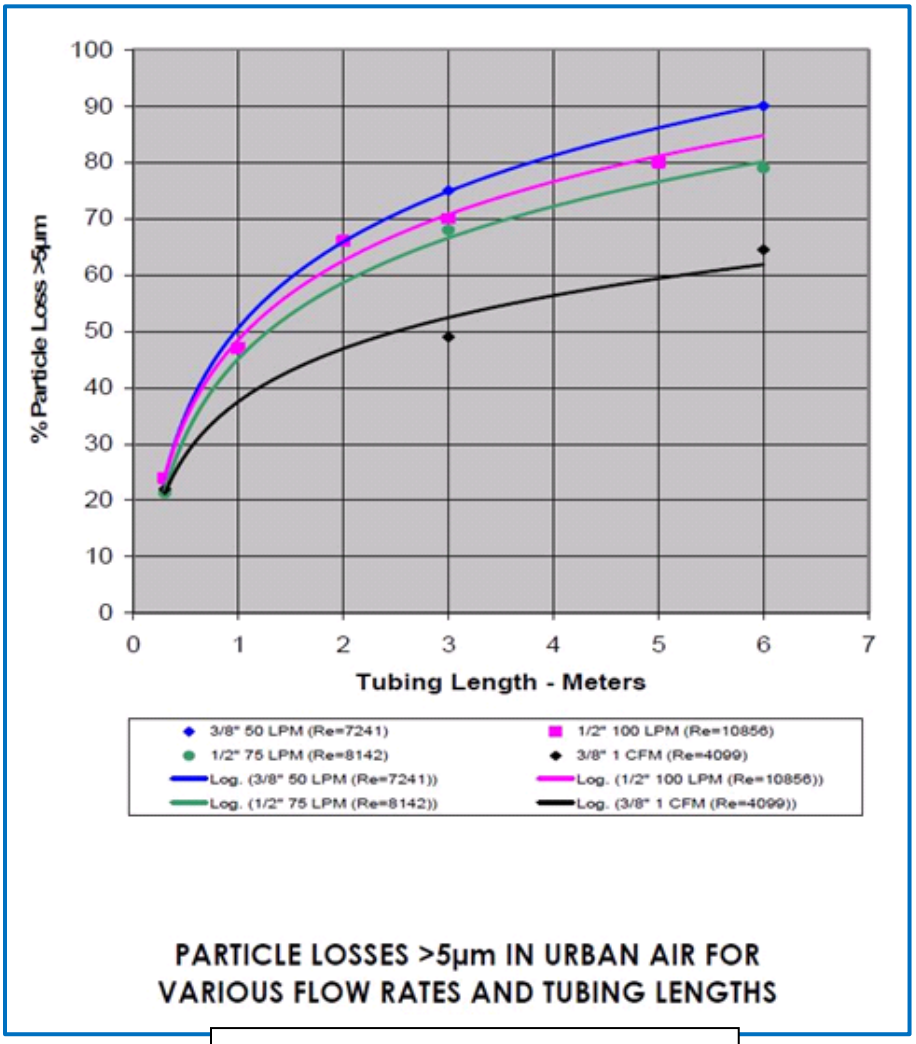
Quality Managers must remember that in 2005, Climet was the first to introduced the 50 LPM particle counter. In 2006, Climet again pioneered the 75 LPM particle counter, and in 2007 Climet again brought to market the world's first useable and reliable 100 LPM particle counter. These all occurred ***after***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

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

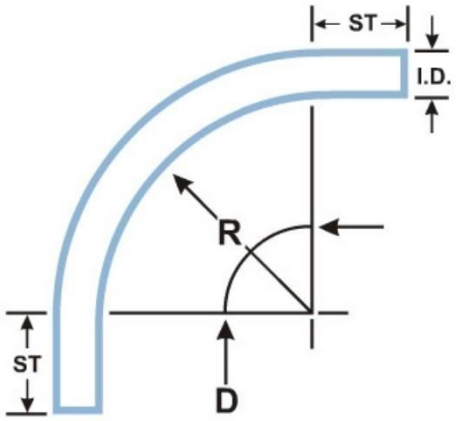
[79] Institute of Environmental Sciences and Technology. IEST Work Group CC100 , "NOTICE OF CANCELLATION FED-STD-209 NOTICE 1", November 29, 2001. And, ISO 14644-1 and ISO 14644-2.



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.[80]

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

[80] USP <1116> page 787

DATA INTEGRITY & USER INTEGRITY

Climet has a web page dedicated to data integrity training, sources, and references: https://www.climet.com/data-integrity/

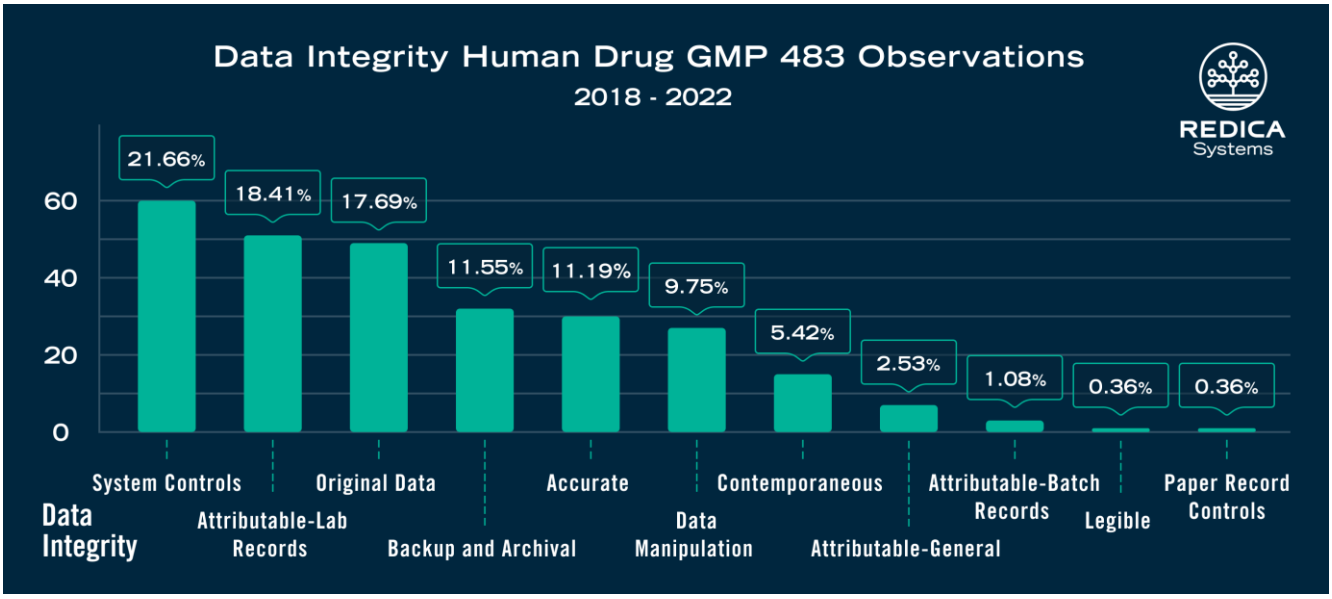
The backbone of data integrity are an organization's processes and procedures (i.e., SOPs).[81] The most commong mistakes that have resulted in FDA 483's and warning letters:

1) **User Integrity common audit observations/findings**
    a. Security not enabled on particle counter or microbial air sampler
    b. Sharing of usernames and passwords (not attributable)
    c. Login credentials being reused or reassigned to other employees
    d. Sample Abort
        i. Failiue to report
        ii. Reporting the aborted sample but losing metadata (i.e., sample counts)
    e. Not periodically checking, recalling or revising passwords
        i. Not renewing passwords (i.e., annually)
        ii. Not maintaining an SOP for password renewals.
        iii. Not deauthorizing terminated employees according to an established SOP
    f. User Access Levels
        i. Not documenting individual access privledges
        ii. Access level MUST be assigned to each user individually
        iii. Assigning all users Admin permission
    g. Not maintaining a log or database of Users: usernames, passwords, date/time issued, data/time last checked/revised, date/time deauthorized, etc.

[81] 21 CFR §11.10 preamble and (e)

2) Data Interigity common audit observations/findings

a. Lack of quality culture in the organization
b. No comprehensive written plan for data integrity controls
c. Falsification of Data
d. Missing critical metadata
    i. Unit ID missing, sample counts on aborted samples, etc.
e. Inability to discern invalid or altered records
f. Particle Counter is not a closed system. Ability to import files into the counter such as pdfs or images.



Per the above chart, if we add all 'attributable' factors, it amounts to 22.02% of all observations. 11.55% of 483's are due to failure to adequately backup or archive metadata, data manipulation still occurs in 9.75% of all FDA 483 observations.

Cyber Security is a key element of Data Integrity, but is not covered by 21 CFR Part 11. What network, cryptographic, and communication protocols are used? Are these protocols the must current? Does the instrument itself have adequate features to limit **Access Control** and **Surface Area** Vulnerabilities. IT Departments are increasing concerned about particle counter cyber security features.