LIGHTHOUSE
WORLDWIDE SOLUTIONS

WEBINAR

Active Air Sampling Technology & ISO 14698

What is Bio-contamination?

Microbiological contamination is the introduction of infectious materials like virus, bacteria, mold, fungi, prions, yeast, protozoa or their by-products onto otherwise clean, sterile or safe environments/surfaces where they shouldn’t be found and at high enough concentrations such that they can be detrimental to our health and the safety/efficacy of medicinal products.

“Hygiene has become increasingly important in many areas of modern society. In such areas, hygiene or biocontamination control methods are, or will be, used to create safe and stable products.

International trade in hygiene-sensitive products has greatly increased. At the same time, the use of antimicrobial agents has been reduced or forbidden, creating a need for increased biocontamination control”

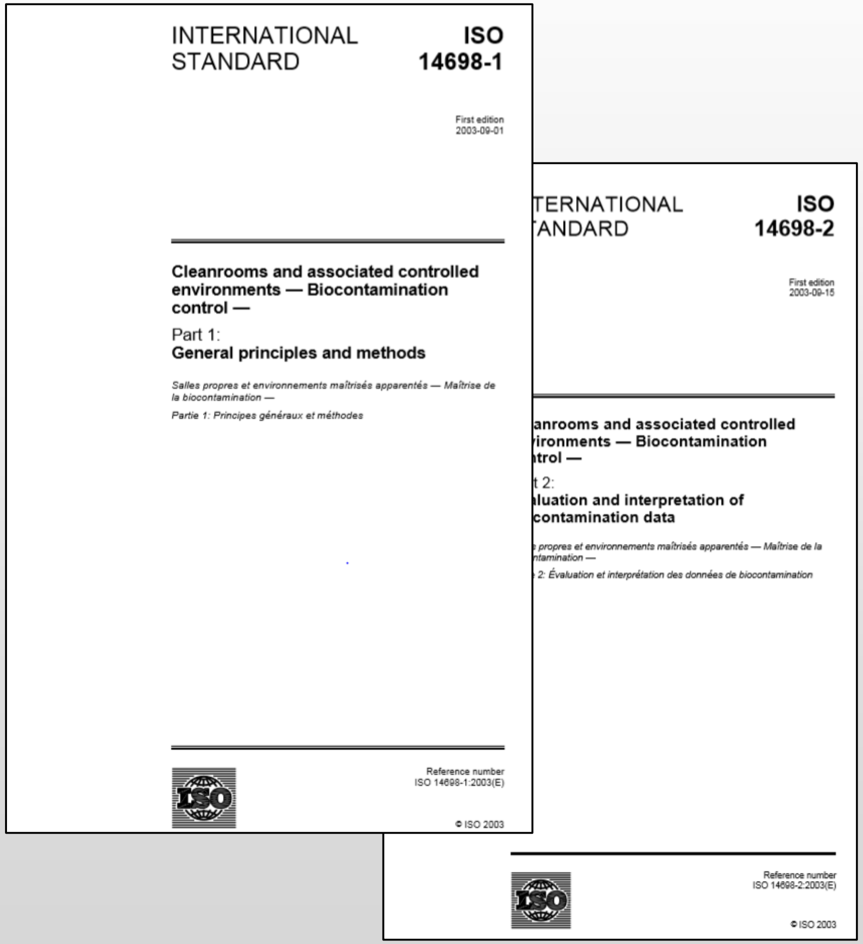
Statement from ISO 14698 – Part 1

What is ISO 14698?

- **ISO 14698 (Released 2003)**

Part 1: General principles and methods.

Part 2: Evaluation and interpretation of biocontamination data.



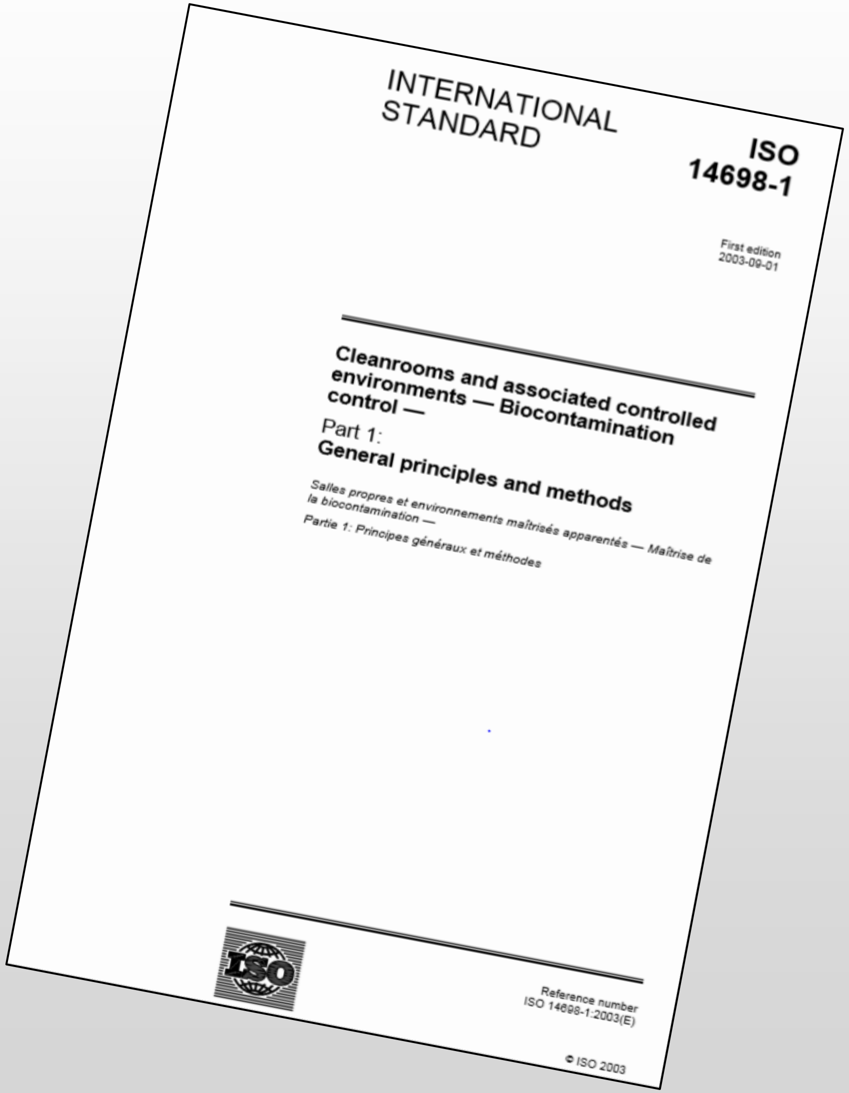
This standard was last reviewed and confirmed in 2014. Therefore this version remains current.

SCOPE

ISO 14698 Part 1

This part of ISO 14698 establishes the principles and basic methodology of a formal system of biocontamination control (Formal System) for assessing and controlling biocontamination when cleanroom technology is applied for that purpose.

This part of ISO 14698 specifies the methods required for monitoring risk zones in a consistent way and for applying control measures appropriate to the degree of risk involved. In zones where risk is low, it can be used as a source of information.



INTERNATIONAL STANDARD

ISO 14698-1

First edition
2003-09-01

Cleanrooms and associated controlled environments — Biocontamination control —

Part 1:
General principles and methods

Salles propres et environnements maîtrisés apparentés — Maîtrise de la biocontamination —

Partie 1: Principes généraux et méthodes

Reference number
ISO 14698-1:2003(E)

© ISO 2003

SCOPE

ISO 14698 Part 2

This part of ISO 14698 gives guidance on methods for the evaluation of microbiological data and the estimation of results obtained from sampling for viable particles in risk zones for biocontamination control. It should be used, where appropriate, in conjunction with ISO 14698-1.

INTERNATIONAL STANDARD

ISO 14698-2

First edition 2003-09-15

Cleanrooms and associated controlled environments — Biocontamination control —

Part 2:

Evaluation and interpretation of biocontamination data

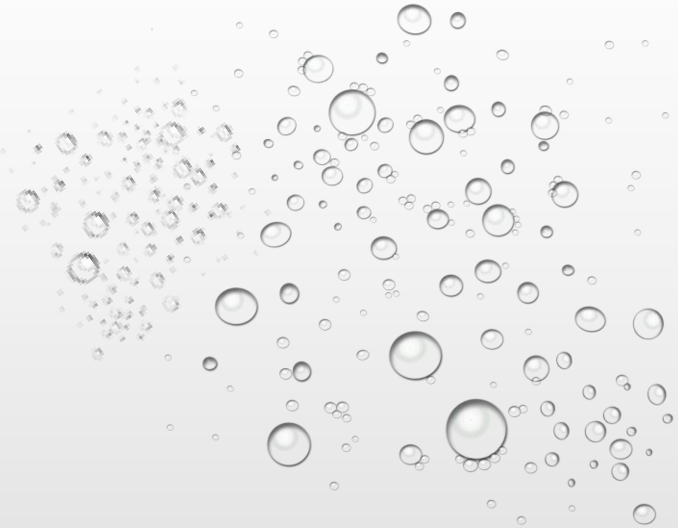
Salles propres et environnements maîtrisés apparentés — Maîtrise de la biocontamination —

Partie 2: Évaluation et interprétation des données de biocontamination

Reference number ISO 14698-2:2003(E)

Types of Contamination

Water Contamination



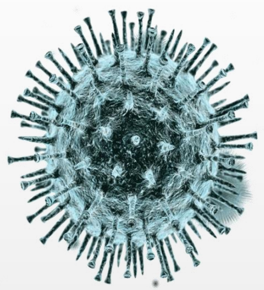
Biological contaminants - Include human fecal matters that introduce disease- causing bacteria like E. coli and many other deadly **pathogens[3]**. For example Contaminated water used for drinking, bathing, washing and food preparation results to various infections, with diarrheal diseases being the most common. The WHO estimates about 1.8 million deaths annually from waterborne diseases.

Surface and Airborne Contamination

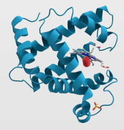
Bacteria - Represent the most important group of **pathogens** within the context of **microbiological contamination**. They are either "commensal" bacteria or **"pathogenic" bacteria**. Commensal bacteria are part of our natural flora and are usually harmless. They even act as protection from the colonization of pathogenic microorganisms

Types of Contamination

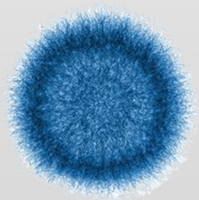
○ Surface and Airborne Contamination



Viruses – These are genetic entities that exist somewhere between living and non-living states, first observed in 1898 by Paul Frosch and Friedrich Loeffler to be smaller than any known bacteria. Viruses exist as capsid or a protein coat (sometimes within a membrane) when found outside of host cells. When they come into contact with host cells, viruses insert their genetic material into the host, where they multiply and literally take over the host's functions.



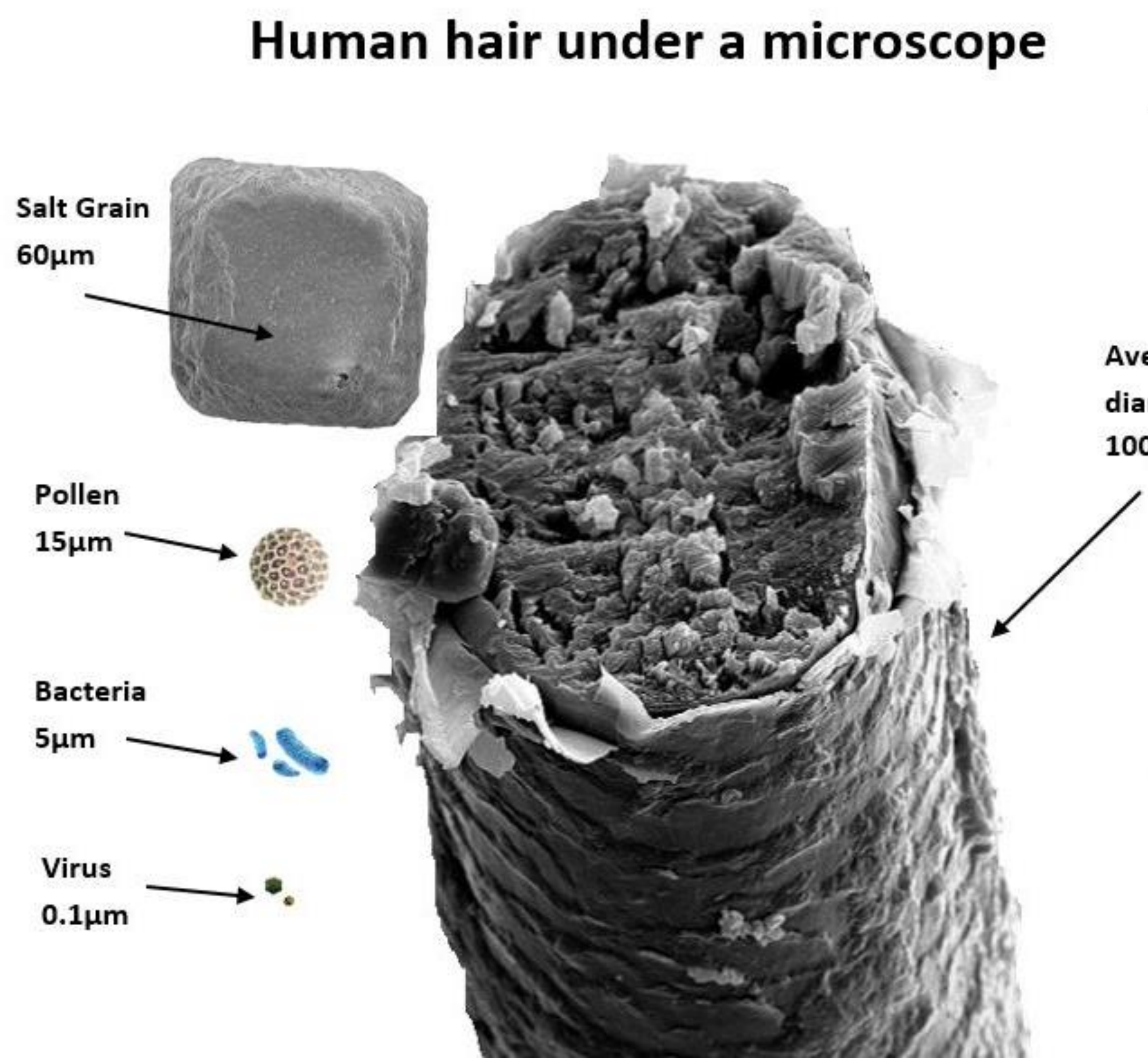
Prions -These are infectious agents made up entirely of protein material. They cause diseases similar to a viral infection.

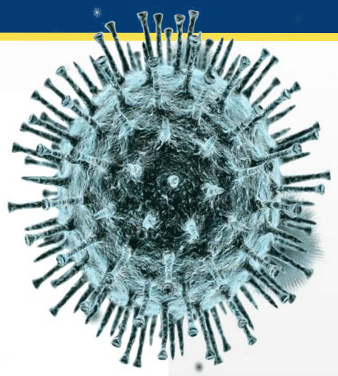


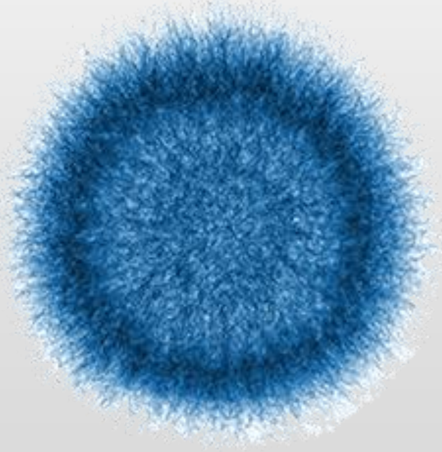
Fungi -These are either single or multi-celled organisms found in any habitat, mostly on plant material and in soil. They cause skin diseases in humans that include ringworm, athlete's foot and thrush. Yeasts and molds are types of fungi

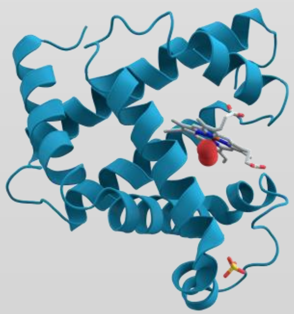
Protozoa - These are single-celled organisms that thrive in moist habitats like soil, marine environments and even fresh water

Types of Contamination

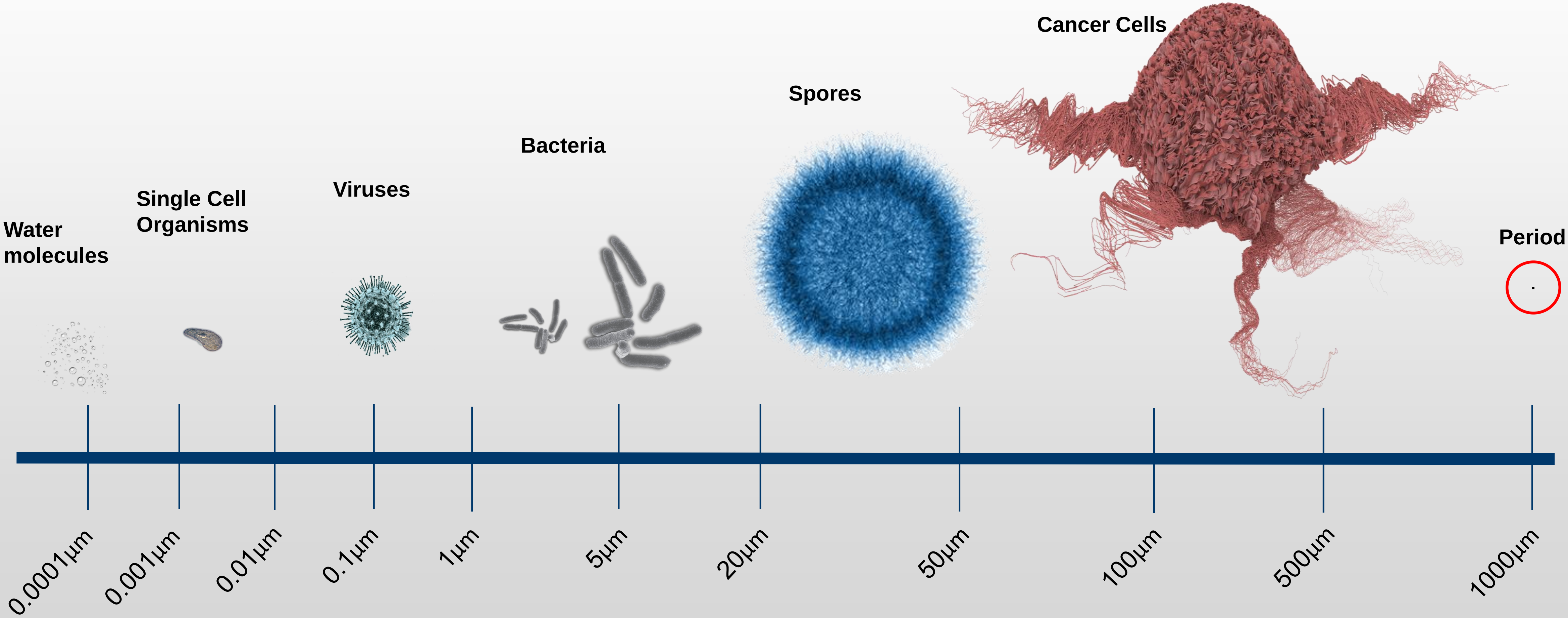




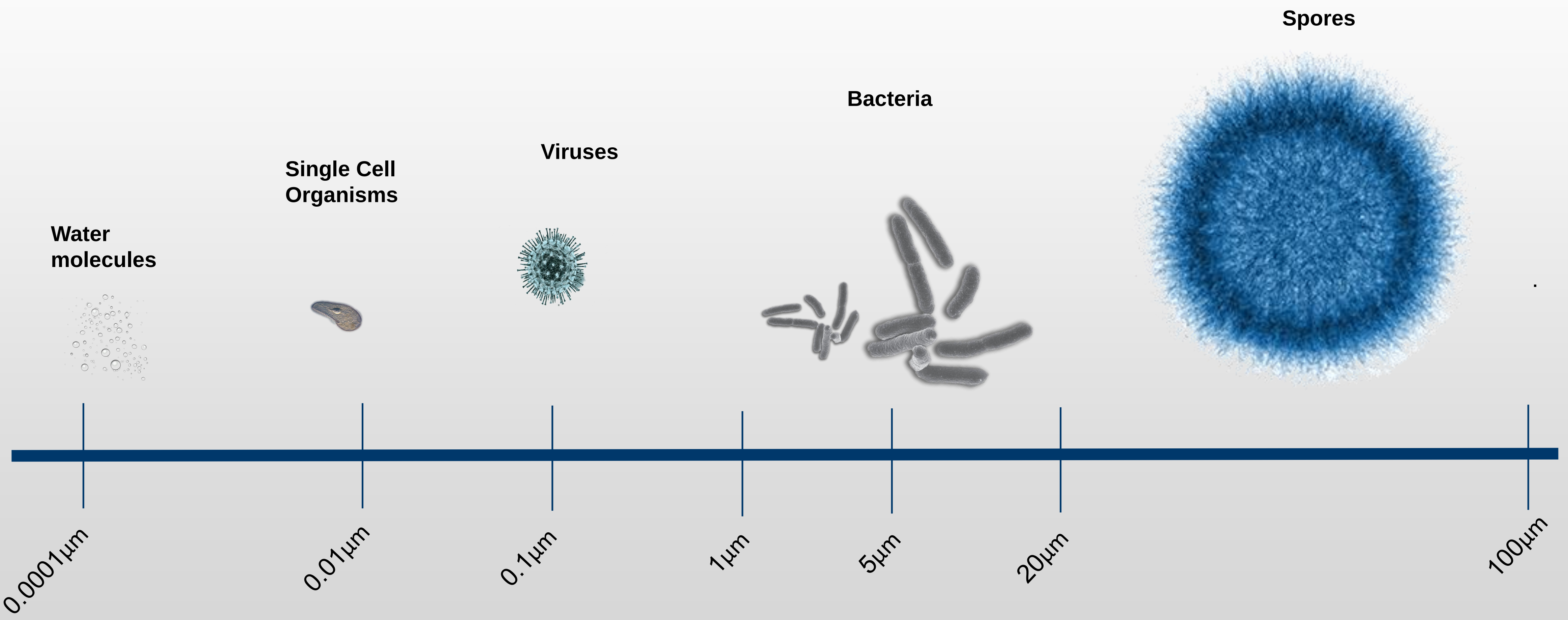




Types of contamination - sizes

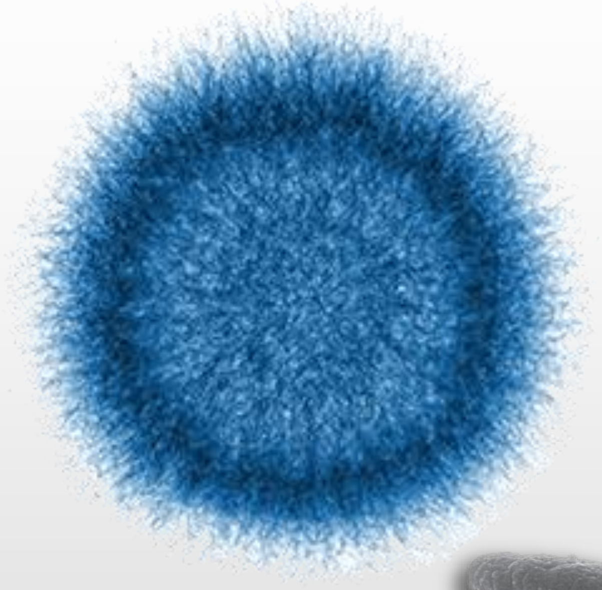


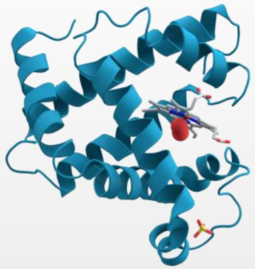
Types of contamination - sizes

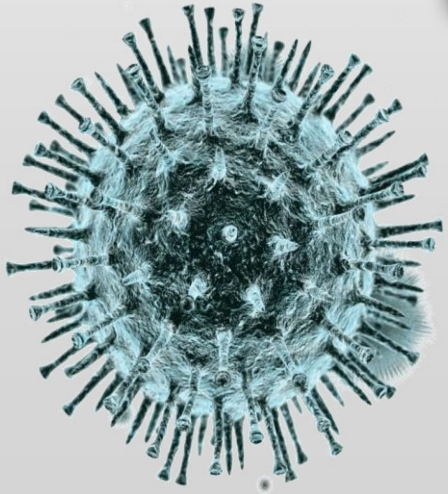


Common Cleanroom Contamination

- **Skin cells**
- **Moisture (liquid molecules)**
- **Spores**
- **Fungi**
- **Microbes**
- **Dust**
- **Dirt**





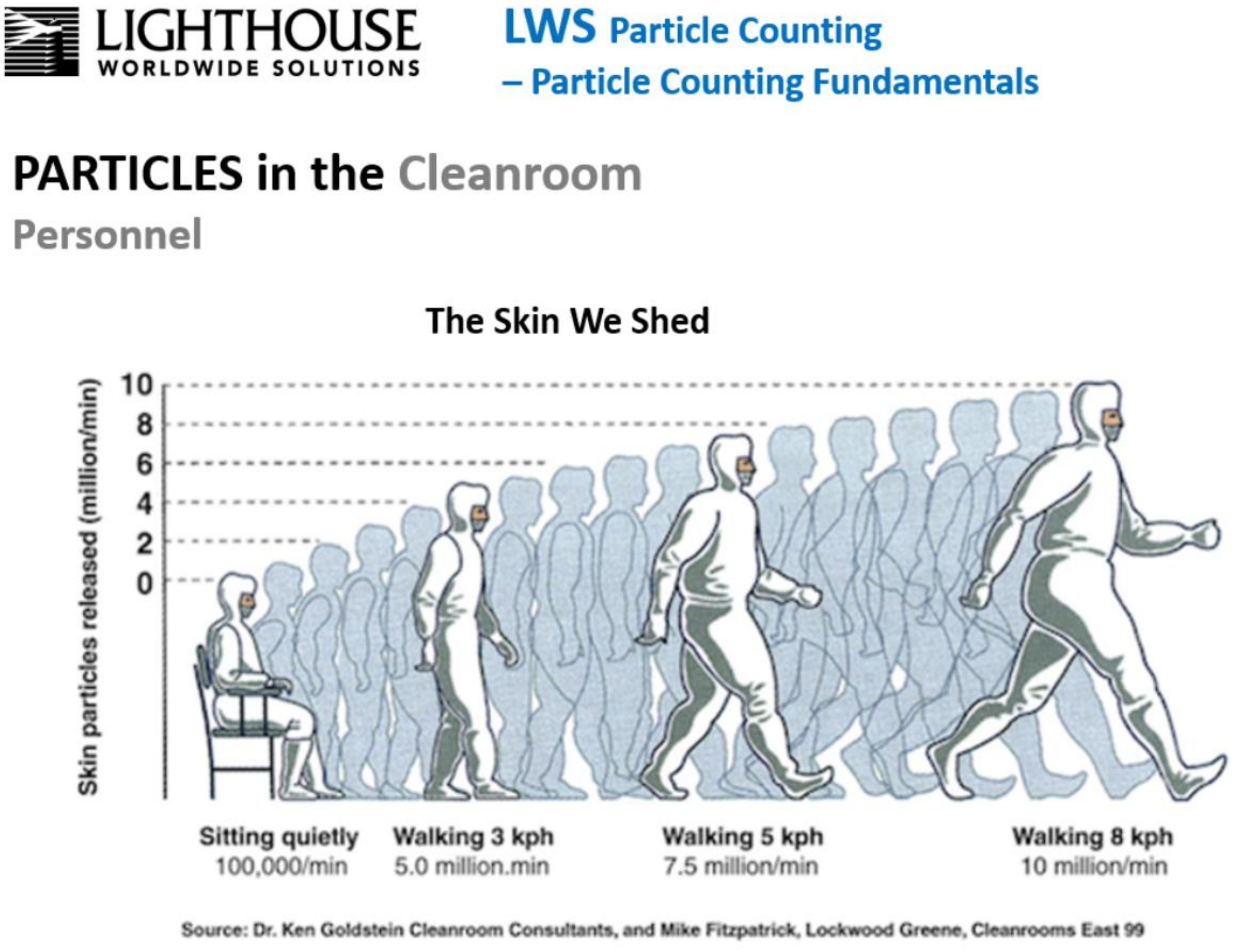


Sources

We are a major source

- A NASA launch was aborted as an Intel chip failed and that failure was traced back to human contamination.

- In 2012 an outbreak of fungal meningitis was reported in the United States. The outbreak was traced to a compounding company based in New England MA and sourced to contaminated injectable product which resulted in more than 64 deaths and infected over 753.



Sources

We are a major source

LIGHTHOUSE
WORLDWIDE SOLUTIONS

did YOU know?

People – related contamination statistics

10,000
Microorganisms
per square inch on hand surface

40,000
Number of skin cells
Shed per minute

100,000
Particles>0.3µm
generated by people when stationary

>5 Million
Particles>0.3µm
generated by people when moving

Overview – Key Points

Access and Control to Microbiological Hazards

1. Identification of potential hazard(s) to the process or product, assessment of the likelihood of occurrence of these hazard(s), and identification of measures for their prevention or control.

2. Designation of risk zones and, in each zone, determination of the points, procedures, operational steps and environmental conditions that can be controlled to eliminate the hazard(s) or minimize the likelihood of their occurrence.

3. Establishment of limits to ensure control.

4. Establishment of a monitoring and observation schedule.

5. Establishment of corrective actions to be taken when monitoring results indicate that a particular point, procedure, operational step or environmental condition is not under control.

6. Establishment of procedures, which may include supplementary tests and procedures, to verify that the chosen Formal System is working effectively.

7. Establishment of training procedures.

8. Establishment and maintenance of appropriate documentation.

8

Controls

Overview – Key Points

"It is the responsibility of the user to develop, initiate, implement and document a Formal System for bio-contamination control that allows detection of adverse conditions in a timely fashion"

ISO 14698- Part 1
Section 5 Establishing the Formal system

GMP Alarm Limits

	Recommended limits for microbial contamination (a)			
Grade	air sample cfu/m^3	settle plates (diameter 90 mm) cfu/4 hours (b)	contact plates (diameter 55 mm) cfu/plate	glove print 5 fingers cfu/glove
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Overview – Key Points

System of bio-contamination control with established and documented procedures – using a

RISK ASSESSMENT

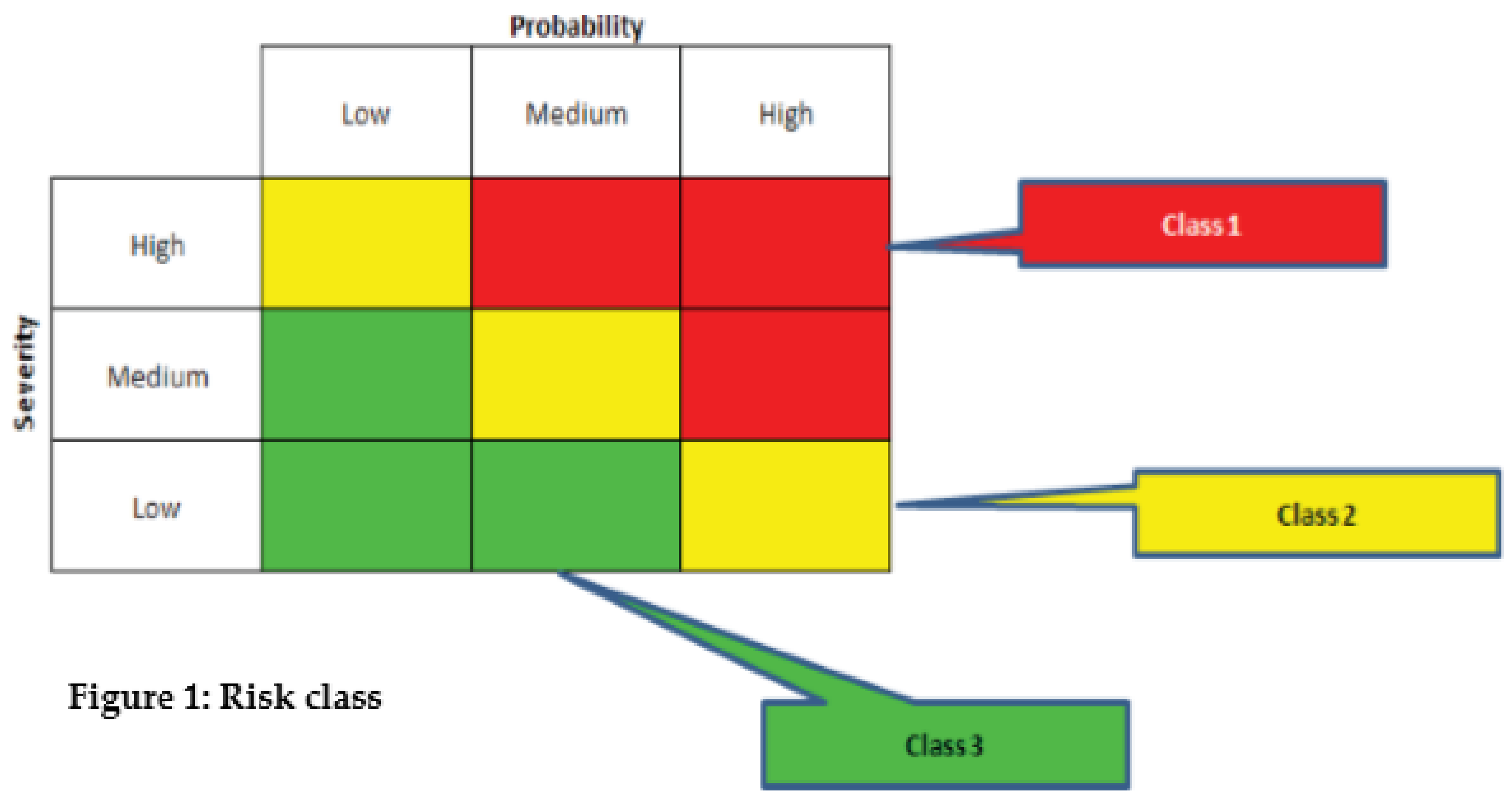


Figure 1: Risk class

Formal System Overview – Key Points

System of bio-contamination control with established and documented procedures – using a

RISK ASSESSMENT

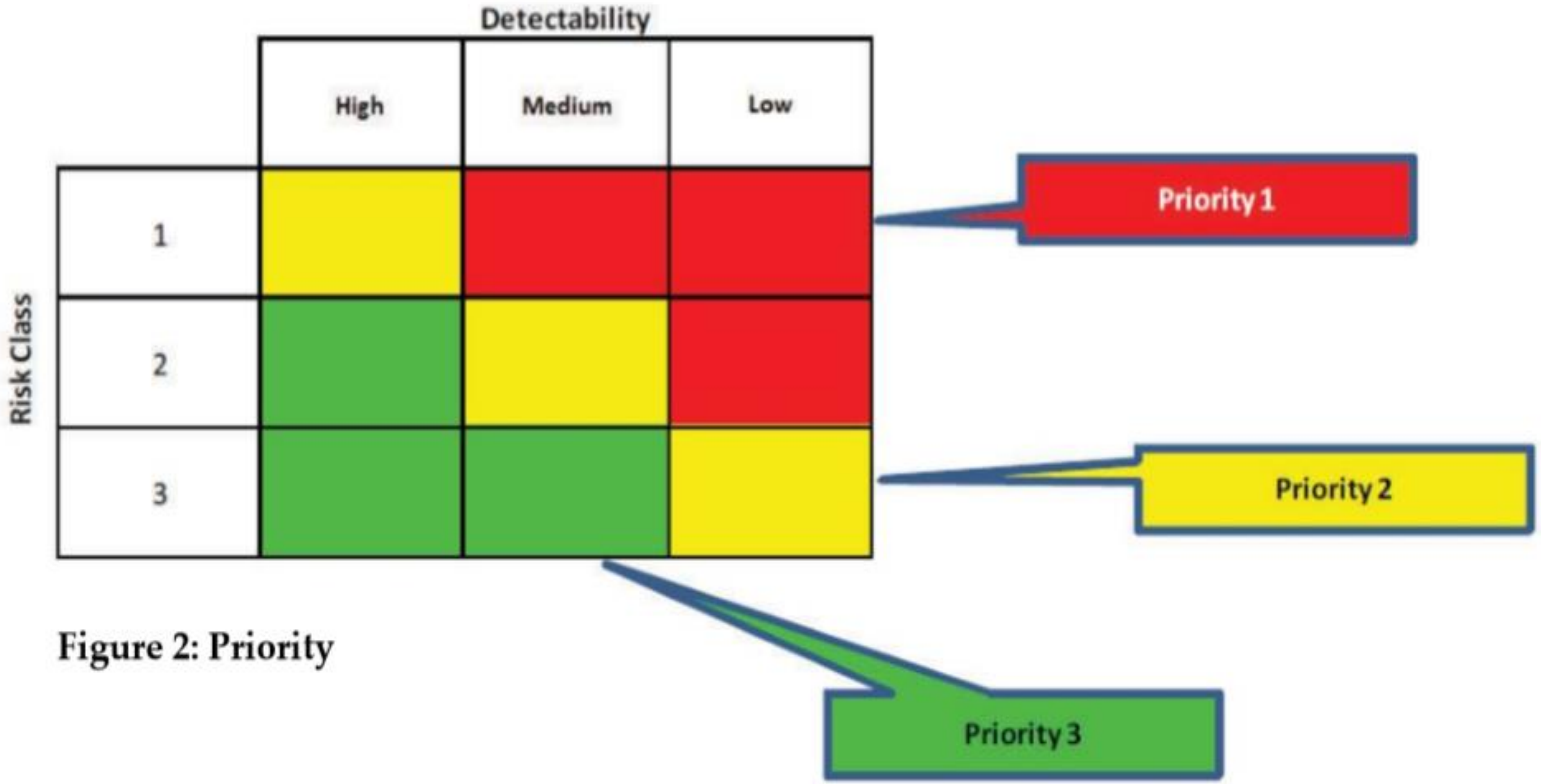


Figure 2: Priority

Formal System

Overview – Key Points

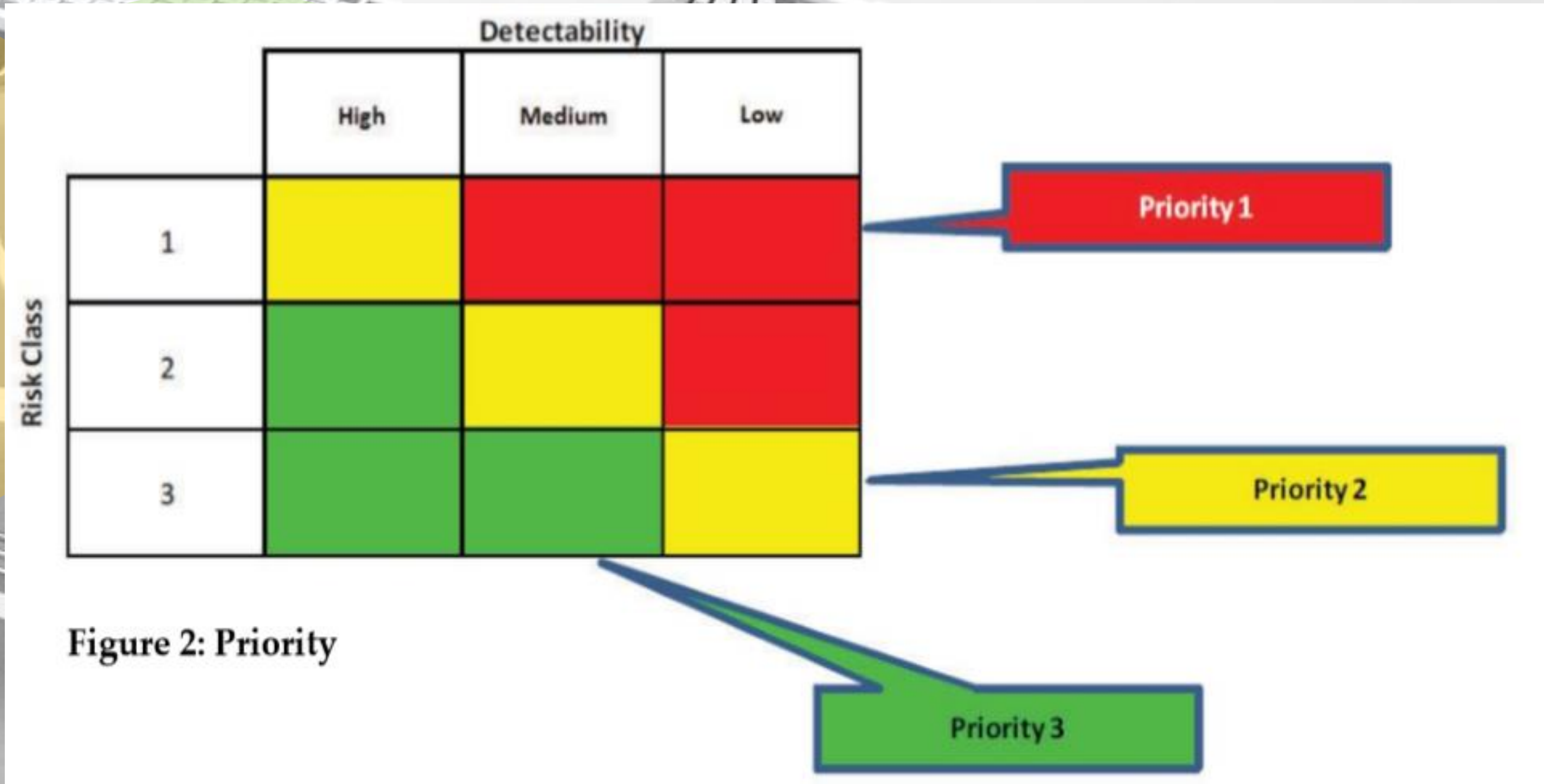


Figure 2: Priority

Overview – Key Points

- Risk zones shall be classified according to relevant guidelines, regulations (where these exist) and the chosen Formal System. Risk zones may also be classified according to the level of aerial and surface biocontamination, for example, low, medium, high or very high risk

- In addition, it is essential that a monitoring program be designed and implemented in a manner that minimizes the possibility of the sampling activities themselves contributing to the contamination of the product or risk zone or both

Overview – Key Points

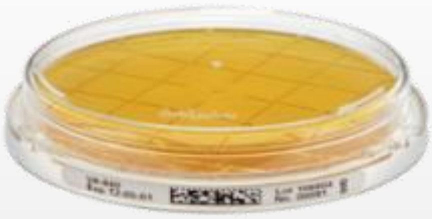
Detection and monitoring of biocontamination in risk zones shall be carried out by sampling and enumerating viable units with appropriate methods in accordance with a sampling plan **Section 5.3.1**

Monitoring of Bio-Contamination

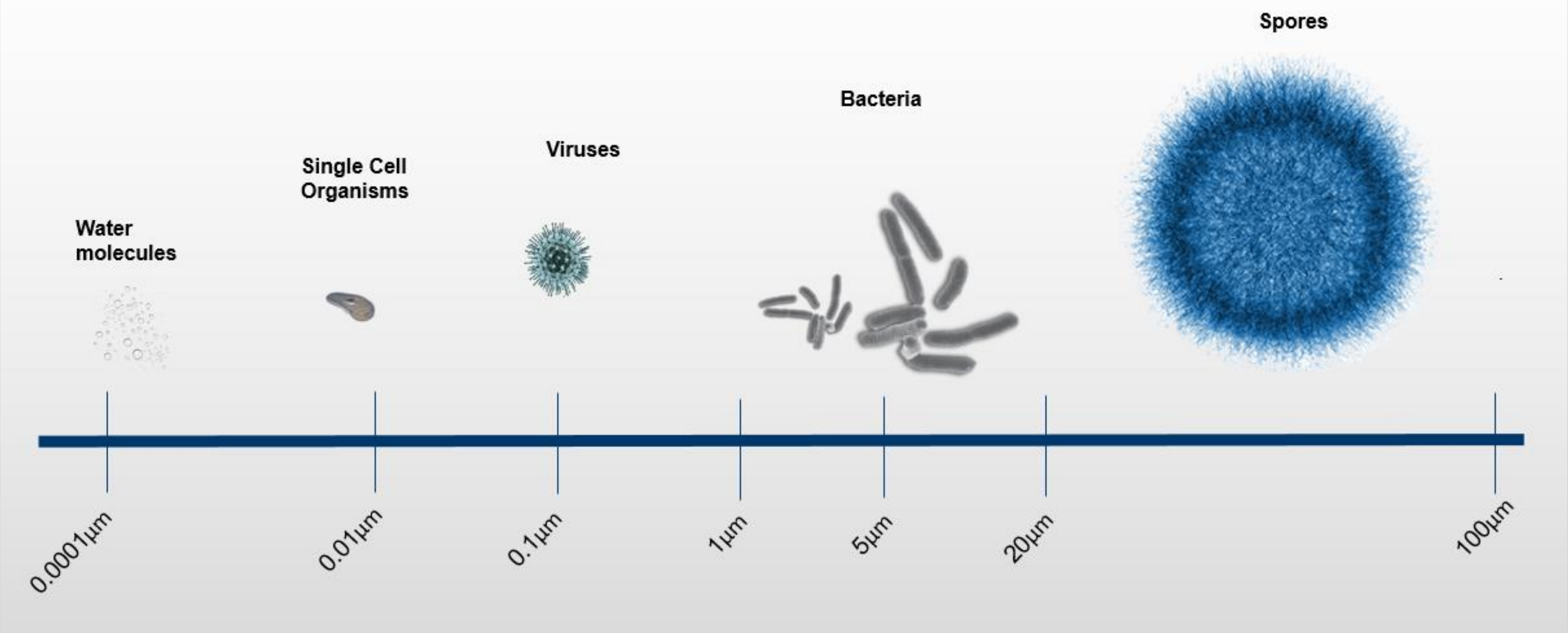
Sampling (Section 5.3.2.1)

The appropriate sampling method and related procedures shall be selected and performed to reflect the complexity and variety of situations. Sampling shall be carried out using a device and method selected in accordance with the written procedure and in accordance with the instructions provided by the device manufacturer.

Sampling Methods



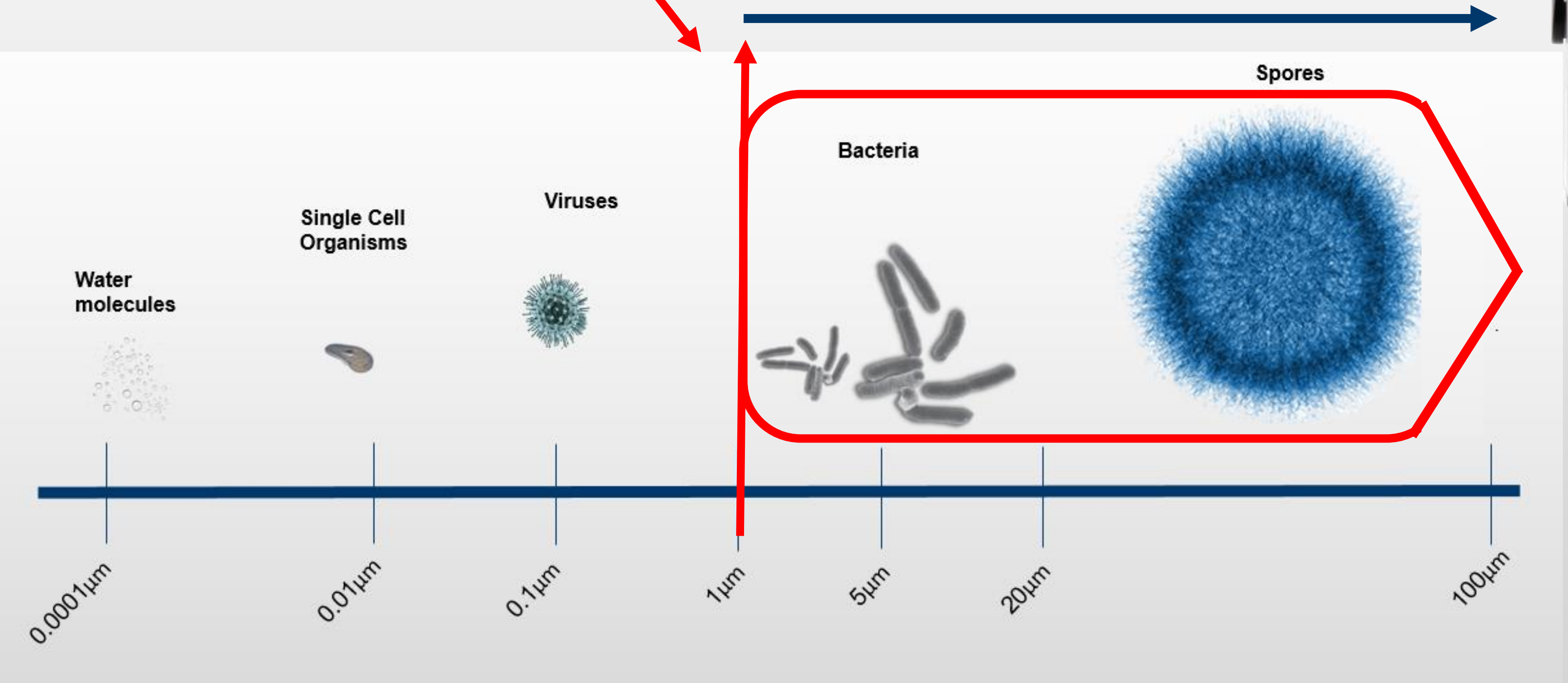
Captures whatever falls into the agar



Sampling Methods

Section A.3.4.2 Impact and impingement samplers

Resolution of 1µm



Critical
Physical Efficiency
Capture Zone

Overview – Key Points

- Setting alert and action limits
- Trend analysis, control charting
- Significance of biocontamination
- Corrective Actions and records
- Sampling and sample tracking
- Collection of results, data recording,
- Evaluation & Validation of results

Overview – Key Points

- **Physical Efficiency:**

  Is the ability of the sampler to collect various sizes of particles.

Overview – Key Points

- **Biological Efficiency**:

  Is the efficiency of the sampler in collecting microbe-carrying particles. The collection process should not invalidate the results.

Types of Air Sampling

Passive Microbial Sampling Devices A.3.3

Passive microbial air sampling devices such as settle plates do not measure the total number of viable particles in the air; they measure the rate at which viable particles settle on surfaces. Settle plates may therefore be used for the qualitative and quantitative evaluation of airborne contamination of products. This can be done by determining the settle plate count per time; then, by relating both the area and time of exposure of the product to that of the settle plate, the possible contamination of the product can be calculated

Filtration A.3.4.3

Filtration sampling devices are widely used, appropriate choice of pump, filter medium and filter size, almost any desired sample quantity can be collected in a given sample period

Active Microbial Sampling Devices A.3.4

- **Impaction**
- **Impingement**

The use of active air sampling devices in risk zones is essential for the assessment of the microbial quality of air. There are several types of active devices commercially available, each having its own limitations.
Based on the principles of sampling, the two main types of apparatus considered suitable for risk zones with normal (low level) biocontamination are impact samplers and filtration samplers.

Focus on Active Air Sampling

- **Active Microbial Sampling Devices** A.3.4
  - **Impaction**
  - **Impingement**

Because there are a variety of impact and impingement samplers available for the detection of viable particles, the device selected for use should have the following characteristics:

a **Impact velocity** of the air hitting the culture medium that is a compromise between

1) being high enough to allow the entrapment of viable particles down to approximately 1 µm, and

2) being low enough to ensure viability of viable particles by avoiding mechanical damage or the breakup of clumps of bacteria or micromycetes;

b **Sampling volume** that is a compromise between being large enough to detect very low levels of biocontamination and being small enough to avoid physical or chemical degradation of the collection medium.

Portable and Continuous Sampling

1

Portable

Certification Monitoring
Spot checks following ISO 14644-2 Environmental Monitoring Plan

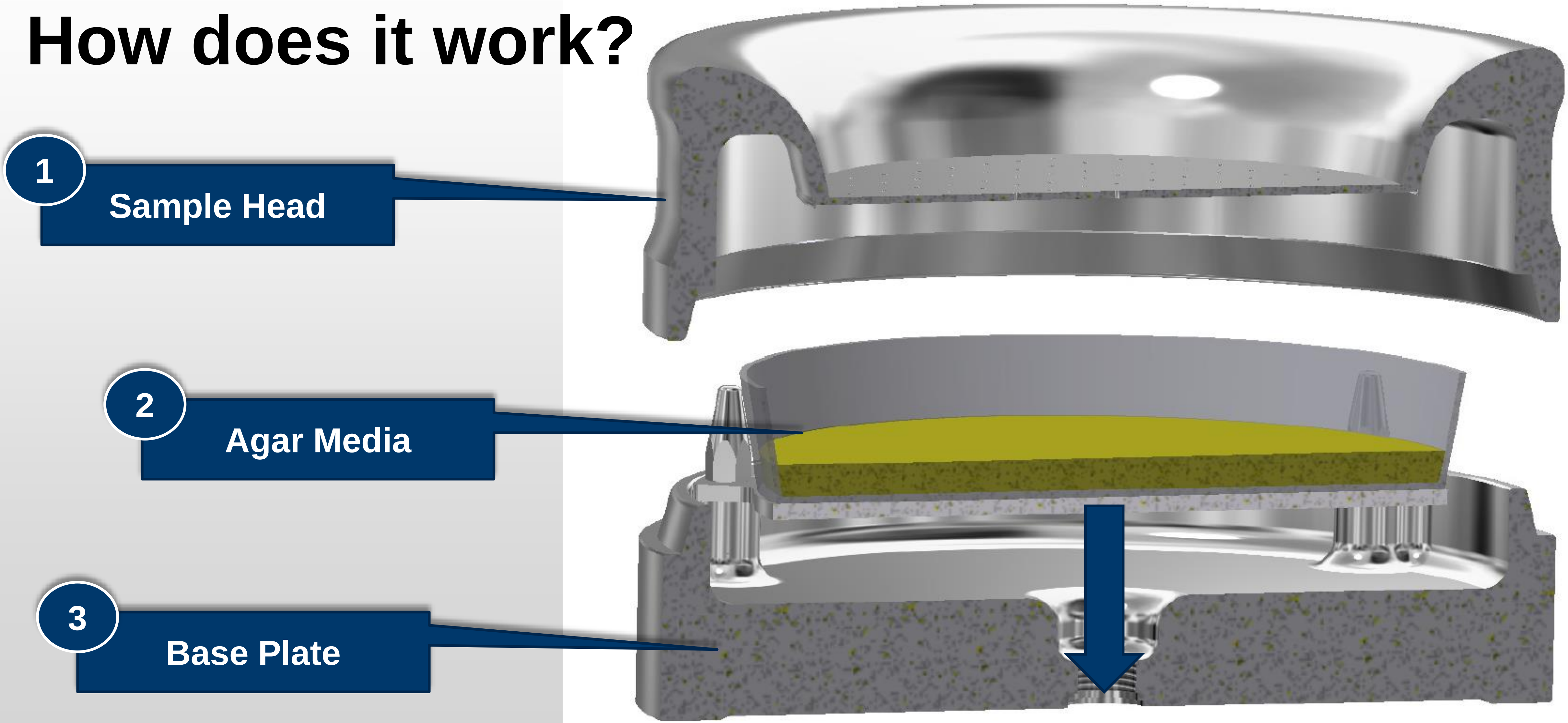
2

Continuous

Production Monitoring
Continuous monitoring during production of sterile medicinal products

Viable Air Sampling Technology

How does it work?

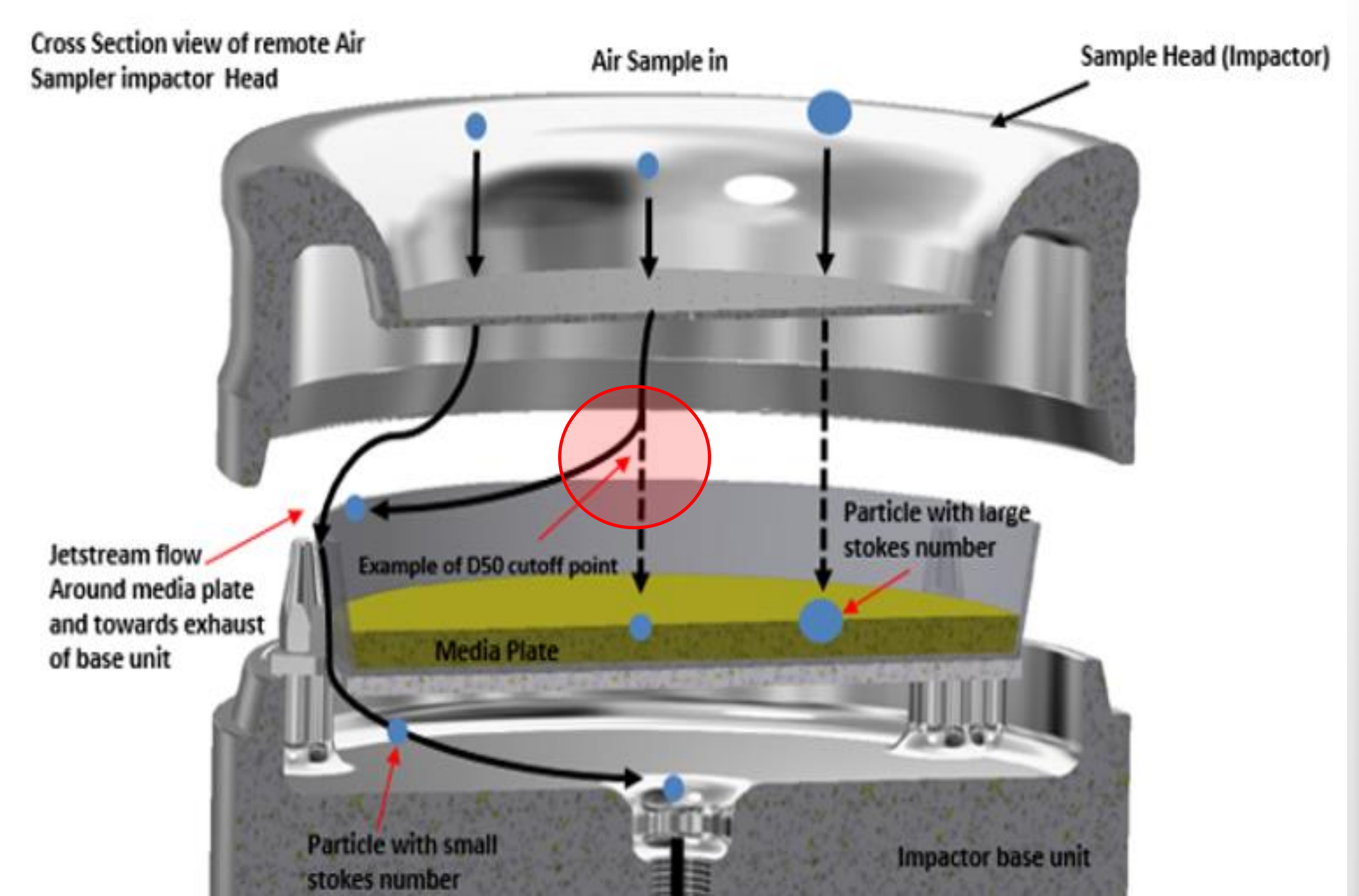


Viable Air Sampling Technology

What is the d50?

Essentially it is the resolution of the air sampler capability of the smallest particle size the system can capture.

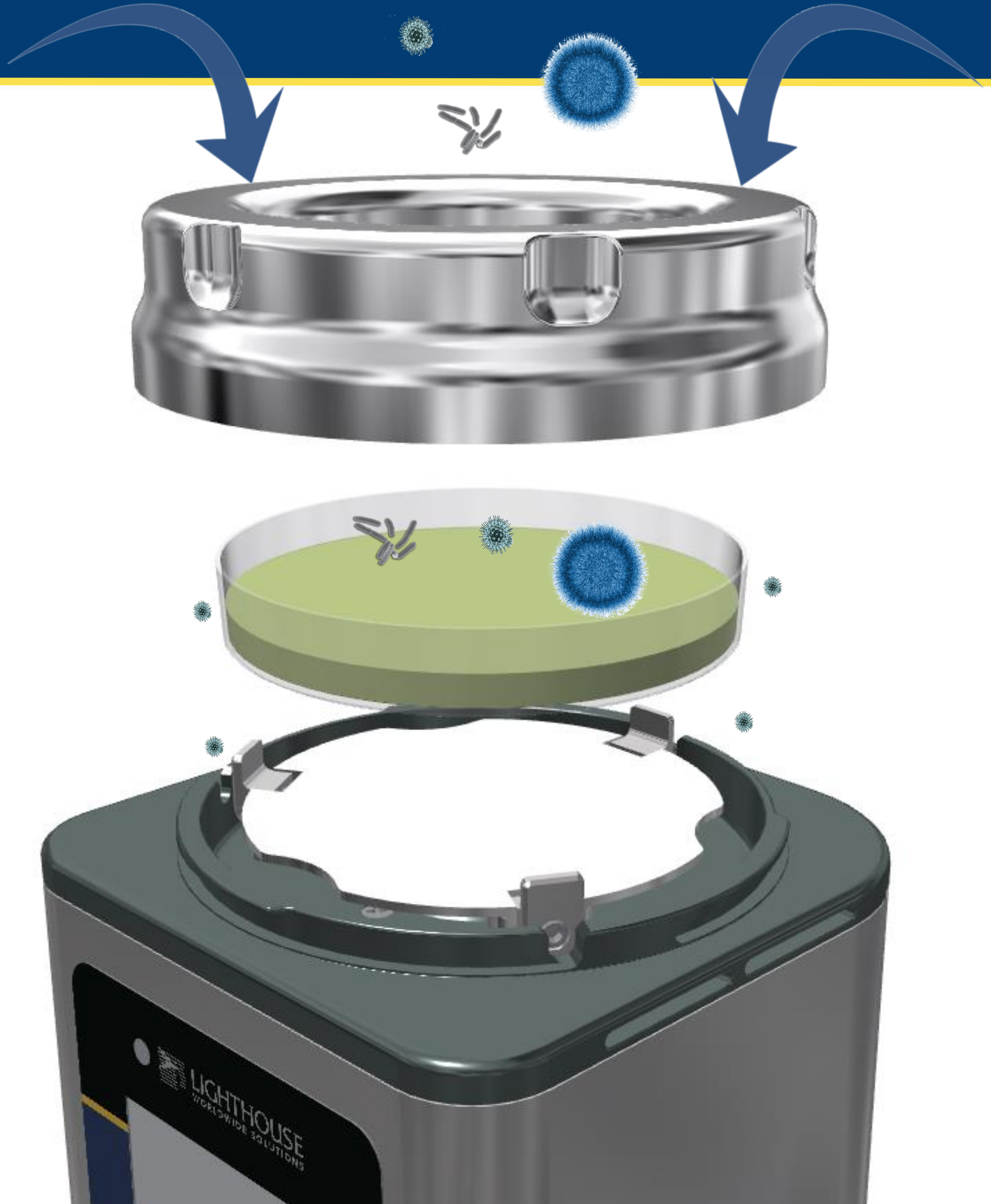
ISO 14698 states that air samplers should have the ability (physical efficiency to capture particles of 1µm



Active Common Cleanroom Contamination

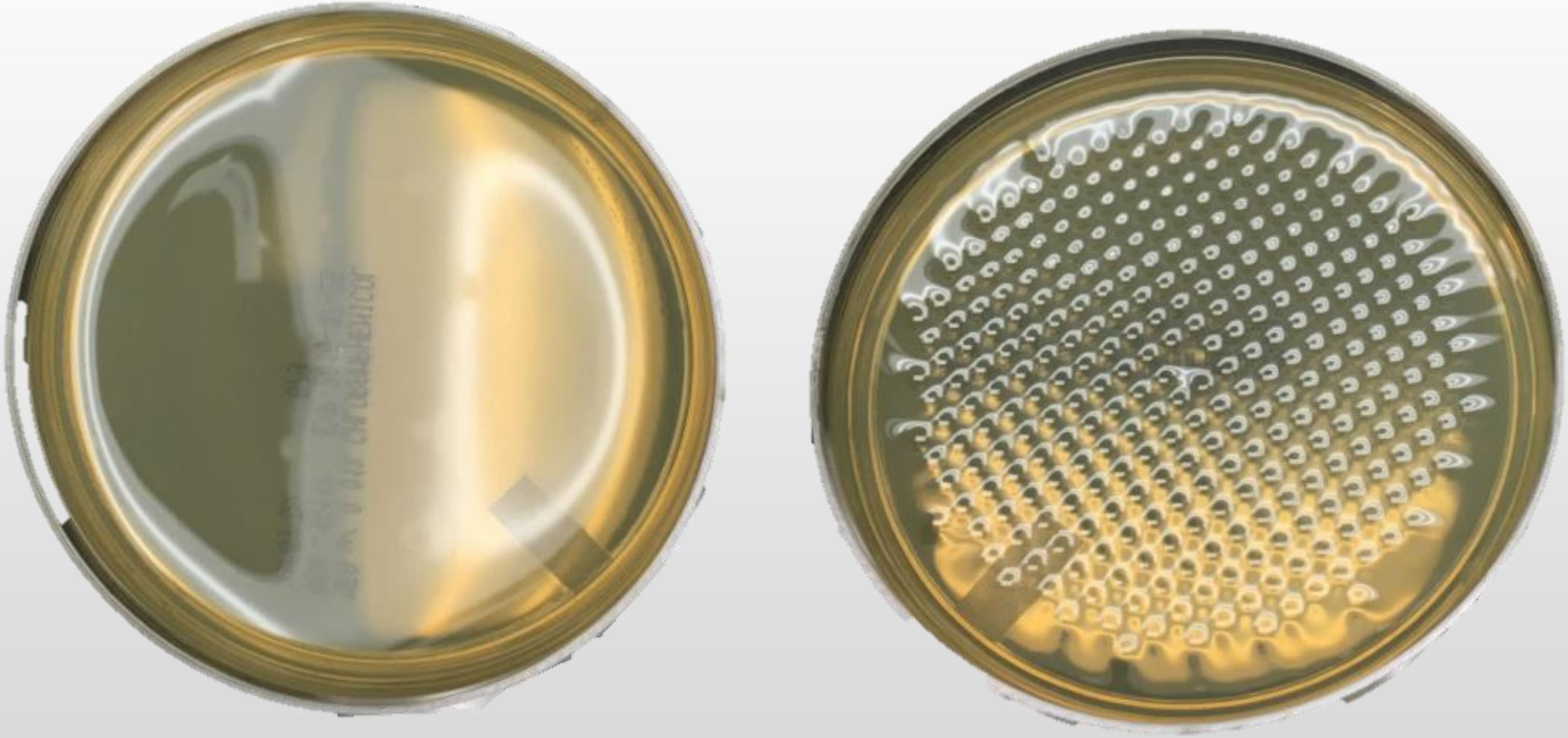
- **Operation**

Example of a Portable Active Air Sampler in action



Notice the smaller particles being pulled away from the sample media

Viable Air Sampling Technology



D50 Explained

Here is the equation for the calculation of the d50 value.

All Lighthouse air samplers have a d50 between 0.97 and 1.10µm

$$D_{50} = \left[\left[\frac{9\eta W}{\rho_p U_o C_c} \right]^{1/2} \right] * (Stk_{50})^{0.5}$$

where: η is the air viscosity [units]

W is the diameter of the impactor Nozzle

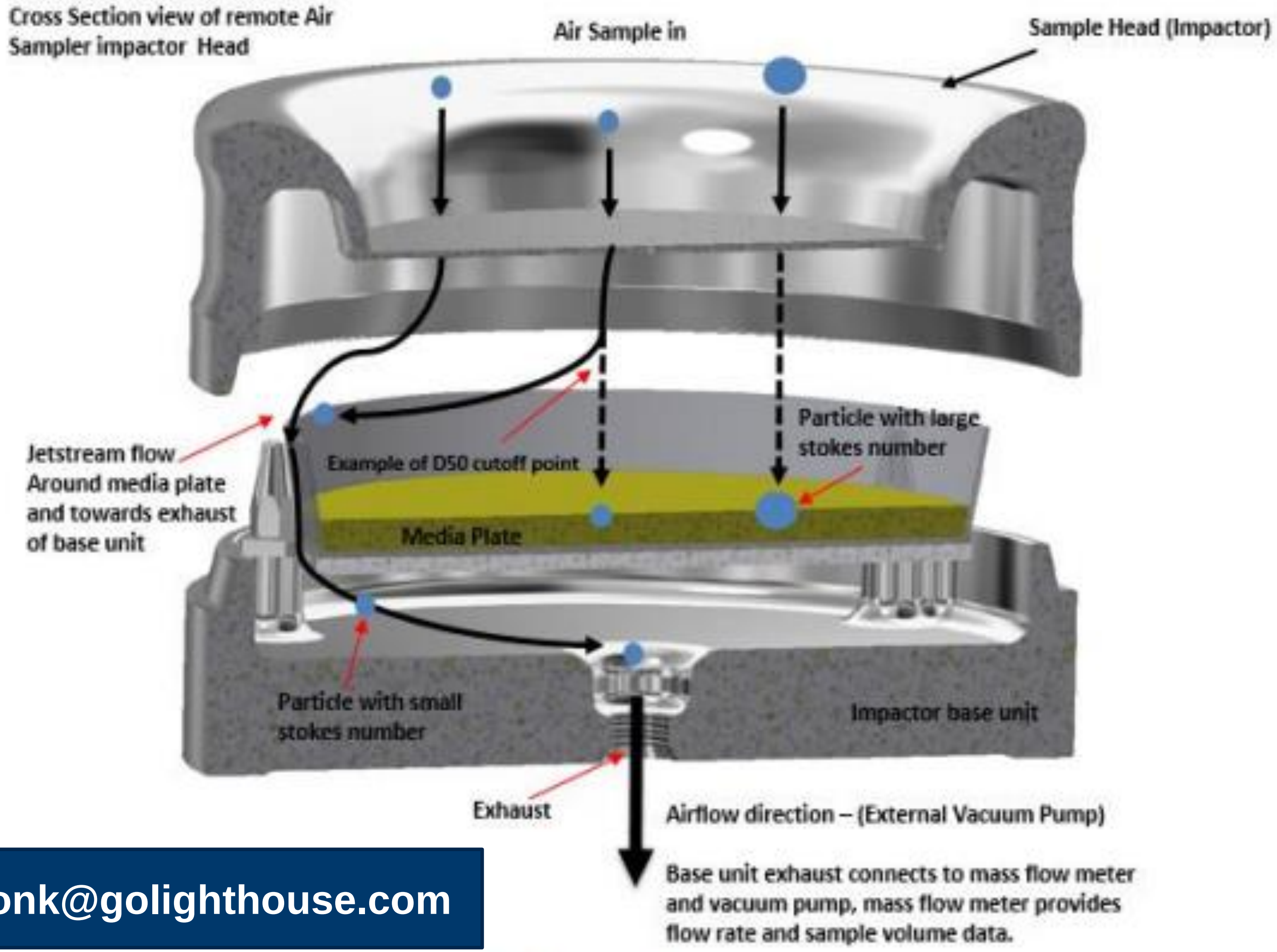
$(Stk_{50})^{0.5}$ is the square root of Stokes number for the collection efficiency of 50%

ρ_p is the particle density

U_o is the jet velocity throught the impactor nozzle

Cc is the Cunningham Correction Factor

Remote Air Sampler Overview



jasonk@golighthouse.com

Fig.4 Remote Portable Air Sampler Overview and Jetstream flow between sample head and media plate

Q&A Session

Question No.1 Why are there different flowrates for air samplers and which one should we choose for our application?

Well it really depends on what your application is?
Like particle counters with different flowrates the reason why some air samplers have different flow rates is based on the sample volume. For example if you need to sample 1 cubic meter for certification purposes to make sure the sample captured at a particular time and location say using a portable air sampler is within the specifications of the cleanroom then you would choose an air sampler with a faster flow rate so this 1 cubic meter sample is captured efficiently as you may have 50 sample to take in various cleanrooms based on your monitoring plan.

So in this case faster is better and I would recommend a portable air sampler that can sample 1 cubic meter in 10 minutes but to still have the physical efficiency to capture a minium of 1um particle sizes as required by ISO 14698

Q&A Session

Question No.2 We only use settle plates for our air sampling in our compounding facility is this sufficient.

The short answer is NO. I would reevaluate your Monitoring Program and conduct a Risk Analysis on just conducting settle plate sampling. Depending on your process and the probability of risk around the product and the product safety I would strongly recommend including active air sampling into your Monitoring Program.

GMP guidance from the FDA requires active air sampling and there have been many FDA citations for lack of air monitoring. Have a look at the FDA guidance on sterile manufacturing published in 2004……

In aseptic processing, one of the most important laboratory controls is the environmental monitoring program. This program provides meaningful information on the quality of the aseptic processing environment (e.g., when a given batch is being manufactured) as well as environmental trends of ancillary clean areas. Environmental monitoring should promptly identify potential routes of contamination, allowing for implementation of corrections before product contamination occurs. Air and surface samples should be taken at the locations where significant activity or product exposure occurs during production.

Q&A Session

Question No.3 How can we tell if our current air sampler meets ISO 14698 requirements?

The best approach is to get a copy of ISO 14698 and review it. One significant part of ISO 14698 Part 1 is in the selection of an appropriate air sampling instrument outlined in Annex A. In Jan 2013 it was voted that this standard including Part 2 were further revised. This is a key indicator as to the importance of this standard to GMP and an indication that it firmly stands as an important GMP guidance document.

According to ISO 14698-1 2003 Annex A.3.2, there are many factors to consider when choosing an Air Sampler. The sampling rate, duration of sample, and type of sampling device can strongly influence the viability of the micro-organisms that are collected, the selection of an air sampler should consider, as a minimum, the following factors:

- Type and size of viable particles to be sampled
- Sensitivity of the viable particles to the sampling procedure
- Expected concentration of viable particles
- Ability to detect high or low levels of bio-contamination
- Appropriate culture media
- Time and duration of sampling
- Ambient conditions in the environment being sampled
- Disturbance of unidirectional airflow by sampling apparatus
- HEPA filtered Exhaust

Q

Question No.3 How can we tell if our current air sampler meets ISO 14698 requirements?

Air Sampler Attributes

- Physical size – small footprint
- Material of construction of enclosure and sample head – Stainless Steel preferable
- Ability to wipe down easily – no crevices, buttons switches or particle traps
- Media plate holder – easy adjustable holder mechanism media dish diameters vary +/- 1mm to 3mm
- HEPA filtered exhaust - captures viable particles that have not impacted
- Touchscreen Interface - reduces contact and potential particle generation
- Battery Operated for better portability on portable units
- Remote sample options – offer more flexibility
- Gas connector options for testing gases to ISO 8573 requirements
- Local or field calibration options from supplier
- Easily autoclave parts
- Capture particles down to 1um to meet ISO 14698 requirements
- Validated for collection efficiency by third party

Q

Question No.4 Can you explain the d50 cut-off point in a little more detail and how can we determine if our current air sampler meets ISO 14698 requirements?

Ok very good question.
We all know with a particle counter in pharmaceutical applications must distinguish and count 0.5um and 5.0um particles as a minimum so we make sure whatever particle counter model is selected it meets that requirement. For an Air Sampler and its ability to capture particle sizes of interest especially when we consider single rod bacteria diameters are potentially as low as 0.3um in diameter its becomes such a critical factor to select an air sampler with the right d50 (Resolution) and ability to collect the smallest particle size physically possible, so you are confident in the results obtained and further more confident in your product batch released.

The d50 is based on particles greater than a certain aerodynamic size collected on the agar media plate and particles less than that size passing through the Air Sampler. This will always happen in current active air sampler designs capabilities which are based on fluid dynamics that we cannot get away from.
With fluid dynamics and Air Sampler impaction technology an indicator of collection efficiency is the Stokes Number. The Stokes number (StK) [1] is a dimensionless number characterizing the behavior of particles suspended in a fluid flow. The stokes number is defined as the ratio of the characteristic time of a particle to a characteristic time of flow or of an obstacle Most well designed impactors can be assumed to be ideal and their efficiency curves characterised by a single number StK50, the Stokes number that gives 50% collection efficiency

Question No.4 Can you explain the d50 cut-off point in a little more detail?

A particle with a low Stokes number follows fluid streamlines, while a particle with a large Stokes number is dominated by its inertia and continues along its initial trajectory.

The d50 is the 50% cutoff particle size where 50% are likely to be impacted and 50% are likely to pass through the Air Sampler. Hence the d50 can be seen as the resolution of the Air Sampler – the smallest particle size that can be physically be captured by the Air Sampler.

Be aware out in the market Air Samplers have varying d50's some as high as 10um – that means anything below 10um is not impacted on the media. Try to explain that to an Auditor who has a firm understanding of ISO 14698 and bacteria size knowledge.

To put all this into simple terms the sample head must be capable of effectively capturing particles in the air and maintaining uni-flow (laminar) conditions between the sample head and the media where the particles impact. Smaller particles are subject to airflow and larger particles maintain their flight path due to higher inertia. The d50 is the point where 50% off the smallest size particles impact on the media, in other words it's really the resolution of the
impactor as the other 50% of these smaller particles will follow the airflow and not impact on the media

Question No.4 Can you explain the d50 cut-off point in a little more detail?

To Summerize

With so many makes and models of Air Samplers on the market it is worth looking deeply into the specifications of each before committing to purchase. Talk to your supplier. Ask them what the d50 is and ask to see 3rd party validation of the physical and biological efficiency of the Air Sampler.

ISO 14698 Part 1 Annex A part A.3.4.2 recommends the Air Sampler impact velocity being high enough to allow the entrapment of viable particles down to approximately 1um and being low enough to ensure viability of viable particles as well has having a filtered exhaust.

A HEPA filtered exhaust is very important.

Time for one more question as we have gone over our allocated timeslot. For the questions I have been unable to get to I will

Question No.5 Continuous Air Sampling using remote samplers is not easy to accomplish and we change out many agar plates during our 4hr production run. Our flowrate is 25L/min yet we spend hundreds of thousands of dollars per year on testing these media plates? We have 20 sampling locations.

Again this is a question of what is needed to be done**.** GMP guidelines say that continuous air sampling is required during sterile manufacturing operations. A flowrate of 25L/min will enable 1000L to be sampled in 40mins so every 40mins you are changing out your media plates and from my math that works out at 6 plates per location per batch run…. right? That's 120 plates per batch run and if you do 2 shifts that's 240 per day so it really starts to add up based on a 5 day week the number of media plates would be 1200 a week that multiplied by 52 = 62,400 … I am using a calculator I am not that good at math.

Ok that is a lot of work and the costs are significant.
I would recommend going for a lower flow rate sample head say 10L/min that will be 100minutes between change outs of media and that would effectively cut your 62,400 per year to 31,200 effectively a 50% reduction. Therefore the cost to swap out your air sampling devices with a lower flow sample head will save your company a lot of work and money and achieve the same result in the monitoring. Sounds like a win – win to me.

That was a quick hour so to wrap up today
Firstly I would like to thank you all for attending and staying awake and for those of you who have submitted questions I will endevour to respond quickly but I will respond and if any of you have any further questions you know where to find me

My email is jasonk@golighthouse.com

What is ISO 14698 used for?

1

Part 1: General principles and methods

Part 2: Evaluation and interpretation of biocontamination data

"ISO 14698 establishes the principles and basic methodology of a formal system of biocontamination control (Formal System) for assessing and controlling biocontamination".


INTERNATIONAL STANDARD

ISO 14698-1

Cleanrooms and associated controlled environments — Biocontamination control —

Part 1:
General principles and methods

ISO

Summary

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ISO 14698 Specifies the methods required for monitoring risk zones in a consistent way and for applying control measures appropriate to the degree of risk involved. In zones where risk is low, it can be used as a source of information.

Provides a method for sampling compressed air for microbial contamination. ISO 14698 highlights the importance in selection of the right air sampling equipment and methods capture microorganisms in the Cleanroom and Clean zones.

Summary

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- A Formal System of biocontamination control shall be established based on Risk
- Risk assessment methods, Hazard Analysis Critical Control Point (HACCP), Fault Tree Analysis (FTA), Failure Mode and Effect Analysis (FMEA).
- Identify potential hazards to the product, the process and likelihood of occurrence
- Establish limits in risk zones to ensure control
- Establish corrective actions when monitoring results indicate lack of control
- Establish Training procedures and SOPs for action and alert limits

ISO 14698 – Selection of the Sampling Device

Summary

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A sampling device shall be selected according to the area being monitored taking into consideration the following factors:

- Type of viable particle to be sampled (d50 (capture resolution) of air sampler is critical)
- Sensitivity of the viable particle to the sampling procedure
- Expected concentration of the viable particles, and the indigenous microbial flora
- Accessibility to the risk zones, ability to detect low levels of biocontamination
- Ambient conditions, time and duration of sample, sampling method
- Effect of sampling device on the process or environment to be monitored
- Collection accuracy and efficiency – Physical and Biological efficiencies

Summary

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- Setting alert and action limits
- Trend analysis, control charting
- Significance of biocontamination
- Corrective Actions and records
- Sampling and sample tracking
- Collection of results, data recording,
- Evaluation & Validation of results

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Alert Level. Indicates when a process might have drifted from normal operating conditions. An investigation may be performed and corrective action may be implemented, but no action is required.

Action Level. Indicates that a process has drifted from normal operating conditions. An investigation must be performed and corrective action must be implemented.

Sample Data with Spike				Sample Data without Spike			
Sample	Aerobic Bacteria	Fungi	Total	Sample	Aerobic Bacte-ria	Fungi	Total
1	21	3	24	1	68	3	71
2	33	3	36	2	95	6	101
3	39	3	42	3	50	3	53
4	694	3	697	4	56	3	59
5	15	6	21	5	55	3	58
6	82	3	85	6	17	3	20
7	33	3	36	7	27	3	30
8	27	3	30	8	26	3	29
9	12	3	15	9	95	3	98
10	36	3	39	10	151	3	154
Mean	99.2	3.3	102.5	Mean	64	3.3	67.3
Bioburden estimate			174.6	Bioburden estimate			114.7
Standard deviation			209.8	Standard deviation			40.9
Recovery efficiency:			0.587	Recovery efficiency:			0.587

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Critical Applications

- Trend Analysis over time period
- Control Charts
- OOT results
  - Requires Evaluation
  - Reliability of Data
  - Event investigations

Summary

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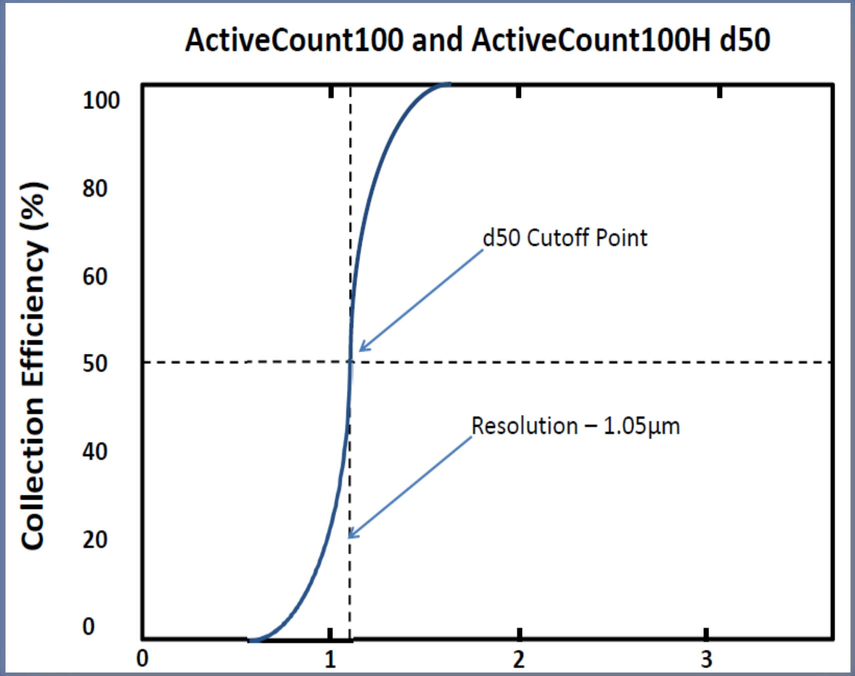
The d50 is the diameter where a particle is 50% likely to impact on the sample media or 50% likely to be manipulated by the sample airstream and not impact on the media.

The d50 for air samplers according to ISO 14698 should be approximately 1.0µm.

The d50 effectively is the resolution of the air sampler.
Poor resolution = poor collection efficiency



Summary

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- **Physical Efficiency:** is the ability of the sampler to collect various sizes of particles.

- **Biological Efficiency:** is the efficiency of the sampler in collecting microbe-carrying particles. The collection process should not invalidate the results.