Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Welcome Speech

Nataša Štirn

Združenje za tehnologijo čistih prostorov (SCS)

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Welcome Speech

Vesna Nahtigal

Gospodarska zbornica Slovenije (GZS)

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Welcome Speech

Hasim Solmaz

Mednarodna konfederacija društev za kontrolo kontaminacije (ICCCS)

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

ISCC 2026

International Symposium on Contamination Control

JACA

Harmonizing various fields beyond the Contamination Control

Tokyo, Japan, 2026

Organized by ICCCS and JACA

Nov. 11 – 13, 2026 Hachioji Tokyo, Japan

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:
INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

The Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15 and the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

30 – 31 MARCH 2026
BRDO PRI KRANJU, SLOVENIA

Bio – Conor Murray

Conor Murray, Principal Consultant
3dimension Cleanrooms
CQV Consultant, Cleanroom & Biosafety Designer, Energy Management
conor@3dimension.ie

ASHRAE
ICS Irish Cleanroom Society

Conor Murray is a Process Engineer by profession, with 4 decades of experience in the design and construction of cleanrooms and biosafety labs; and since 2007 as an independent CQV Consultant. Conor represents Ireland as Head of Delegation for NSAI (national standards body) and is a SME in working groups on cleanroom standardisation in ISO/TC 209, since the early 2000s.
Conor is Chair of the Irish Cleanrooms Society (ICS), and a past Chair of the International Confederation of Contamination Control Societies, (ICCCS).
Conor is a past President of the ASHRAE Ireland chapter and currently Regional Vice Chair for the European Region on Technology Transfer, a member of the global EHC (Environmental Health Committee) and RAC (Research Administration Committee). He is a member of various TCs, including 2.4 Air Cleaning Devices, 2.9 Far UV-C and 9.11 Clean Spaces. Conor lectures internationally and gives cleanroom training and education courses on Annex 1 applications as well as ISO Cleanroom standards.

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Adopt best available technology in Annex 1 for Predictive Quality, Regulatory Alignment - Constant Compliance!

Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Presentation Content

- Challenges in Life Sciences
- Trends & Drivers in Society & Challenges in Life Sciences
- Annex 1 is a “Call to Action” – adopt new Tech & ARMM
- Sustainability and UN SDGs
- Pharma 5.0 vs 4.0 (a la Industry 5.0 vs Industry 4.0)

Challenges in Life Sciences

- Life Science companies are coming under increasing emphasis by regulators (and we the people) due to:
  - Variety – combinational devices, drug product forms
  - Complexity – toxicity,
  - Shelf Life - CGT/ATMP
  - Therapeutic targets - Orphan drugs
  - Cost and Access to medicines
  - Access to distributed manufacturingacross the globe
  - Risk to Patient Safety and above all “Wellbeing”

Challenges in GMP Cleanrooms

- Data Integrity and Uncertainty in Traditional EM sampling (growth based)
- For many years the traditional work of microbiology has had to deal with:
  - inherent uncertainties around living matter, and
  - the blunt instrument of microbiological sampling using the lag indicator of CFU enumeration after incubation, and hopefully recovery
- In Terminally Sterilised products and non sterile products the emphasis is on Bioburden and objectionable microorganisms
- In an aseptic manufacturing environment a huge amount effort and focus is spent on: **"counting Zeros”**

The Key Challenge in manufacturing Safe Drugs!

False Positives – "Fools Errand" & Cost of Drugs Rise

vs False Negatives – Puts Patients at risk

Conor Murray, 3dimension Cleanrooms

"Take Home" Message – Know your Process!

Demand Based

Contamination Control Strategy (CCS)

Predict if we are in control (MES - PAT leads to RTRT)

Know the limits of our prediction (Digital Twin & DI)

"Take Home" Message – Annex 1 is a "Call to Action"

We need to adopt best available technology and tools to monitor the Environment & Process for Constant Compliance

Predict if we are in control (MES - PAT leads to RTRT)

Know the limits of our prediction (Digital Twin & DI)

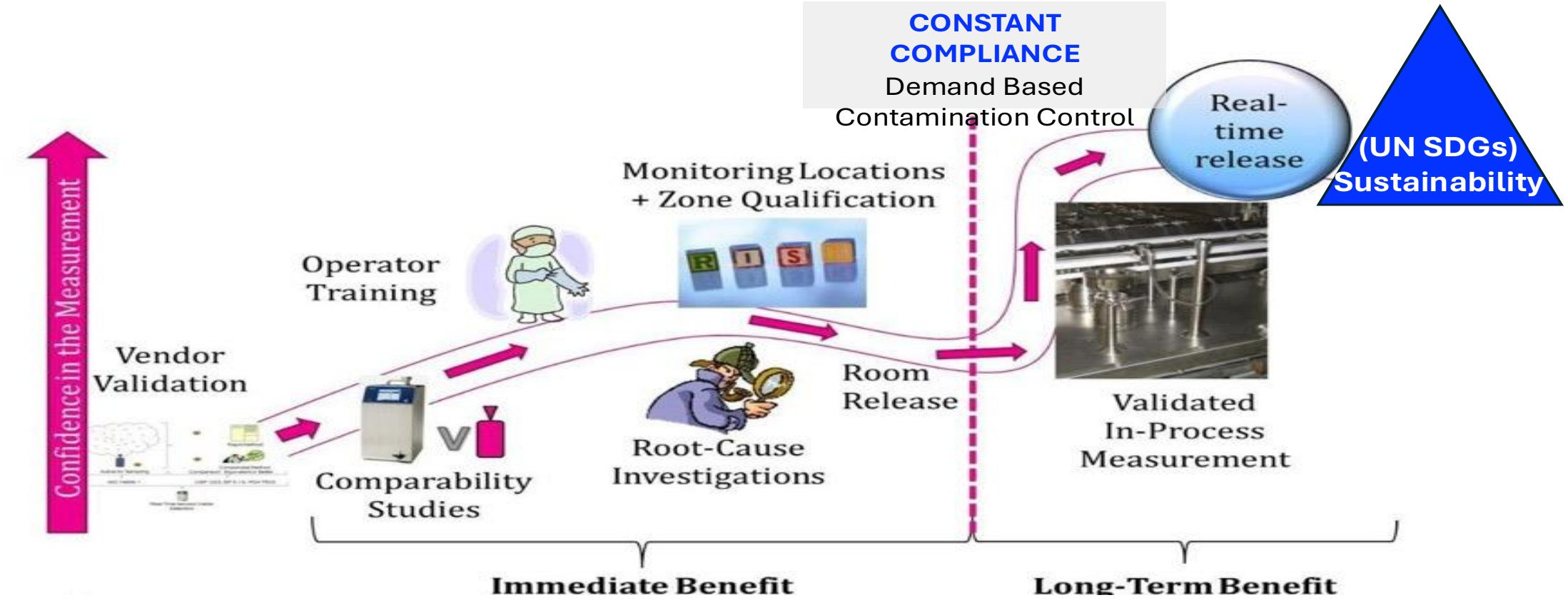
Impact - Increasing Demands on LS Facilities

- Facilities performance not optimised
- Project costs are too high & Cost estimates are not reliable
- Regulatory requirements create long project cycle times and high cost
- Technical risks of the product approval process
- Pricing pressures
- Market variability & Speed to market pressures
- Incomplete product and process development
- Politics

Climate Challenge – Cleanrooms Future!

- Cleanrooms are at the **heart of the solution** - part of a Circular Economy
- Must focus on creating Cleanrooms of the future that are based on **sustainability, agility and flexibility, and can be repurposed**
- A central part of this is the latest ISO 14644 standards which promote better science, smarter designs and both engineering & operational controls - "**doing more with less**"
- Predictive Quality & Digital Twins – under dynamic adaptive control
- AI, Robotics and Automation will play an increasing role in demand based contamination control - sustainability within a circular economy

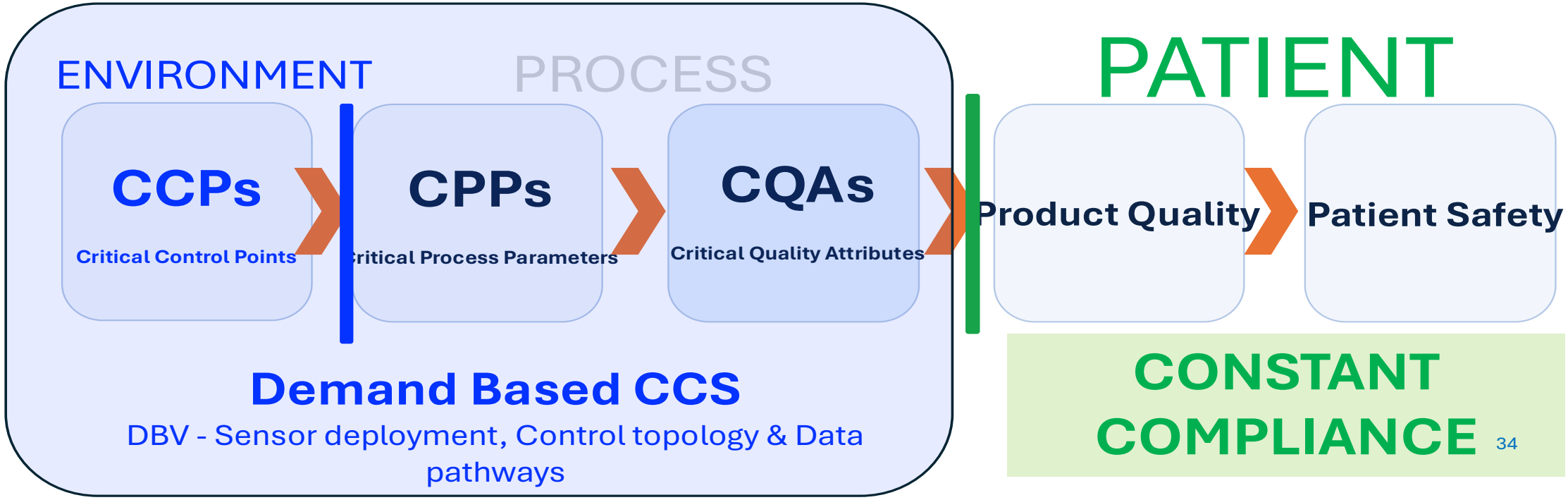
Journey to Constant Compliance & Sustainability



Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Link between Environment Control and Patient Safety



The Data to Intelligent Decision making Revolution for CONSTANT COMPLIANCE

Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Trends & Drivers in Society

- Lifestyle Changes & Impact on Life Sciences

Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

A More Demanding Consumer - Living Longer!

- Increasing life-spans = ↗ Medicines
- Higher expectations = ↗ R & D
- Global population growth = ↗ demand
- More people movements = ↗ exposure
- Increasing Wealth = ↗ waste byproducts
- Tighter Safety laws = ↗ investment
- Litigation Costs = ↗ Quality Control

Increasing Pace of Change!

- ↗ Lifestyle Choices
- ↗ Lifestyle Behaviour
- ↗ Tech Advances – AR, VR, AI, Wearables, Drones & Driverless EVs
- ↗ Technological Convergence – BioMedical Engineering – Prosthetics,
- ↗ Medical Devices Variety & Complexity – Tissue & Stem Cell Therapies, Organ Regeneration
- ↗ Harmonisation & Globalisation
- ↗ Climate Rebalancing and need to move to a circular economy - ESG

We want to Feel better than Well!

- Gene for obsessive Behaviour!
- Brain switch to control Fear!
- Medication as hope for anxiety Patients!
- A pill for every ill!
- A pill to cut down Stress!
- An injection to help you lose weight!
- A pill to boost brain Power!
- A pill to stop you Shopping!
- “Clockwork Orange” drug now Possible!

Can we have our Cake and Eat it?

- Ageing baby Boomers – Self Medicators
- Life Style Drugs VS Life Saving Drugs
- $20b R&D in 400 new Lifestyle drugs
- Botox vs Vaccine injections
- Viagra vs AIDS relief
- Antidepressants ↗
- Diabetes and Obesity (e.g. Ozempic & Wegovy) ↗

- There are both Life threatening and non Life threatening ethical issues
- Non life threatening conditions such as baldness, wrinkles, erectile dysfunction, or acne or just overweight
- Vanity is part of our nature!
- Mental health is a key part of our well being

Impact - Increasing Demands on LS

- Facilities performance not optimised
- Project costs are too high & Cost estimates are not reliable
- Regulatory requirements create long project cycle times and high cost
- Technical risks of the product approval process
- Pricing pressures
- Market variability & Speed to market pressures
- Incomplete product and process development
- Politics

Fundamental principles of QRM and CCS

- what does this mean in practice and what do I need to do in order to comply with the new Annex 1?

Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Cleanroom Contamination Control

Fundamental principles of a Cleanroom contamination control strategy:

first

1. ESTABLISH a “state of control” and then
2. DEMONSTRATE a “state of control” (i.e. the effectiveness of your CCS)

CCS and Qualification & Validation

Control	Qualification/Validation	Establish Control	Demonstrate
Facility	Clean Room area and HVAC System	Sanitization; Revision of Barriers, Traffic Patterns, or Air Balance	Monitoring (EM)
HVAC	Qualification of the Clean Room area and HVAC System	Certification and Preventative Maintenance (PM) of System; Repair of HEPA Filters	EM
Water	Qualification of Water System	Certification and PM Regular Sanitization of System	Bioburden Monitoring of Water System
Equipment	Qualification of the Equipment as Suitable for its Intended Use	Certification and PM Regular Sanitization	EM Finished Product Release Testing
Sanitization	Validation of Cleaning, sanitization and sporicidal treaments	Regular cleaning and sanitization of facilities and equipment	EM
Personnel	Proficiency Criteria Participation in Media Fills Trending Data by Operator	Training Discipline	Personnel Monitoring Trending Data by Operator
Process	Process Validation	Acceptance Testing of Raw Materials and Containers	In-process Bioburden Monitoring Finished Product Release Testing

CCS and Qualification & Validation

		Qualification/Validation	Establish Control	Demonstrate Control
	Facility	Qualification of the Clean Room area and HVAC System	Maintenance of Facilities Sanitization; Revision of Barriers, Traffic Patterns, or Air Balance	Environmental Monitoring (EM)
	HVAC	Qualification of the Clean Room area and HVAC System	Certification and Preventative Maintenance (PM) of System; Repair of HEPA Filters	EM
NOT IN ISO TC/ 209 CR SCOPE	Water	Qualification of Water System	Certification and PM Regular Sanitization of System	Bioburden Monitoring of Water System
NOT IN ISO TC/ 209 CR SCOPE	Equipment	Qualification of the Equipment as Suitable for its Intended Use	Certification and PM Regular Sanitization	EM Finished Product Release Testing
	Sanitization	Validation of Cleaning, sanitization and sporicidal treaments	Regular cleaning and sanitization of facilities and equipment	EM
	Personnel	Proficiency Criteria Participation in Media Fills Trending Data by Operator	Training Discipline	Personnel Monitoring Trending Data by Operator
NOT IN ISO TC/ 209 CR SCOPE	Process	Process Validation	Acceptance Testing of Raw Materials and Containers	In-process Bioburden Monitoring Finished Product Release Testing

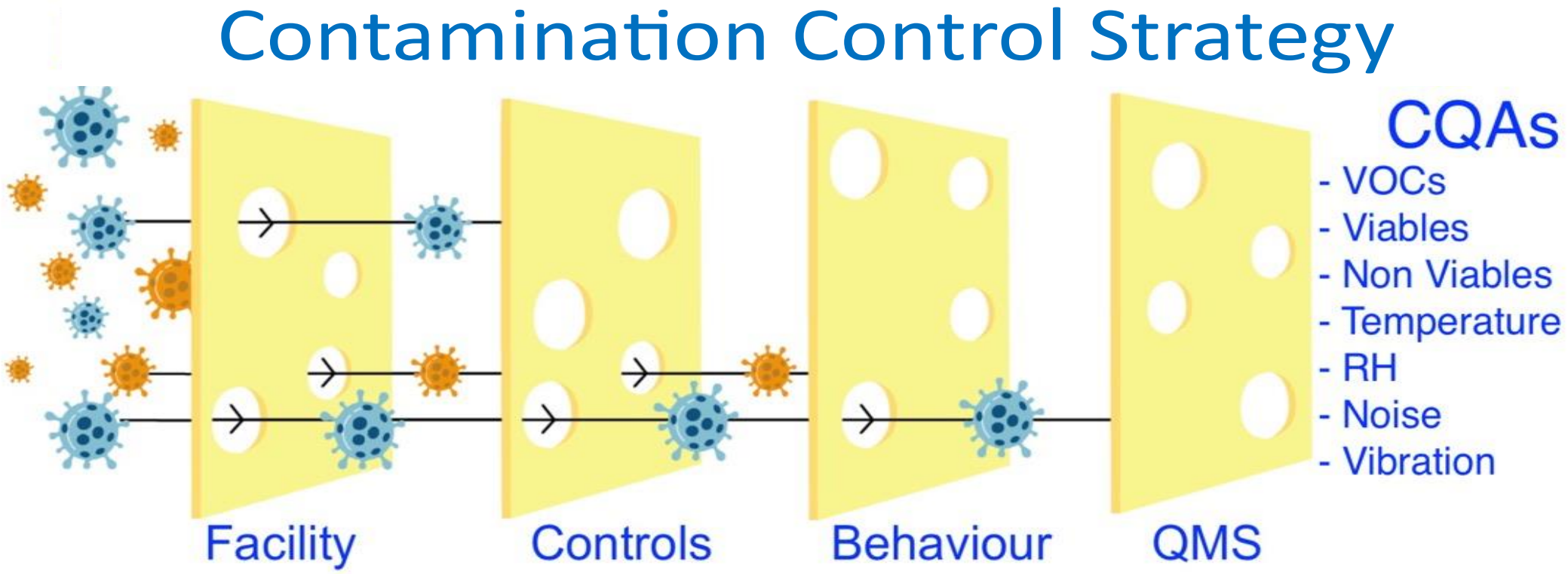
Contamination Control Strategy

1. ESTABLISH a “state of control” i.e. CCS
   - Risk Assessment - ID Critical Control Points (CCPs) in the cleanroom environment
   - Assess GMP Regulatory & ISO Classificationreq’ts for total particles
   - Set Performance Requirementsi.e. not to exceed LIMITS during EM– Total and Viable, **noting ARMM inputs (inc BFPC tech and AFUs, not = to CFUs)**
   - Set Alert & Action LEVELS in the EM Plan– Total and Viable
   - Formulate a Contamination Control Strategy

Contamination Control Strategy

2. DEMONSTRATE a "state of control" i.e Effectiveness of CCS
   - Monitor Critical Control Points (CCPs) against the Environmental Monitoring (EM) Plan
   - **Evaluate Data from ARMM (inc AFUs from BFPC)**
   - Evaluate Data and Watch/Act on EM Trends (Total & Viable)
     – "the trend is your friend"
   - Review Effectiveness of the EM Plan (Total & Viable)
   - Update EM Plan as required based on trends and changes to the cleanroom or process

QRM for Cleanroom Facilities



Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

QRM & Hazard/Risk Assessment

- Everything starts with QRM (ICH Q9)
- Understand your process and product characteristics
- What are the Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs)– leading to Product Quality & Patient Safety
- Carry out QRM - Hazard/Risk Assessment - what contaminants are important to establish a “state of control”
- IDENTIFY Critical Control Points (CCPs) in the cleanroom environment that impact on CPPs & CQAs

Annex 1 - A “Call to Action” to adopt new technology and mitigate risks in support of better Quality products

Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Annex 1 GMP is essentially about Microbiological Contamination Control - an EMA and PIC/s joint effort

Conor Murray, 3dimension Cleanrooms

New Annex 1 has 59 (PIC/s 58) pages vs 16 in 2008 version!

European Commission

Public Health

European Commission > Public Health > Latest updates > Revision - Manufacture of Sterile Medicinal Products

NEWS ANNOUNCEMENT | 25 August 2022 | Directorate-General for Health and Food Safety

Revision - Manufacture of Sterile Medicinal Products

GENERAL PUBLICATIONS | 25 August 2022

20220825_gmp-an1_en.pdf

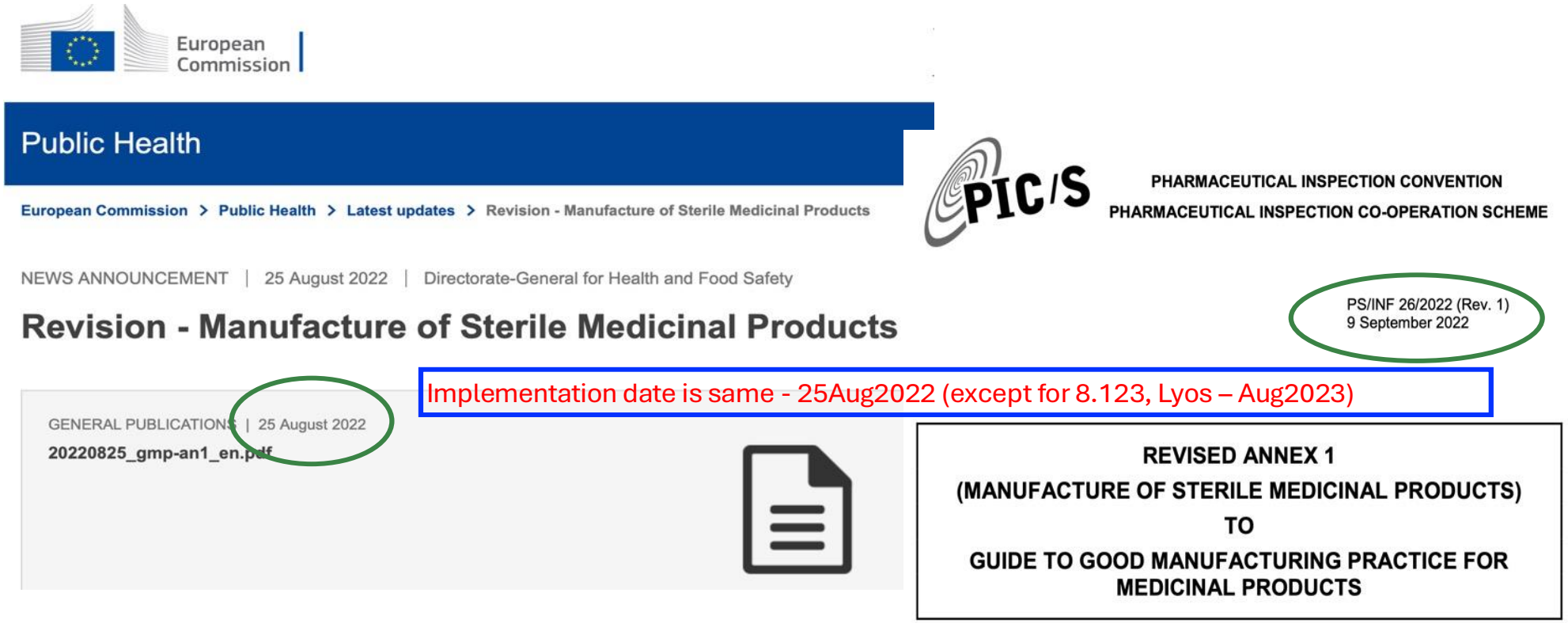
PIC/S

PHARMACEUTICAL INSPECTION CONVENTION
PHARMACEUTICAL INSPECTION CO-OPERATION SCHEME

PS/INF 26/2022 (Rev. 1)
9 September 2022

REVISED ANNEX 1
(MANUFACTURE OF STERILE MEDICINAL PRODUCTS)
TO
GUIDE TO GOOD MANUFACTURING PRACTICE FOR MEDICINAL PRODUCTS

New Annex 1 has 59 (PIC/s 58) pages vs 16 in 2008 version!



Annex 1 is a "Call to Action" – Carpe Diem

- Pharma 5.0 – Add a circular economy for sustainability to Pharma 4.0
- Predictive QUALITY with Data Integrity
  - PAT - Process Analytical Technology
  - RTRT - Real Time Release Testing
- ICH Q9 QRM – understand the process and risks/hazards
- QRM drives the CCS (Contamination Control Strategy) - based on Good Science & GEP
- Annex 1 encourages adoption of New Technology & ARMM
- Digital Twin gives DI as part of MES/Process Control for QP Batch Release

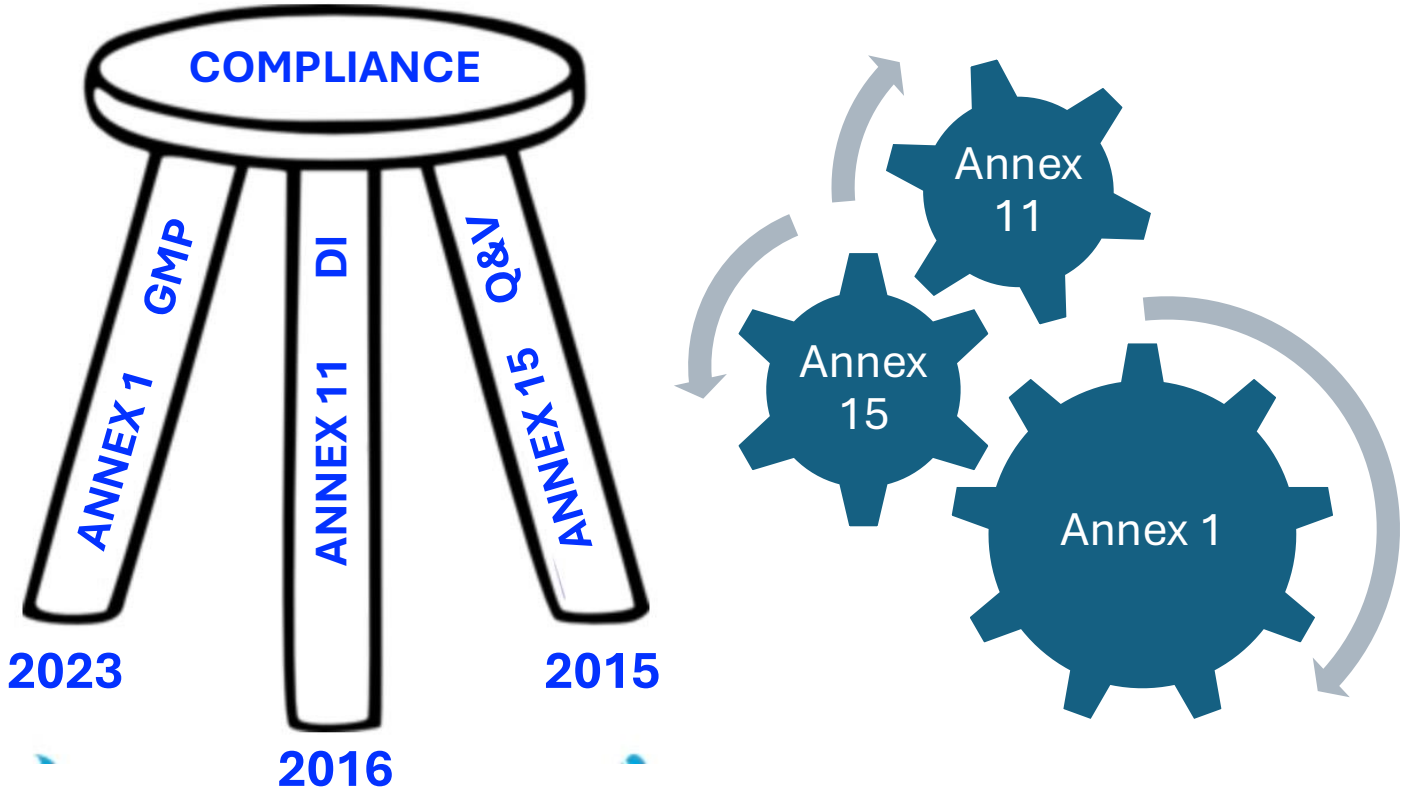
Life Science Regs – EMA & PIC/S Guidance

- **Annex 1 – GMP 2023 (inc Lyos)**
- **Annex 11 – Data Integrity 2016**
- **Annex 15 – Qualification and Validation 2015**
- ATMPs (CGT) now have their own dedicated GMP

"Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products"

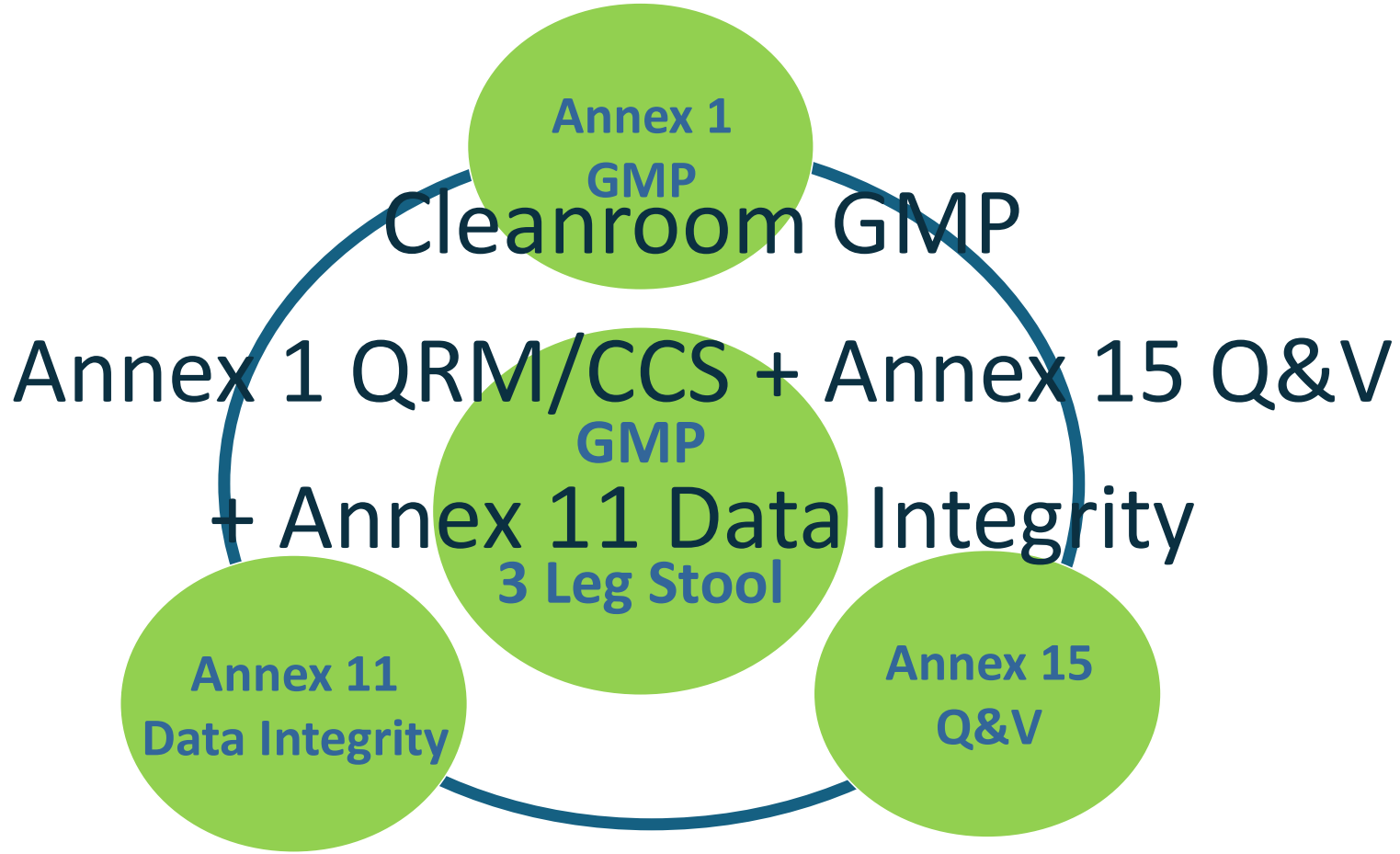
QRM and CCS fundamental to all of the above

3-Legged Stool/Cogs of Life Science Compliance



Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms



Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Ref. Ares(2015)1380025 - 30/03/2015

EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Medicinal Products – Quality, Safety and Efficacy

Brussels, 30 March 2015

As simple as you can

As complex as you must

EudraLex

Volume 4

EU Guidelines for
Good Manufacturing Practice for
Medicinal Products for Human and Veterinary Use

Annex 15: Qualification and Validation

Legal basis for publishing the detailed guidelines: Article 47 of Directive 2001/83/EC on the Community code relating to medicinal products for human use and Article 51 of Directive 2001/82/EC on the Community code relating to veterinary medicinal products. This document provides guidance for the interpretation of the principles and guidelines of good manufacturing practice (GMP) for medicinal products as laid down in Directive 2003/94/EC for medicinal products for human use and Directive 91/412/EEC for veterinary use.

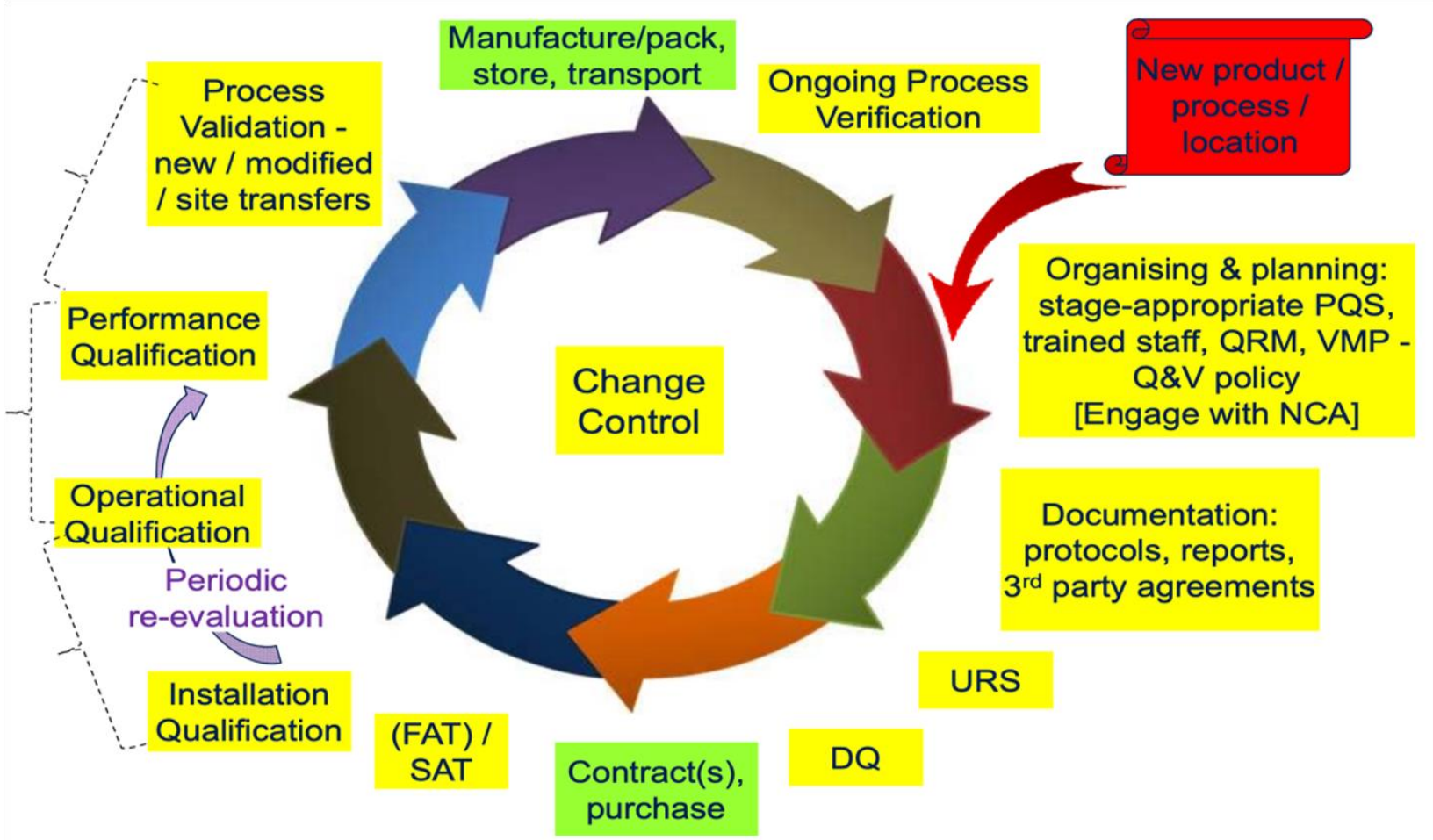
Status of the document: Revision

Reasons for changes: Since Annex 15 was published in 2001 the manufacturing and regulatory environment has changed significantly and an update is required to this Annex to reflect this changed environment. This revision to Annex 15 takes into account changes to other sections of the EudraLex, Volume 4, Part I, relationship to Part II, Annex 11, ICH Q8, Q9, Q10 and Q11, QWP guidance on process validation, and changes in manufacturing technology.

Deadline for coming into operation: 1 October 2015

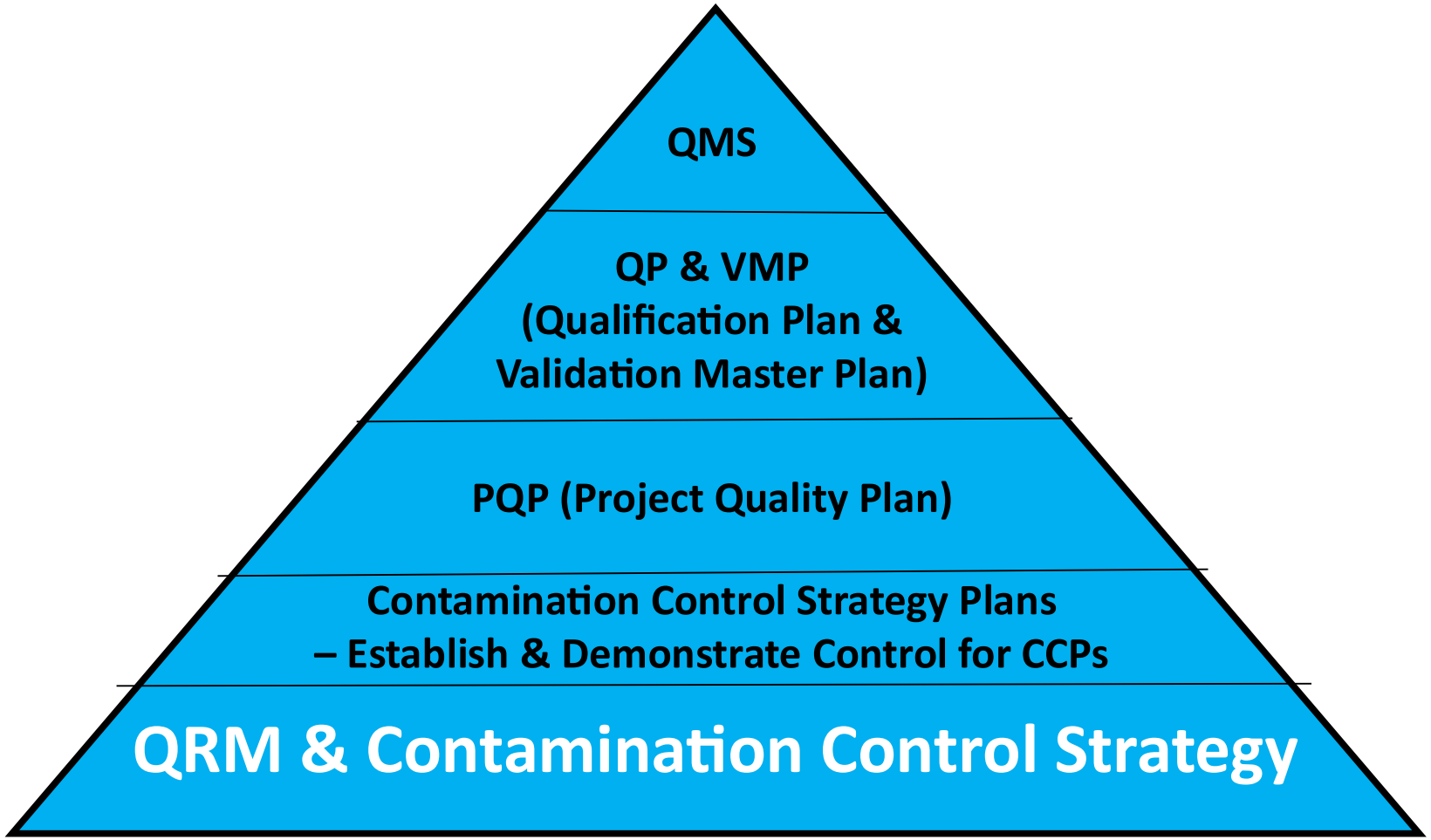
Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms



Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms



Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Annex 1 GMP – Back to Basics

- GMP principle:
  - Simply testing a product after manufacture is not sufficient to ensure product quality and sterility
  - Quality must be built in during all stages

- GMP covers:
  - How products are made, packaged, labelled & stored
  - How products are tested to ensure they are of a suitable quality, inc final evaluation and QP approval for release of each batch

Cleanroom Technology & GMP

- meeting the challenges of Pharma 5.0 PAT & RTRT

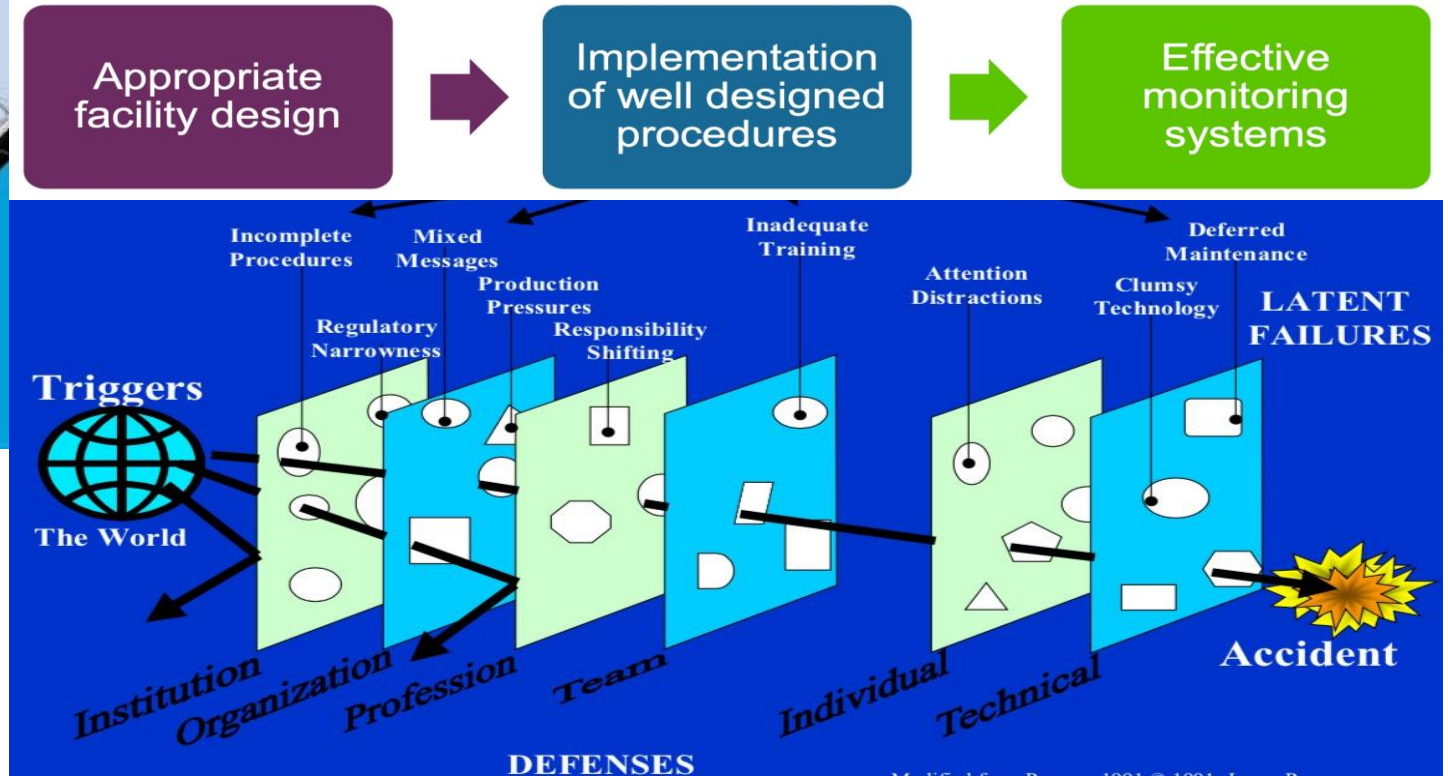
“Predictive Quality with DI”

Digital Twin to MES

Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

QRM for GMP CR Facilities – Swiss Cheese!



Bridge to Constant Compliance with GMP Annex 1, DI Annex 11 and Q&V Annex 15,the road to Pharma 5.0 and Sustainability

Conor Murray, 3dimension Cleanrooms

Digital Twin & Predictive Quality

- Digital Twin link to MES (Manufacturing Enterprise System) & PROCESS
- Annex 1 encourages adoption of New Technology & ARMM

Predict if we are in control (allied to RTRT)

+

Know the limits of our prediction (allied to DI)

How does this fit in with Pharma 5.0 – Why Not?

Pharma 5.0 – Sustainability (SDGs)

Pharma 4.0 – PAT and RTRT (QP Batch Release)

Data to Knowledge based Decision Making

Connected Information MES - Manufacturing Enterprise System

Process Control = PAT = Predictive Quality & Digital Twins

Annex 1 (GMP), Annex 11 (DI) & Annex 15 (Q&V)

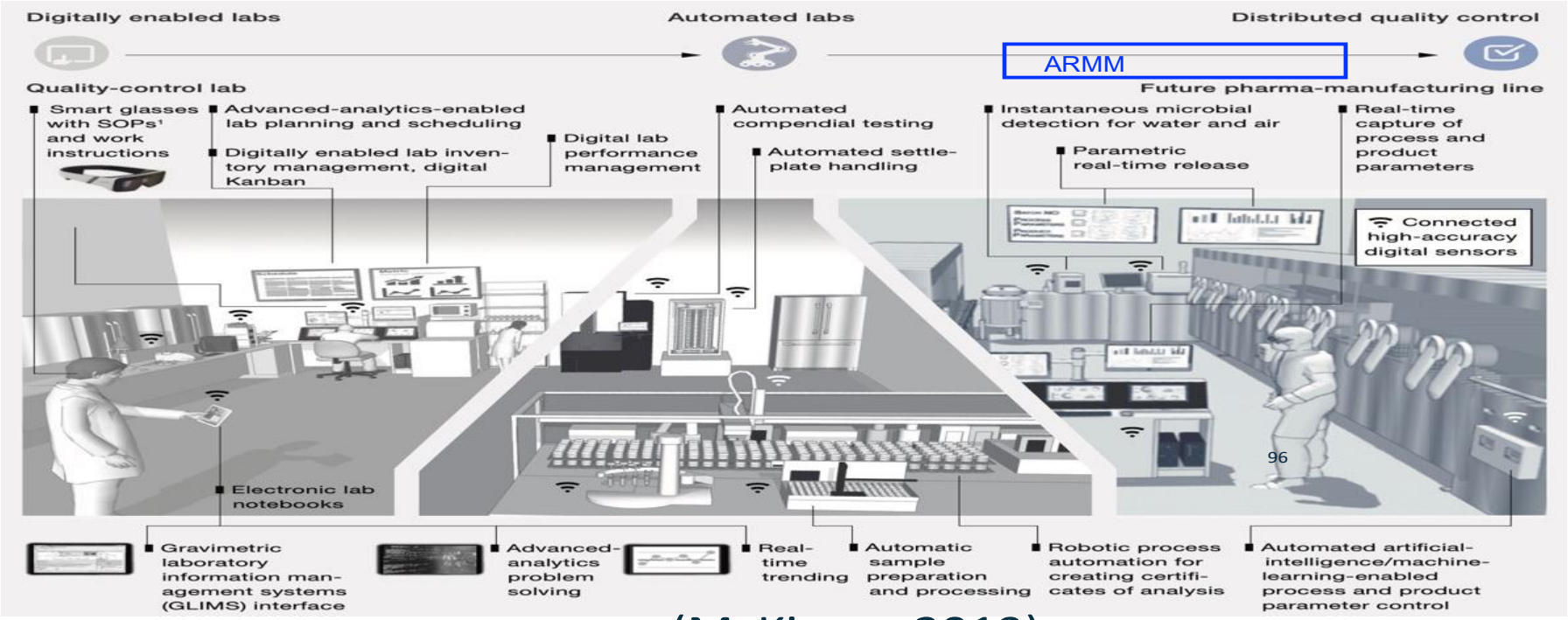
QRM & CCS - Real-Time ARMM (BFPC)

Why Pharma 5.0 – Why Not? & link to UN SDGs

- Industry 5.0 reflects a greater emphasis on societal value and wellbeing, not just economic value
- This is a shift in focus from welfare to wellbeing, based on UN SDGs (Sustainability Development Goals)
- **This is NOT new** - think CSR (Corporate Social Responsibility) and ESG (Environmental Sustainability Goals)
- Industry 5.0 leverages Industry 4.0 with its focus on AI, Robotics and Automation, same as Pharma 4.0 - "**Data to Knowledge based Decisions**"
- Key elements are still automation, robotization, big data analytics, smart systems, virtualization, AI, machine learning and Internet of Things
- **BUT BUT BUT - based on Sustainabiliy**

Pharma 5.0 PAT & RTRT – Future Sustainability

AI, Robotics & Automation - Predictive Quality –Digital Twin - Data Integrity and ARMM



(McKinsey 2019)

Climate Challenge – **CR Energy/Resources Footprint**

- Cleanrooms are complex projects - Semiconductors, Microelectronics, Data Centres, Giga Batteries, Life Sciences and Nuclear – same issues and challenges
- Among largest consumers of energy and resources – run 7/24/365!
- Unfortunately, risk averse design means large CAPEX and inflexible controls
- Until energy costs rose no need to look at OPEX - now forced to do so
- Current “climate crisis” will force us to change and incentivise us to manage energy and resources more efficiently
- **Answer – Do More with Less! The 3 R’s of ReUse, RePurpose & ReCycle**

The Next 50 Years – **The Challenge Ahead**

- Cleanrooms are huge consumers of Energy and our Natural resources
- Climate Change is our biggest challenge – must work together, its our future
- Climate Rebalancing & Move to a Circular Economy is the ONLY solution
- Cleanrooms are getting bigger and smaller at the same time
- Increasing number of CC applications in more and more countries
- Promote application of ISO Standards – across all applications
- "Awareness leads to Behavioural Change" - **Education is key**

Climate Challenge – The Future of Cleanrooms

- **CLEANROOMS of the future will focus more on:**
  - Cleanroom Risk/Hazards, potential to do HARM
  - In CAPEX, Demand Based Contamination Control (DBV in HVAC systems) Air Change Effectiveness (ACE) and Contamination Removal Effectiveness (CRE)
  - In OPEX, reuse materials and components, especially in consumables

ReUse, RePurpose and ReCyclex

Thank you for your attention!

Slovenian Clearoom Society
Dimičeva street 13 I 1504 Ljubljana
T: +386 1 5898 312 I F: +386 1 5898 317 I E: ptz@gzs.si
https://scs.gzs.si

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:
INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Contamination control in practice – From Construction Site to Manufacturing Excellence

Alenka Koblar, Lek Pharmaceuticals d.d.

30 – 31 MARCH 2026
BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

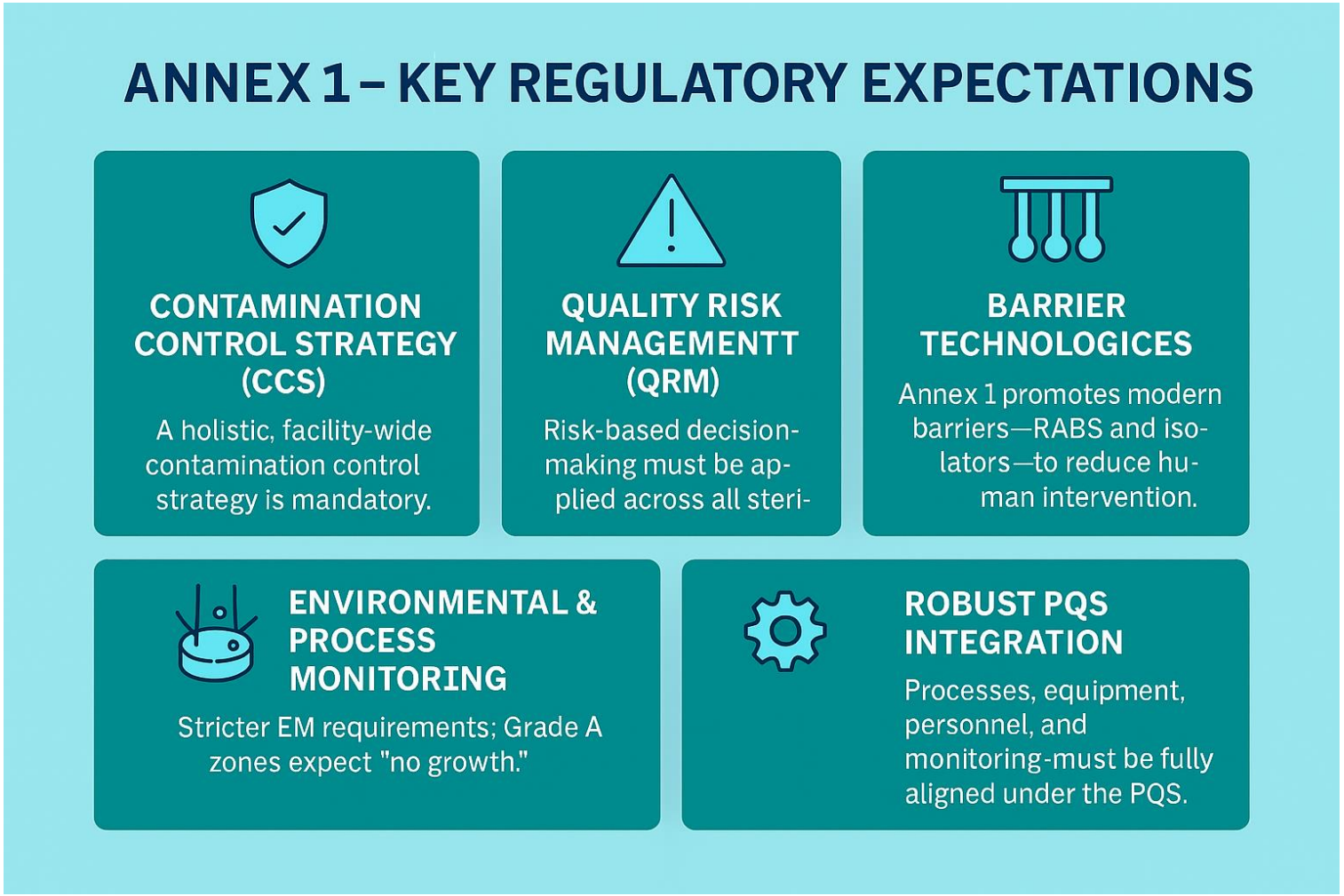
"This is how it started — one of Sandoz's major investments in Slovenia: a new sterile manufacturing facility, Bioinjectable production at Brnik"

Contamination control in practice – From Construction Site to Manufacturing Excellence

Alenka Koblar, Lek Pharmaceuticals d.d.

A VISION WITHIN A REGULATORY FRAMEWORK

- Strict regulatory framework
- EU GMP Annex 1 requirements
- Understanding the process
- Risk-based and justified decisions



Contamination control strategy by design – CCS implemented from the earliest concept.

Contamination control in practice – From Construction Site to Manufacturing Excellence

Alenka Koblar, Lek Pharmaceuticals d.d.

FROM VISION TO REALITY: STARTING FROM ZERO

- No historical data
- New technologies
- Complex material flows
- High regulatory expectations

Contamination control in practice – From Construction Site to Manufacturing Excellence

Alenka Koblar, Lek Pharmaceuticals d.d.

FROM SOURCES TO RISKS - QRM

- Identify contamination sources
- Evaluate severity & probability
- Define control measures
- Verify effectiveness (later)

QRM – TOOL FOR CHOOSING TECHNOLOGY

"We defined clear decision criteria."

- Risk reduction potential.
- Alignment with Annex 1 expectations.
- Operational robustness.
- And long-term sustainability.

EXAMPLE 1: Isolator vs RABS

- Barrier choice
- Isolators cut human interaction
- Annex 1 favors strong barriers
- Interfaces = main challenge
- QRM → isolator preferred

EXAMPLE 2: Introduction of RTU into isolator

- RTU reduces open handling
- Maintaining sterility during transfer
- Multiple transfer concepts evaluated
- QRM asked: where/impact/likelihood
- Highest risks → highest controls

EXAMPLE 3: Manual vs automatic material transport

- Manual vs. automatic transfer
- Human handling = contamination risk
- Automation lower variability
- New cleaning/disinfection challenges
- Risk not removed — transformed

KEY TAKEAWAYS

CCS is about connections

Biggest risks hide between systems

Annex 1 & FDA = design framework

Technology works only inside CCS

Risk management drives every decision.

We built a strong foundation —but the real test is coming...
Theory guides us... practice will confirm it.

Contamination control in practice – From Construction Site to Manufacturing Excellence

Alenka Koblar, Lek Pharmaceuticals d.d.

Thank you for your attention!

Slovenian Clearoom Society
Dimičeva street 13 I 1504 Ljubljana
T: +386 1 5898 312 I F: +386 1 5898 317 I E: ptz@gzs.si
https://scs.gzs.si

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:
INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Evolving qualification requirements under Annex 1: A case study in cleanroom performance and re-design

Meta Resnik, Lek d.d., a Sandoz company

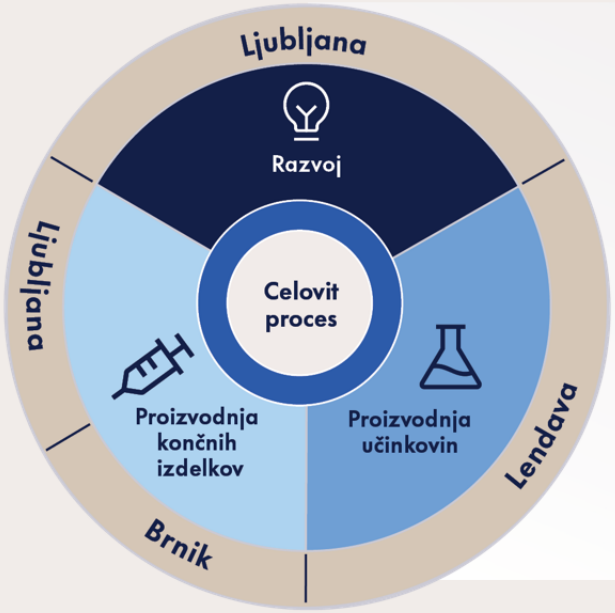
SANDOZ | lek

80 LET LEKA
YEARS OF LEK

30 – 31 MARCH 2026
BRDO PRI KRANJU, SLOVENIA

Vodilno evropsko razvojno-proizvodno središče za podobna biološka zdravila v Sloveniji

SANDOZ | lek



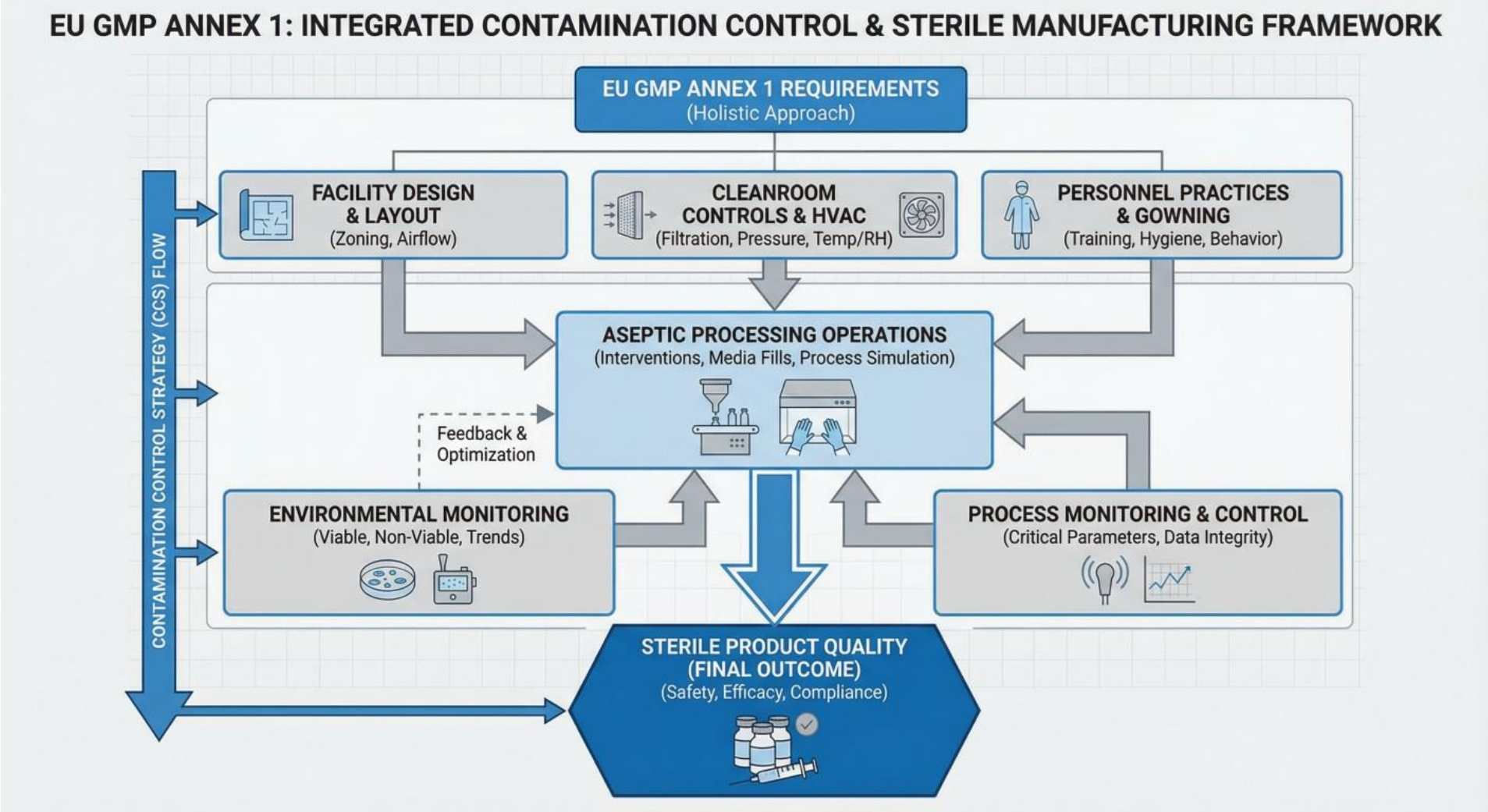
- Sandoz uresničuje svojo **globalno strategijo** na področju podobnih bioloških zdravil s **ključnimi naložbami v Sloveniji**.
- Na **treh lokacijah** vlaga **več kot 1 milijardo evrov** v širitev vertikalno integrirane mreže za razvoj in proizvodnjo.
- S tem utrjuje svoj **vodilni globalni položaj** na področju podobnih bioloških zdravil.
- Krepi se **zanesljivost in trajnost oskrbe** s podobnimi biološkimi zdravili, izdelanimi v Evropi.
- Naložbe bodo **ustvarile 800 novih delovnih mest**.

Evolving qualification requirements under Annex 1: A case study in cleanroom performance and re-design

Meta Resnik, Lek d.d., a Sandoz company

SCOPE

- 2022 EU GMP Annex 1 revision and its impact on aseptic manufacturing
- Impact of Annex 1 revision on new and legacy facilities
- Case study which reflects how expanded expectations lead to new findings in legacy facility
- Lessons learned from case study



ISO 14644

CCS

Evolving qualification requirements under Annex 1: A case study in cleanroom performance and re-design

Meta Resnik, Lek d.d., a Sandoz company

EU GMP Annex 1 2022

4.25 Cleanroom and clean air equipment qualification is the overall process of assessing the level of compliance of a classified cleanroom or clean air equipment with its intended use. As part of the qualification requirements of Annex 15, the qualification of cleanrooms and clean air equipment should include (where relevant to the design/operation of the installation):

i. Installed filter system leakage and integrity testing.

ii. Airflow tests - volume and velocity.

iii. Air pressure difference test.

iv. Airflow direction test and visualisation.

v. Microbial airborne and surface contamination.

vi. Temperature measurement test.

vii. Relative humidity test.

viii. Recovery test.

ix. Containment leak test.

Reference for the qualification of the cleanrooms and clean air equipment can be found in the ISO 14644 series of standards.

- Many information are gathered during cleanroom initial qualification for new facilities
- What about the legacy facilities? How do we reach compliance and where can it be detected?

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

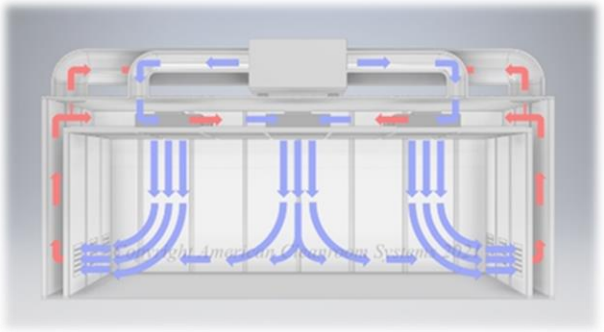
Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Detection of adverse trend in operator‘s EM results

HVAC re-design

Material transfer process

Air visualization studies



Evolving qualification requirements under Annex 1: A case study in cleanroom performance and re-design

Meta Resnik, Lek d.d., a Sandoz company

PERSONNEL CONTAMINATION MANAGMENT

- **Gowning certification program**
- **Operator‘s routine sampling frequency:**

After critical operations (Grade A requirements)

After routine work, before each exit (Grade B requirements)

1 Forehead

2 Mouth area

3 Collar

4 Chest

5, 6 Fingertips

7, 8 Forearms

- **Requirements:**

Grade A: 0 CFU/plate

Grade B: ≤5 CFU/plate

- **Trending**

Contamination recovery rate is the rate at which environmental samples are found to contain any level of contamination. For example, an incident rate of 1% would mean that only 1% of the samples taken have any contamination regardless of colony number (*USP<1116>)*

Grade A: RR < 1%

Grade B: RR < 5%

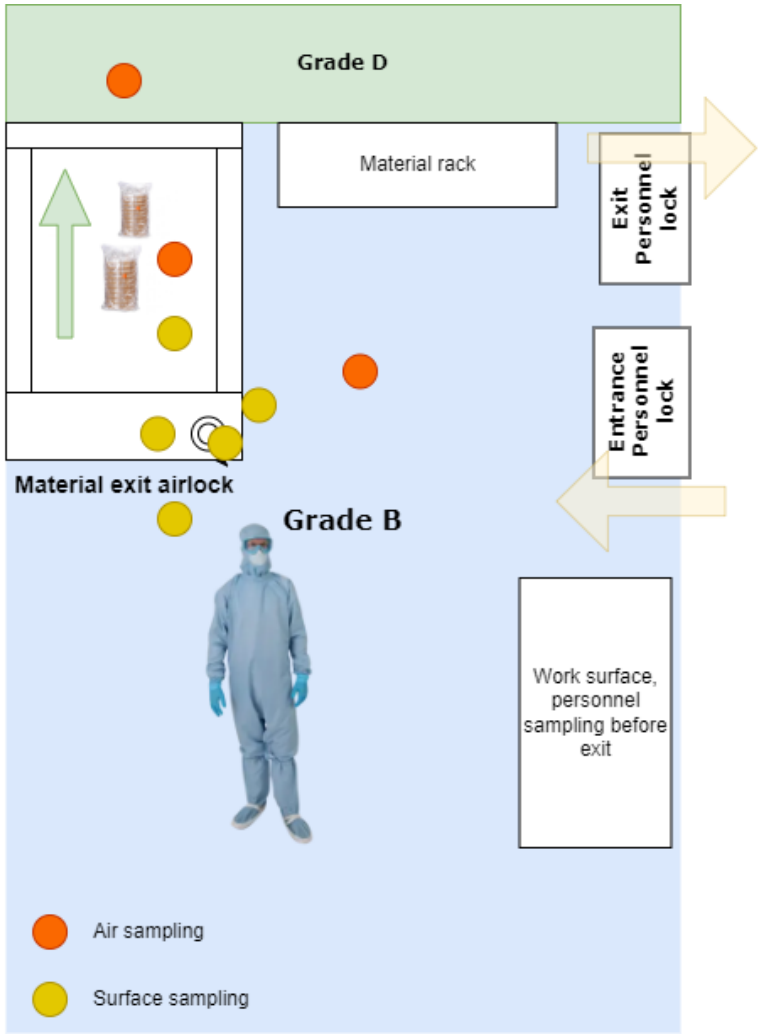
MAINTAINANCE OF OPERATOR‘S CERTIFICATION TO OPERATE IN GRADE A/B

- **Supervised and monitored gowning session annualy**
- **Annual theoretical workshop**
- **Compliant annual trend review:**
  - EM individual operator‘s results
  - Aseptic techniques and behaviours
- **Aseptic process simulation (APS) attendance**

1 Forehead
2 Mouth area
3 Collar
4 Chest
5, 6 Fingertips
7, 8 Forearms

EM individual operator‘s results:

- EM results review for an individual operator for past year
- USP recommended grade B recovery rate < 5%, internally defined alert limit **< 2.5%**
- Further investigation was initiated



1. Adverse annual EM personnel trend detected for an operator 5% > RR > 2.5%
2. No microbial growth detected in grade A for the operator
3. Detailed investigation on growth occurances
4. All growth detections during glove sampling performed immediately prior to exiting the cleanroom, specifically at the point of material handover into material airlock (MAL)
5. Intensive sampling around the MAL, incresed QA oversight for the process
6. Microbial detections occured only on grade B air around airlock
7. Minor MAL malfunction was identified
8. Airflow visualization studies around MAL were performed. Less efficient air flushing of the area was detected, local HVAC re-design followed.

Evolving qualification requirements under Annex 1: A case study in cleanroom performance and re-design

Meta Resnik, Lek d.d., a Sandoz company

TAKEAWAYS & LESSONS LEARNED:

- Only few growth detections resulted in exceedances, therefore it is important to setup and identify trends, alert and action limits
- Deep in-process knowledge and understanding of CCS connections is crucial to identify trends
- Operator(s) were performing adequately, because glove disinfection must not be performed after MAL handling if it is followed by glove sampling
- Complete initial cleanroom qualification is not deemed necessary for legacy facilities, however it is wise to use individual tests to identify potential issues

TAKEAWAYS & LESSONS LEARNED:

- Expanded requirements by Annex 1 revealed contributing factors to potential issue
  - *4.25 Cleanroom and clean air equipment qualification is the overall process of assessing the level of compliance of a classified cleanroom or clean air equipment with its intended use. As part of the qualification requirements of Annex 15, the qualification of cleanrooms and clean air equipment should include (where relevant to the design/operation of the installation): … iv. Airflow direction test and visualisation.*
  - *9.25: …Particular consideration should be given to monitoring personnel following involvement in critical interventions (at a minimum gloves, but may require monitoring of areas of gown as applicable to the process) and on each exit from the grade B cleanroom (gloves and gown).*
  - *9.10 Alert levels for grade A (total particle only) grade B, grade C and grade D should be set such that adverse trends (e.g. a numbers of events or individual events that indicate a deterioration of environmental control) are detected and addressed.*

Thank you for your attention!

Slovenian Clearoom Society
Dimičeva street 13 I 1504 Ljubljana
T: +386 1 5898 312 I F: +386 1 5898 317 I E: ptz@gzs.si
https://scs.gzs.si

Sources, references:

1. https://pharmuni.com/2025/05/11/ultimate-eu-gmp-annex-1-checklist-are-you-truly-audit-ready/
2. Volume 4 EU Guidelines for GMP for Medicinal Products for Human and Veterinary Use / Annex 1: Manufacture of Sterile Medicinal Products
3. USP <1116> Microbiological control and monitoring of aseptic processing environments
4. PDA Technical Report No. 13, 2022, Fundamentals of an Environmental Monitoring Program
5. ISO 14644-1/2015: Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Contamination Control Blind Spots

Missed Risks in a Contamination Control Strategy

Ziva Abraham, Microrite, Inc.

Ziva Abraham, Ph.D
President, Microrite, Inc.
Phone: 408-445-0507
Fax: 408-445-1236
Email: zabraham@microrite.com
www.microrite.com

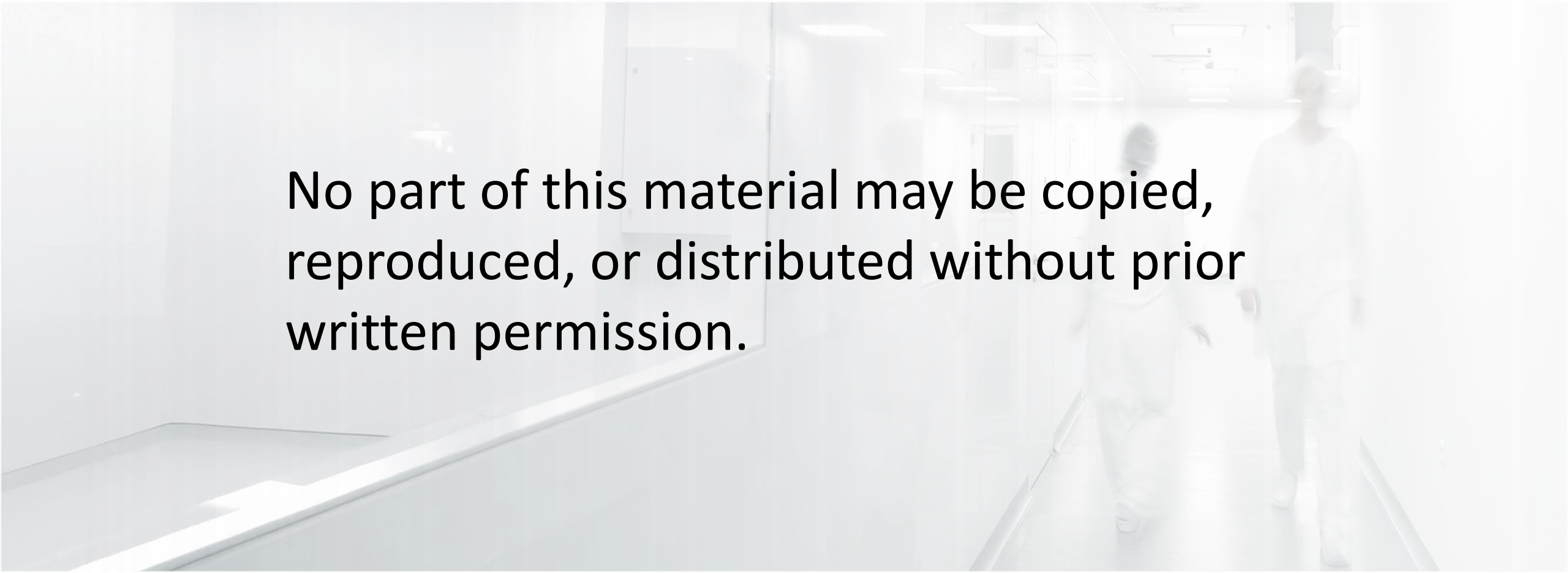
Ziva Abraham *has over 35 years of academic, research, clinical and industrial experience in Microbiology, and Quality Assurance. Ziva has received her Master's Degree in Microbiology and has conducted research on developing Microbial Insecticides using entomogenous bacteria and fungi towards her Ph. D degree.*

She worked as a clinical scientist and established clinical laboratory systems in Israel. In this capacity Ziva evaluated the first automated microbial identification system, introduced new technologies for HIV testing, and rapid methods into the group of laboratories she helped establish for Maccabi Medical. Her laboratory system conducted clinical testing in microbiology, chemistry, parasitology, hematology and immunology.

She is a passionate microbiologist and mycologist and provides training worldwide on various microbiology and contamination control topics. Her clinical experience has made helps her evaluate and teach the clinical implications of microbial contamination to patients.

Ziva started Microrite in 1998 as an independent microbiology consultant. Ziva used her extensive research experience with fungi and clinical organisms and their impact on human health to build an internationally renowned contamination control team of experts. Her team consists of facilities, cleanroom, airflow, static charge, validation, quality and microbiology professionals with practical knowledge in their field and involvement in industry standards and guidance organizations.

Ziva's team work congruently to resolve client issues, prevent contamination through design and help making safe products for human and veterinary use.



Why an Update to GMP Annex 1?

- **To Remove Ambiguities: (**previous guidance created loop-holes & misunderstandings)
- Emergence of New Technologies in Sterile manufacturing
- Improved Isolators and RABS designs
- Changes in ISO Cleanroom Standards (ISO 14644-1&2:2015)

Observations of:

- Inadequate root cause analysis
- Ineffective use or application of CAPA

Emphasis

- Over a instances of the word “Risk”
- 18 instances of the word “Risk Assessment”
- 38 instances of the word “Airflow”
- Multiple instances of the word “Airflow Visualization”

CCS Blindspots
Personnel
Gown choice, management, gowning and aseptic behavior

Personnel

Annex 1: Reusable clean area clothing should be cleaned in a laundry facility adequately segregated from production operations, using a qualified process ensuring that the clothing is not damaged and/or contaminated by fibres or particles during the repeated laundry process. Laundry facilities used should not introduce risk of contamination or cross-contamination. Inappropriate handling and use of clothing may damage fibres and increase the risk of shedding of particles. After washing and before packing, garments should be visually inspected for damage and visual cleanliness. The garment management processes should be evaluated and determined as part of the garment qualification programme and should include a maximum number of laundry and sterilization cycles.

No control of laundry cycles

Unsuitable Goggles?

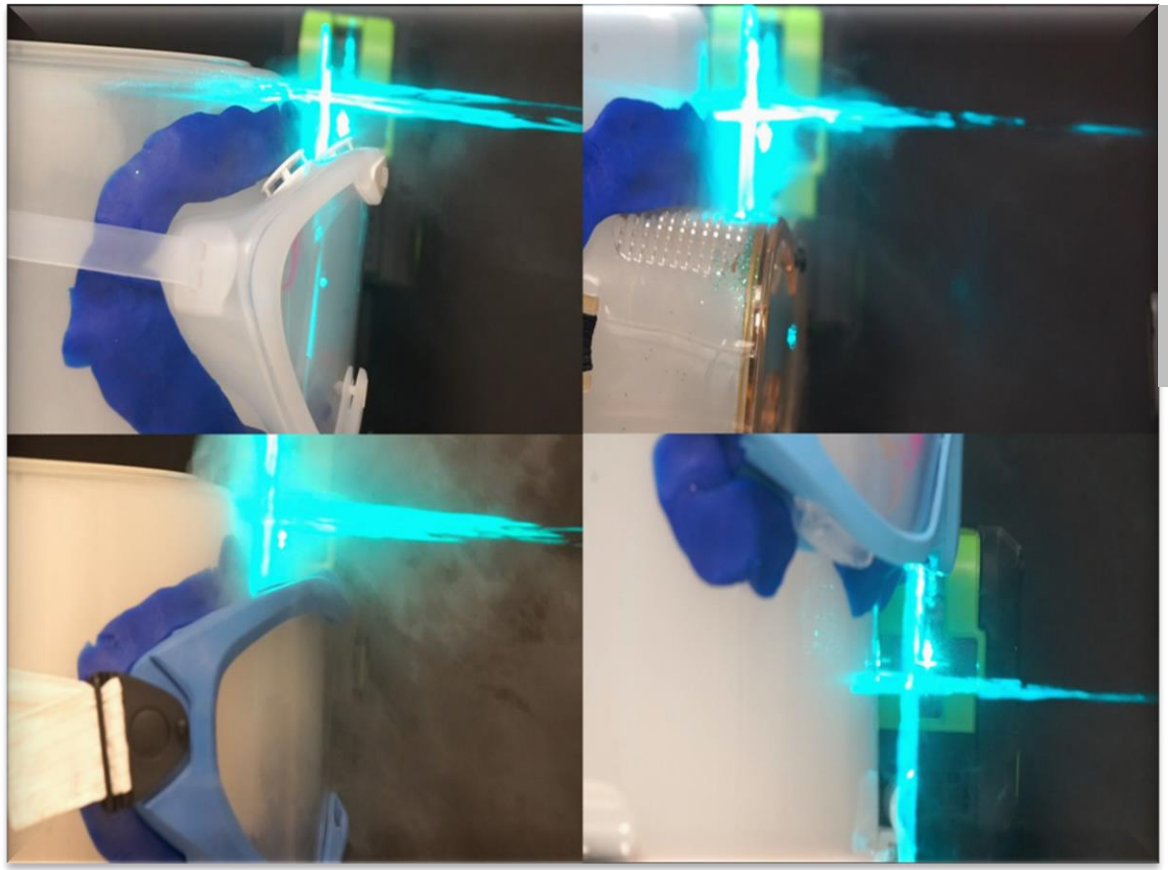
OBSERVATION

Procedures designed to prevent microbiological contamination of drug products purporting to be sterile are not established.

For Example:

a) Operators were observed with goggles having (unprotected) holes in the top and bottom. Goggles used by operators to protect against exposed skin while working in the grade (b)(4) areas during aseptic operations having unprotected holes creates a lack of defense against a source of particles generated by, and microorganisms shed from, the face.

Propionibacterium source and transport

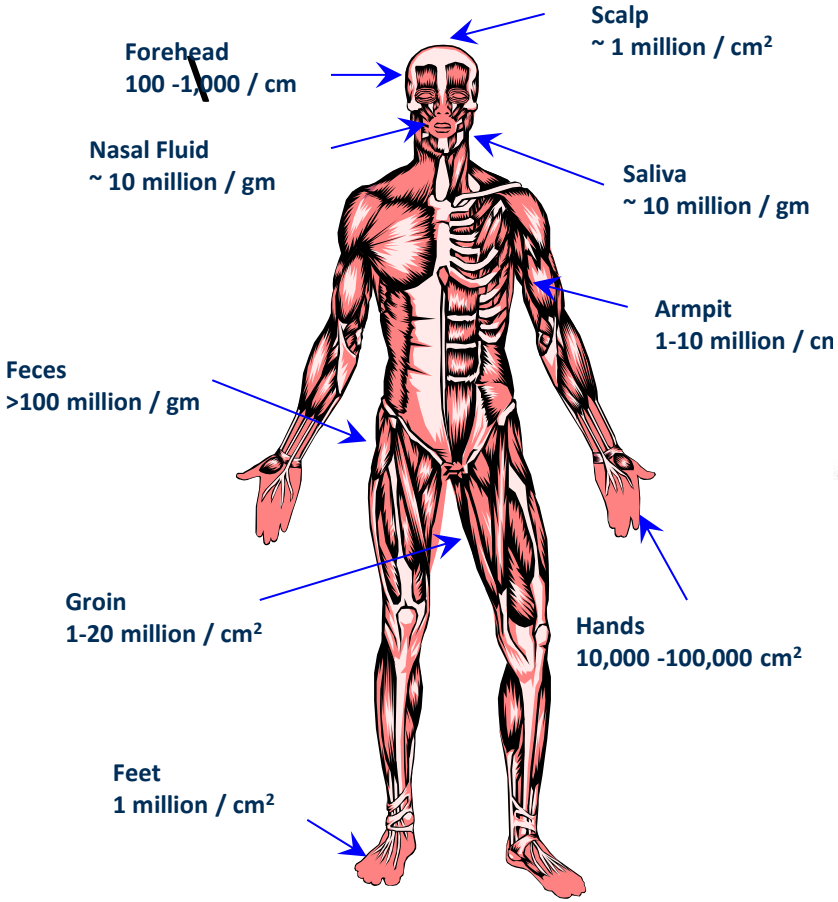


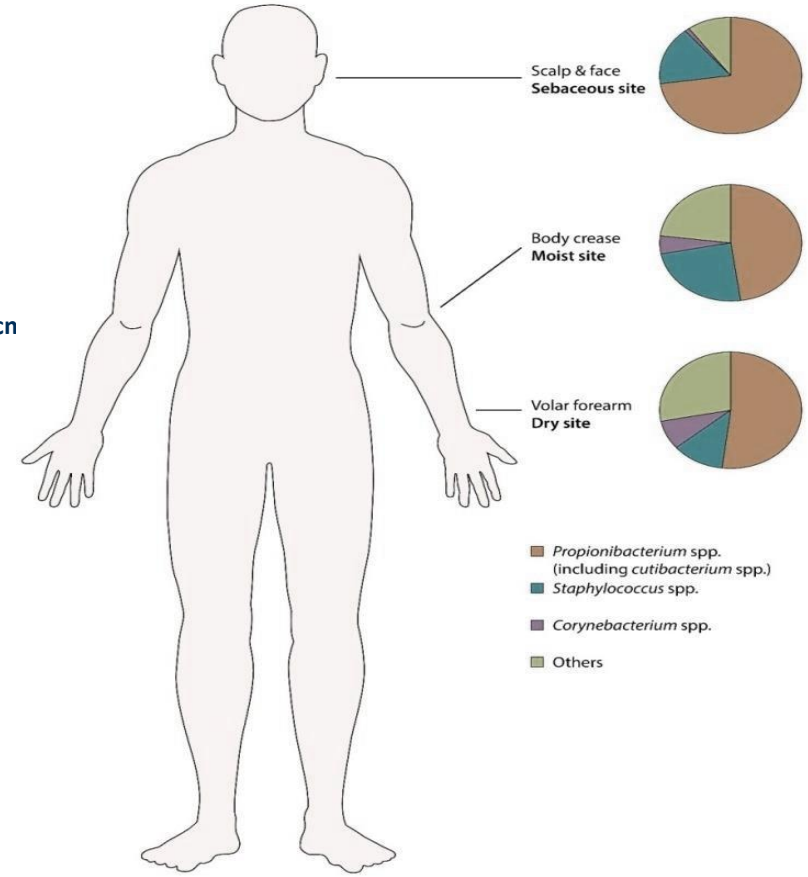
Video Capture: Smoke Study evaluating vents of 4 different Goggles. Laser is used to illuminate smoke particles escaping via goggle vents.

How a goggles vent can generate human borne contamination

Neutrally buoyant smoke escapes via goggle vents

Human Borne Contamination

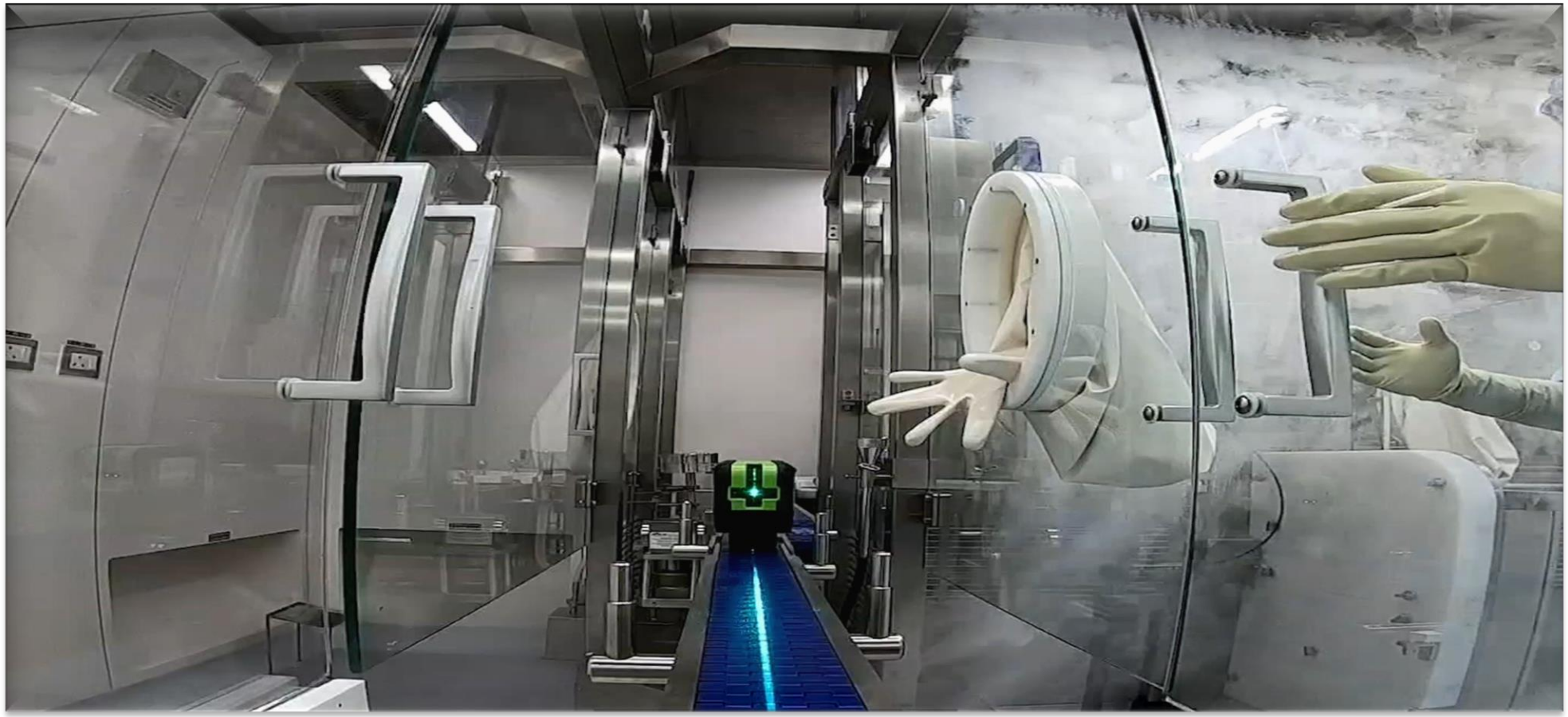




Viable but non-culturable organisms (VBNCs) may not be detected through routine personnel monitoring, yet they can still appear in media fills and sterility testing

CCS Blindspots
Facility Design, Qualification and Maintenance

Bacillus and Human Borne Contamination in RABS



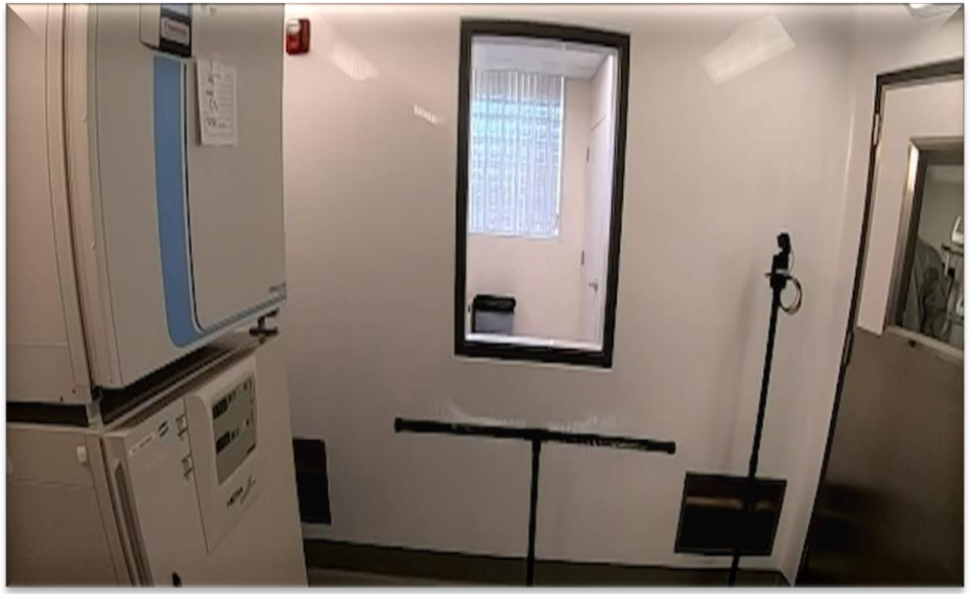
Airflows-Transport Method of the Contaminants

Placement of Equipment is Important

Uncleanable Surfaces in RABS over the Filling Needles

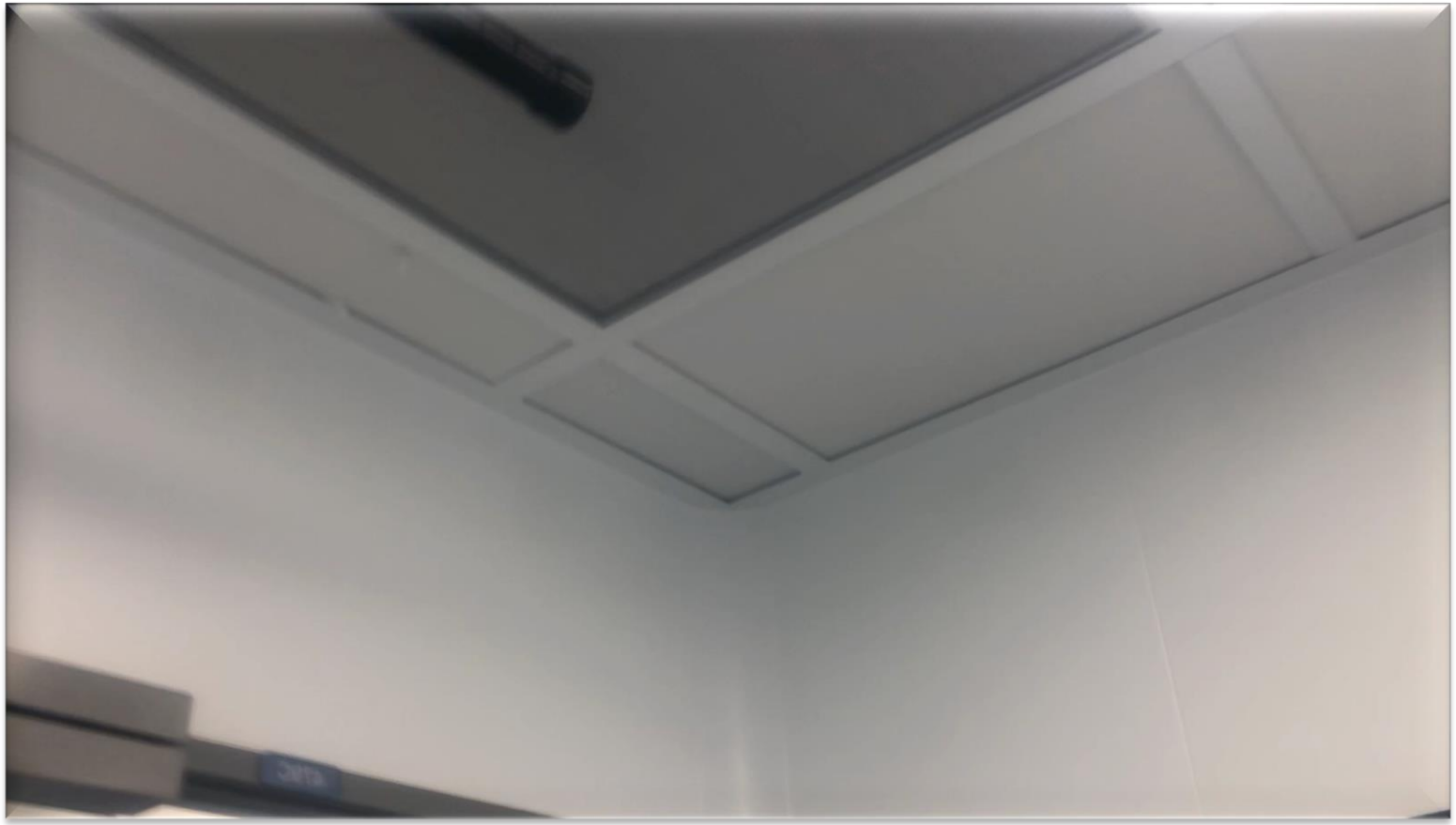
Intermittent EM excursions in BSC and Product Failures-Design Issue?

Transport of Mold into Incubators



When the door to the gowning room is opened the air seeks to exit through returns.
Return proximal to incubators is not optimal for contamination control.

Maintenance ignored?



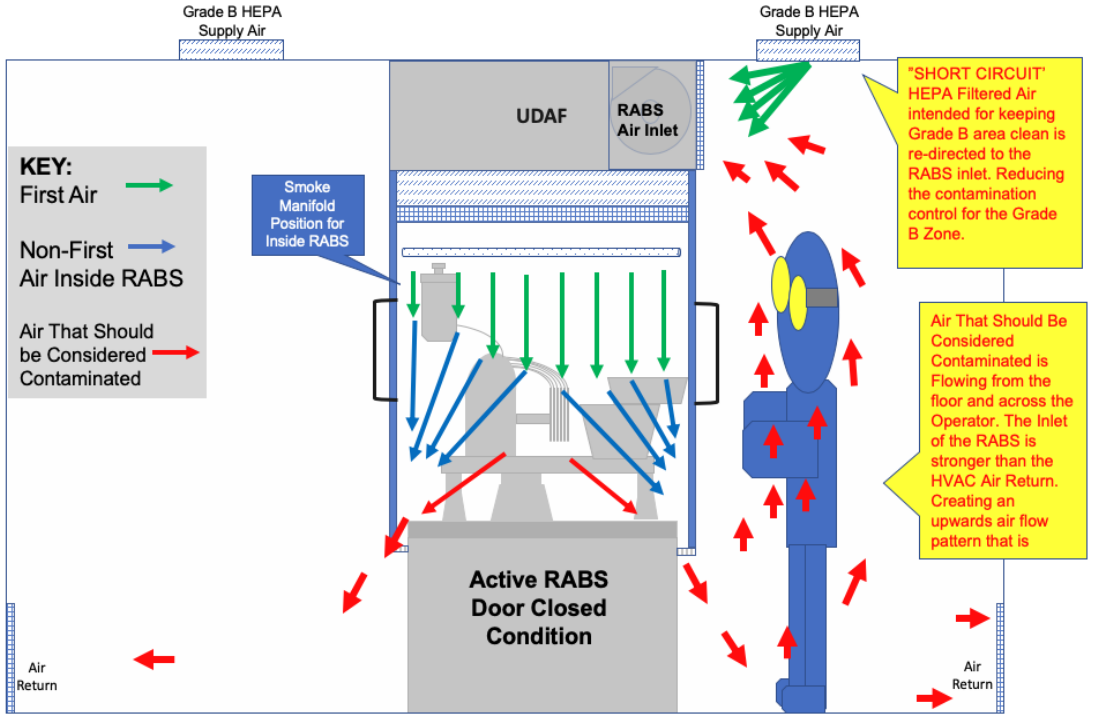
Predominance of Fungi due to blocked retuned ducts

CCS Blindspots
Barrier Design and Integration

Microbial Contamination in RABS- Peter Stealing from Paul-Grade B starved of clean air

Barrier System: Peter Stealing from Paul

Holistic View of Air Flow Patterns:



What is wrong with this picture?

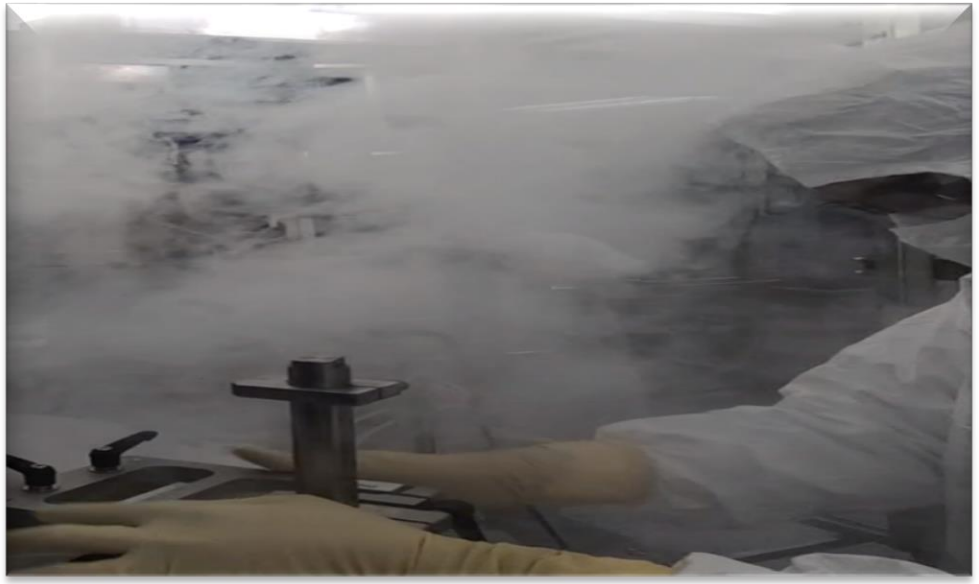
"It is useful to assume that the operator is always contaminated while operating in the aseptic area.

If the procedures are viewed from this perspective, those practices which are exposing the product to contamination are more easily identified."

Hank Avallone – 1989

"a gowned operator may release as many as 10,000 CFU/hour or more…

(Reinmuller and Ljungqvist).



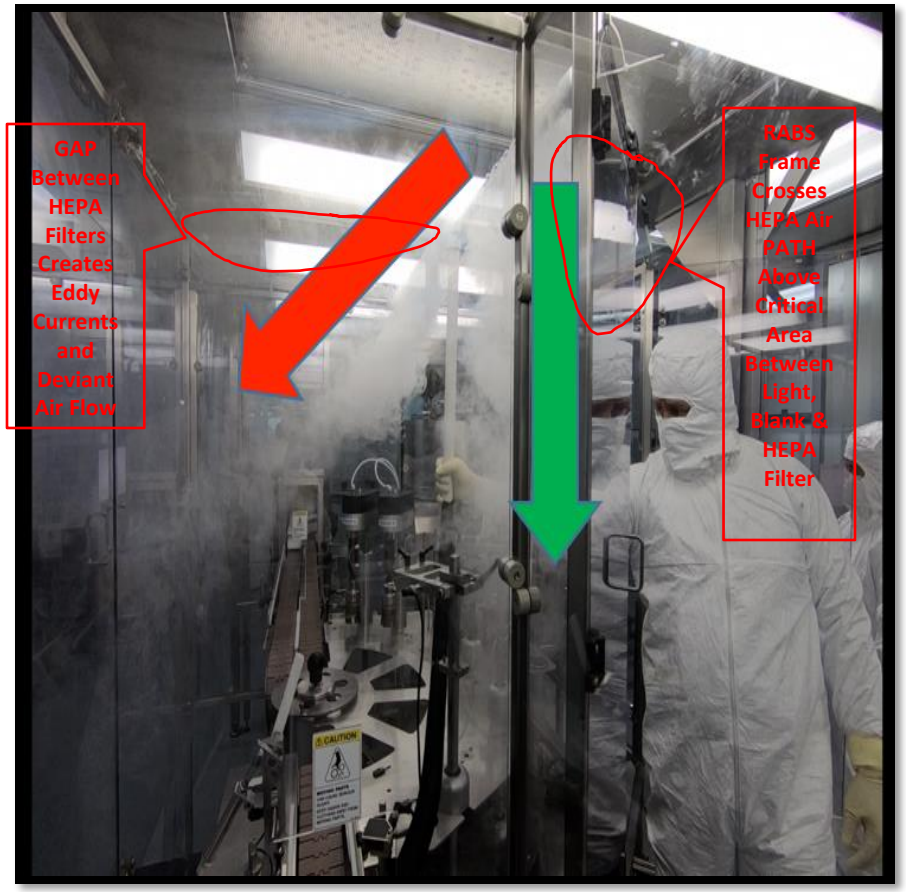
Smoke Study Video Capture: Smoke Flows over the operator onto the filling area during simulation of a corrective intervention. "Needle Adjust"

P.acnes is an VBNC, may not be captured in environmental monitoring but may show up during media fill and sterility

What is wrong with this picture?

483 Regarding Smoke Study for EM

- You do not have a scientific rationale for the environmental monitoring sampling locations in aseptic filling Suites (b)(4). You did not include factors such as smoke study findings, number and location of operators, and historical microbial data in your assessment of hazardous points.
- For example, we found that settling plates are not appropriately placed in critical areas. Your smoke study showed that during set-up and filling, air flows toward the front (when the (b)(4) is open) or back of the RABS. However, two relevant sampling points were recently eliminated. As a result, these points of increased risk are not monitored.



CCS Blindspots
Cleaning and Disinfection

Annex 1

More than one type of disinfecting agent should be employed to ensure that where they have different modes of action, and their combined usage is effective against all bacteria and fungi. Disinfection should include the periodic use of a sporicidal agent. **Monitoring should be undertaken regularly in order to assess the effectiveness of the disinfection program and to detect changes in types of microbial flora** (e.g. organisms resistant to the disinfection regime currently in use). Cleaning programs should effectively remove disinfectant residues

Understanding Disinfectant Label Claim

Label Claim Testing for Bactericidal Activity

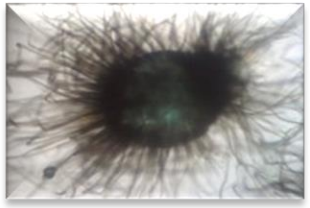
- *Salmonella choleraesuis*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*

Label Claim Testing for Sporicidal Activity

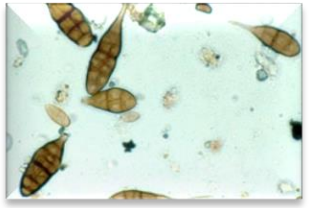
- *Bacillus subtilis*

Label Claim Testing for Fungicidal Activity

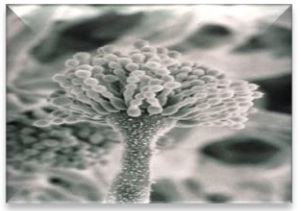
- *Trichophyton mentagrophytes*
- *Aspergillus niger*



Ascomycota



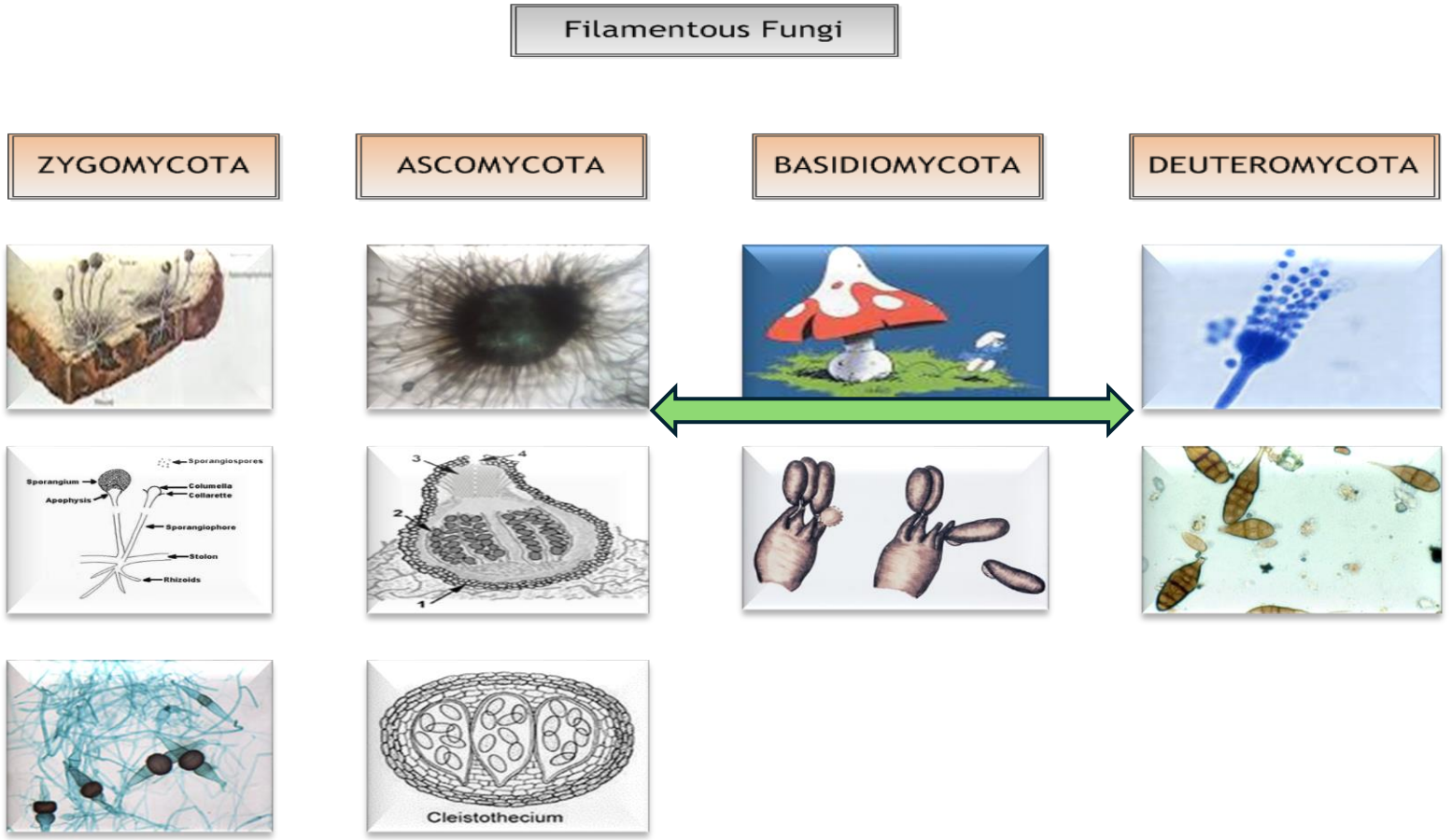
Colored Deuteromycota



Colorless Deuteromycota

Note fungicidal activity of disinfectants is tested using colorless deuteromycotous fungi which are easy to kill. Ascomycota and colored Deuteromycota are hard to kill.

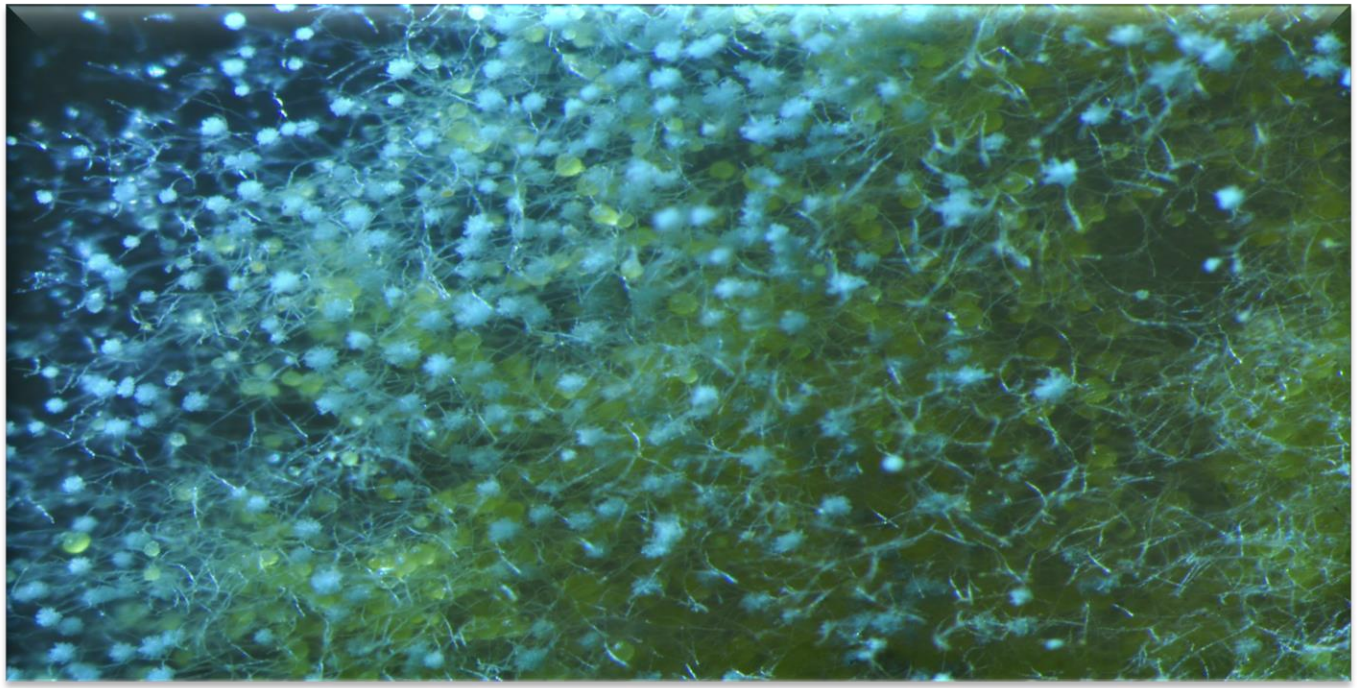
Understanding Fungi



Understanding Mold

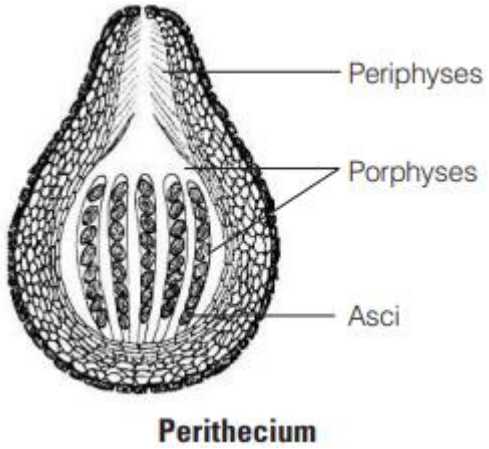
Anamorph and teleomorph on same substrate

Switching the mode of reproduction!

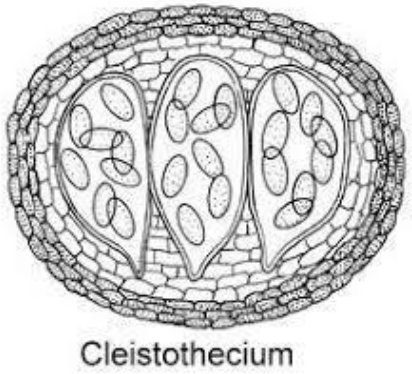


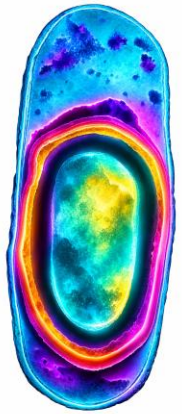
Disinfectant Resistance

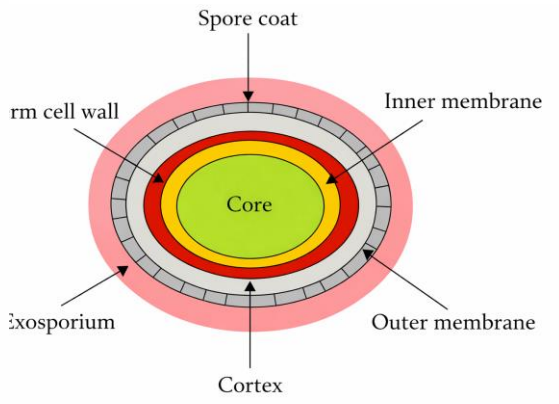
- Different groups of bacteria vary in their susceptibility to biocides, with bacterial spores being the most resistant, followed by mycobacteria, then Gram-negative organisms, with cocci generally being the most sensitive.
- Intrinsic resistance (intrinsic insusceptibility) is found with bacterial spores, mycobacteria and Gram-negative bacteria.
- This resistance might, in some instances, be associated with constitutive degradative enzymes but in reality, is more closely linked to **cellular impermeability.**



Perithecium

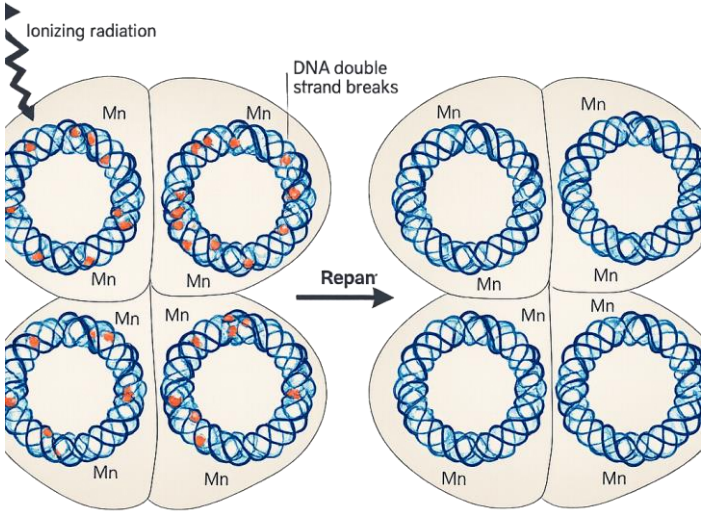

Cleistothecium



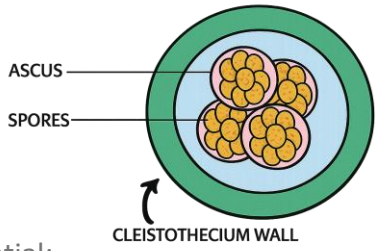


Radiation Resistance

Deinococcus radiodurans
Nicknamed **"Conan the Bacterium."**
Radiation and Desiccation Resistance

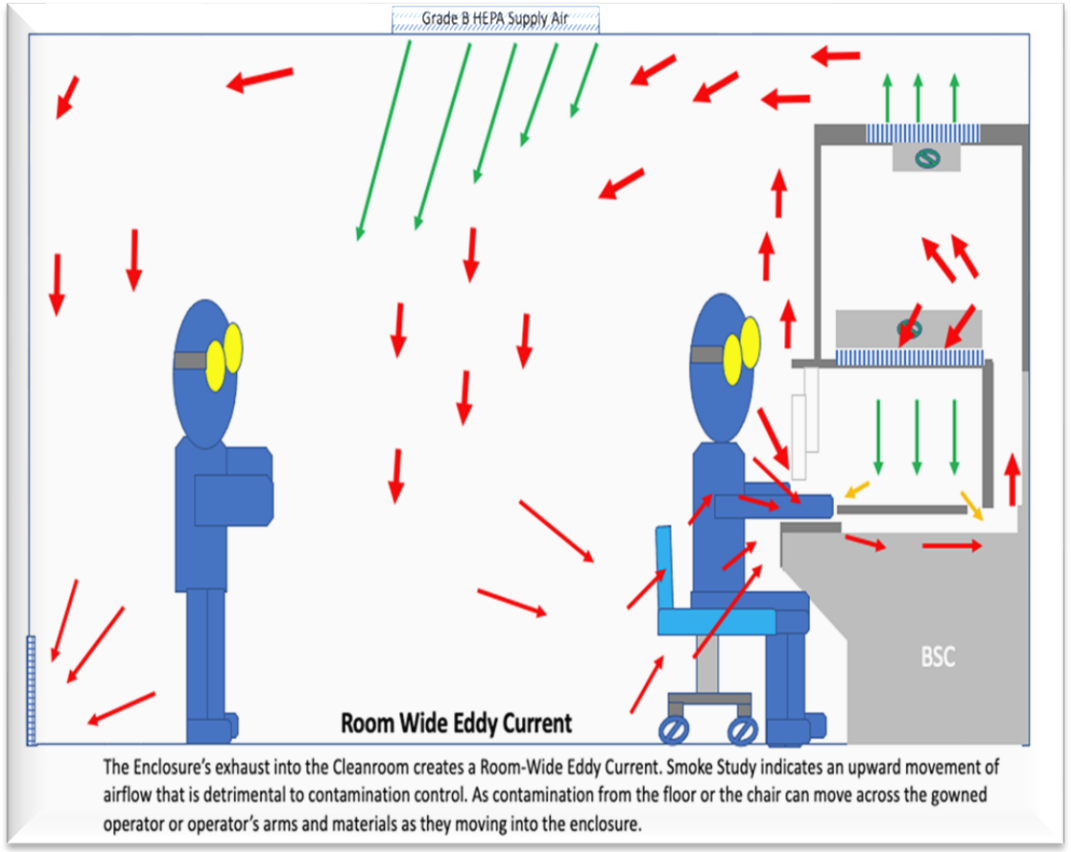


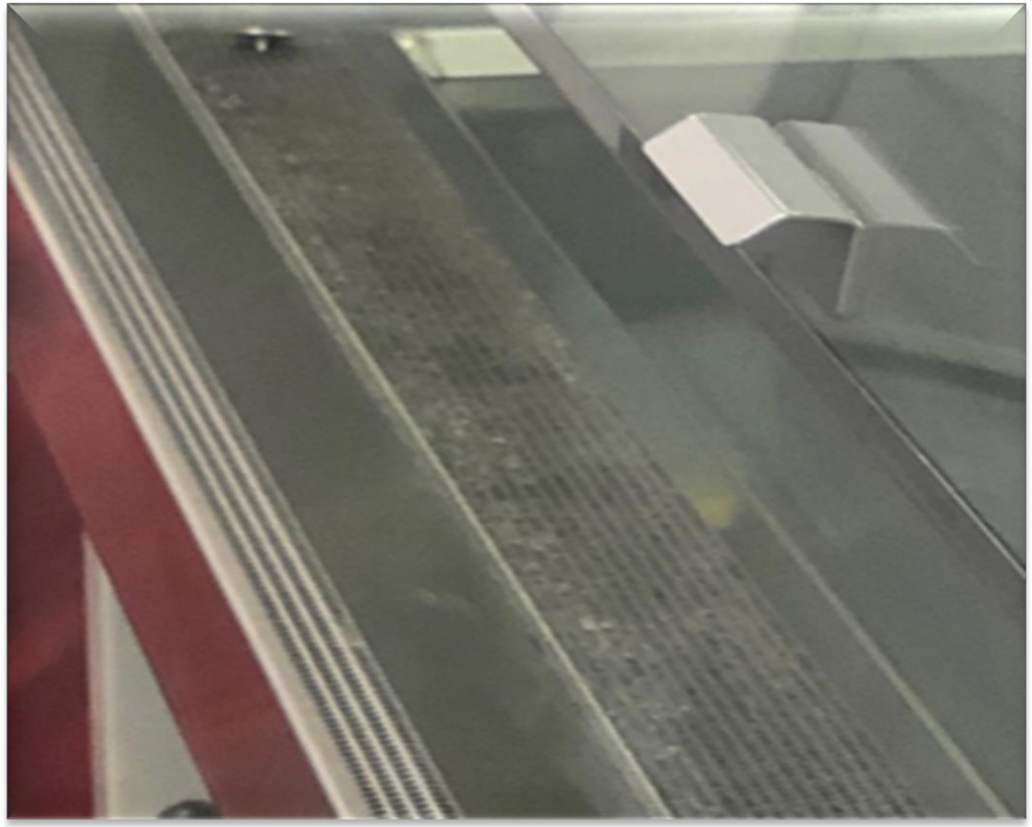
- Melanins is their ability to absorb all types of electromagnetic radiation which endows them with the capacity for both energy transduction and shielding.
- The findings from Chernobyl, the space station, Antarctic mountains show melanins have functions analogous to other energy harvesting pigments such as chlorophylls.
- The telepmorphic stages of mold have shown radiation resistance, e.g. *Pyronema* and many more



Supplies Matter

- No bucket system is good if:
- The dilution of disinfectant is not correct
- The mops don’t allow removal and retention of dirt, debris and contamination
- Cotton mops are cellulous, will promote mold growth
- String mops don’t dry easily



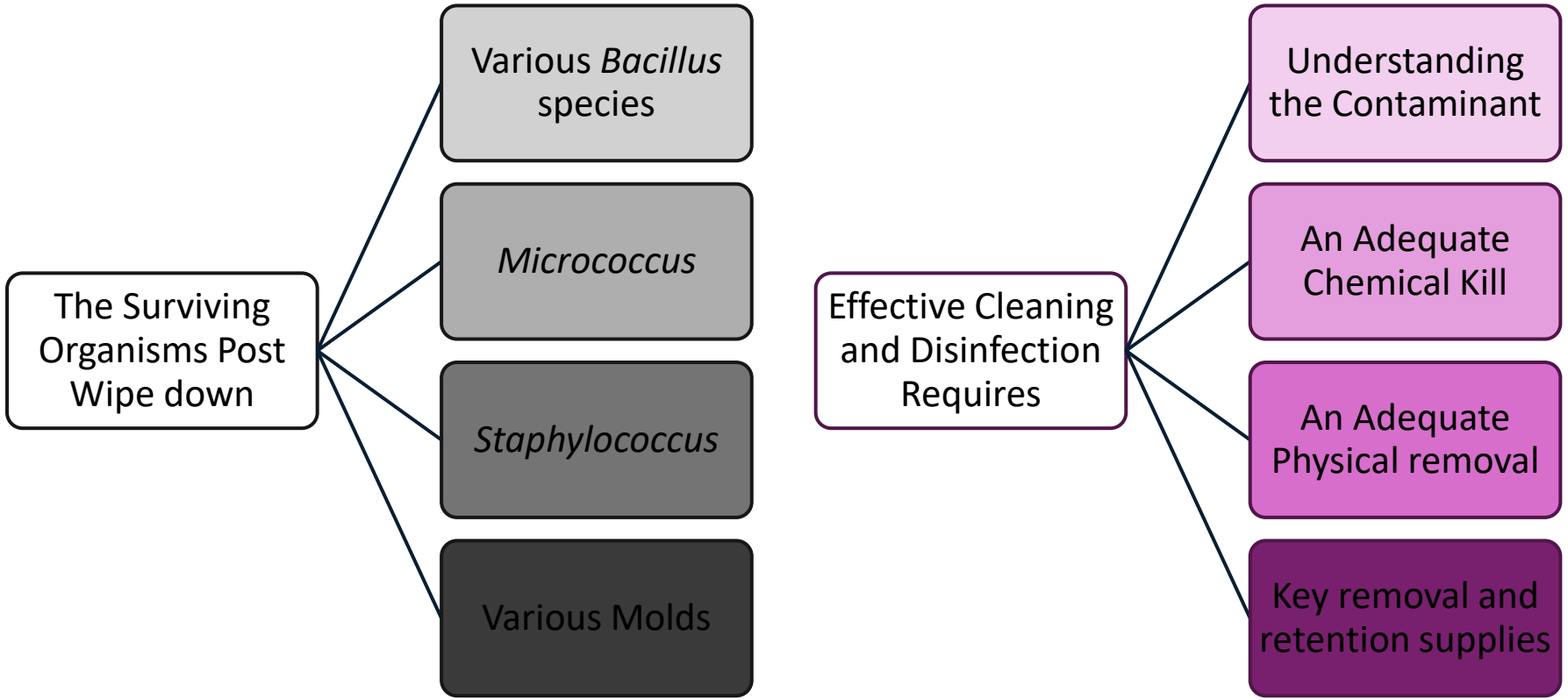


Cleaning and airflow connection

CCS Blindspots
Material Transfer

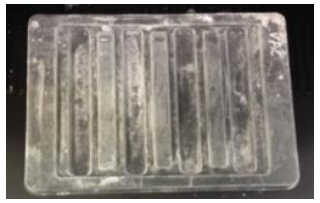
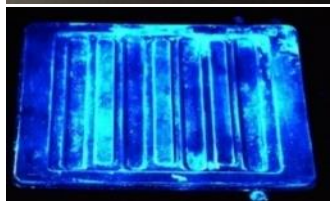
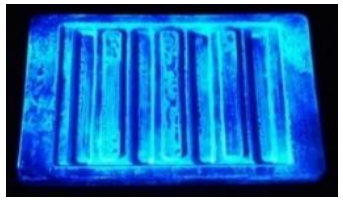
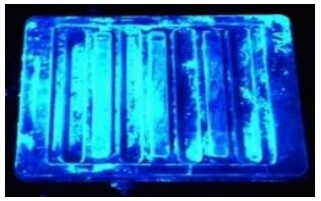
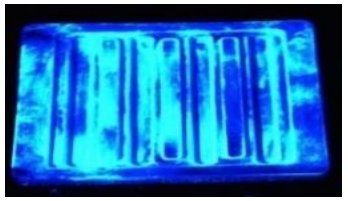
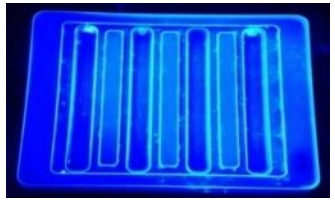
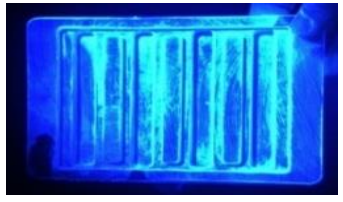
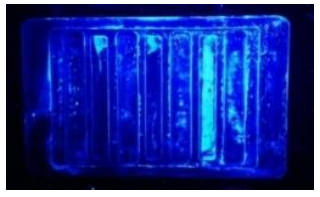
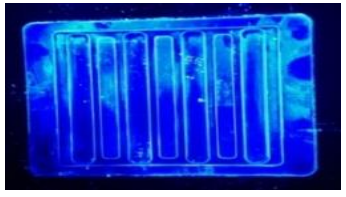
Tote cleaning

Case Study: Organisms recovered after wipedown with PAA Chemistry



Under Fluorescent & UV Light

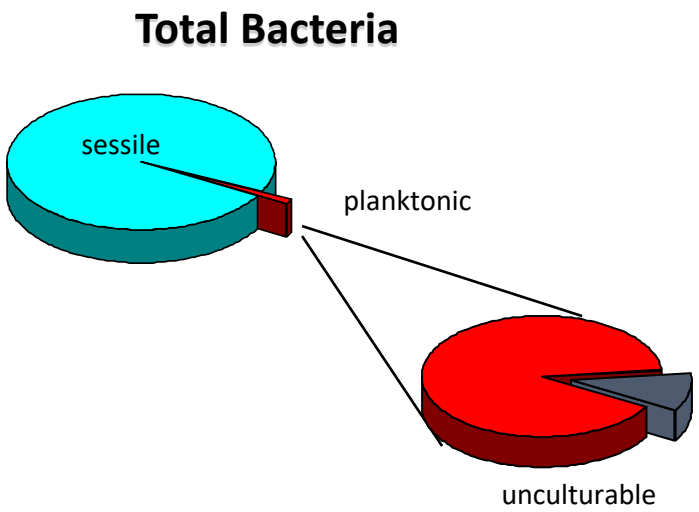
Best Results

	Aqueous Cleaner	Wiper / DI-IPA	Vacuum Wand	CDA Blow-off
Fluorescent Lighting **Before Cleaning**				
Fluorescent Lighting **After Cleaning**				
UV Lighting **Before Cleaning**				
UV Lighting **After Cleaning**				

Note: Based on the surface roughness, the powder may leave more or less residue on the surface after cleaning. It's assumed that these surfaces had the same surface roughness

CCS Blindspots
Water Systems

Water Systems



- Sessile organisms (i.e., biofilms) are dominant form in a system
- Organisms recovered from a system may not grow in culture
- Result: grab samples may underestimate the true population of a system

White, Manual of Environmental Microbiology, 91-101 (1997)

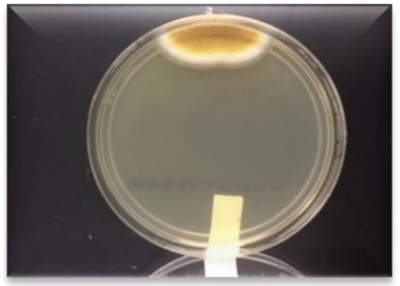
Mycobacterium chelonae is a rapidly growing opportunistic bacterium commonly found in a variety of water systems. As a nontuberculous mycobacterium (NTM), it exhibits resistance to many commonly used disinfectants. Additionally, this pleomorphic organism is capable of passing through certain filtration systems.

CCS Blindspots
Media and Microbial Identification

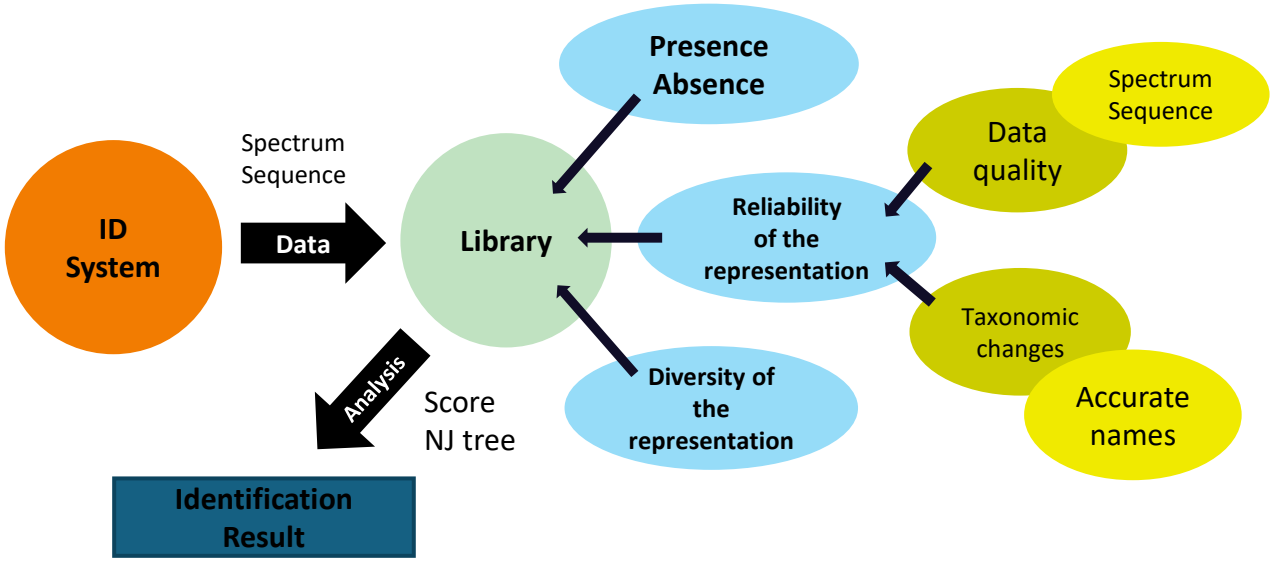
Microbial testing is dependent on the quality of media

Accuracy of viable monitoring results depend upon media quality and incubation. Common causes for erroneous results :

- Discoloration or hemolysis
- Storage location
- Integrity of packaging
- Broken or cracked petri dishes
- Quality and accuracy of labeling
- Condensation in petri dishes
- Retracted medium
- Dried and cracked media
- Sloped or uneven filling of petri dishes
- Contamination
- Gel strength
- Pitted surface or large bubbles
- Presence of leakage….



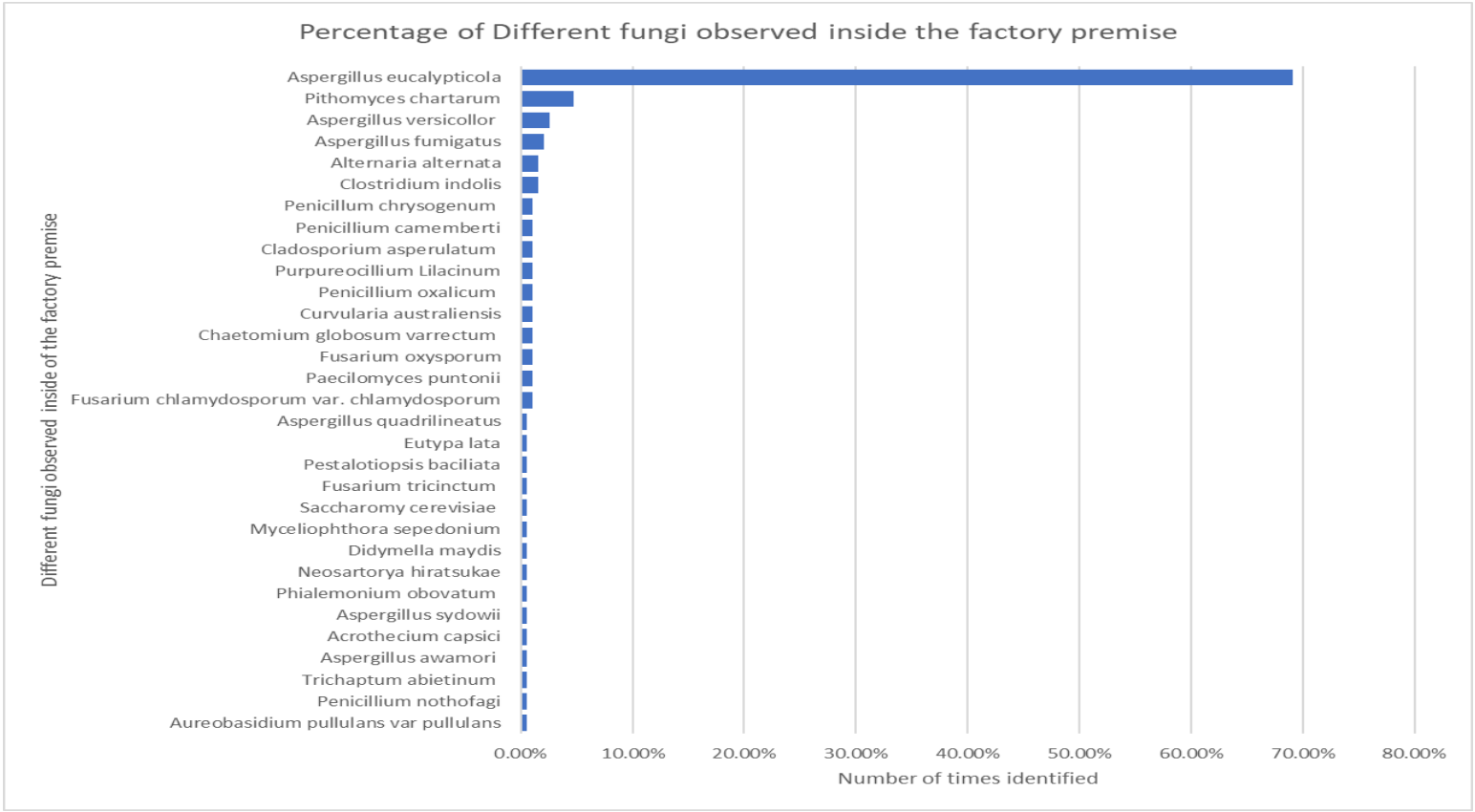
The Importance Of The Library



Quality System for Compliance

CCS Blindspots
Investigations and Trends

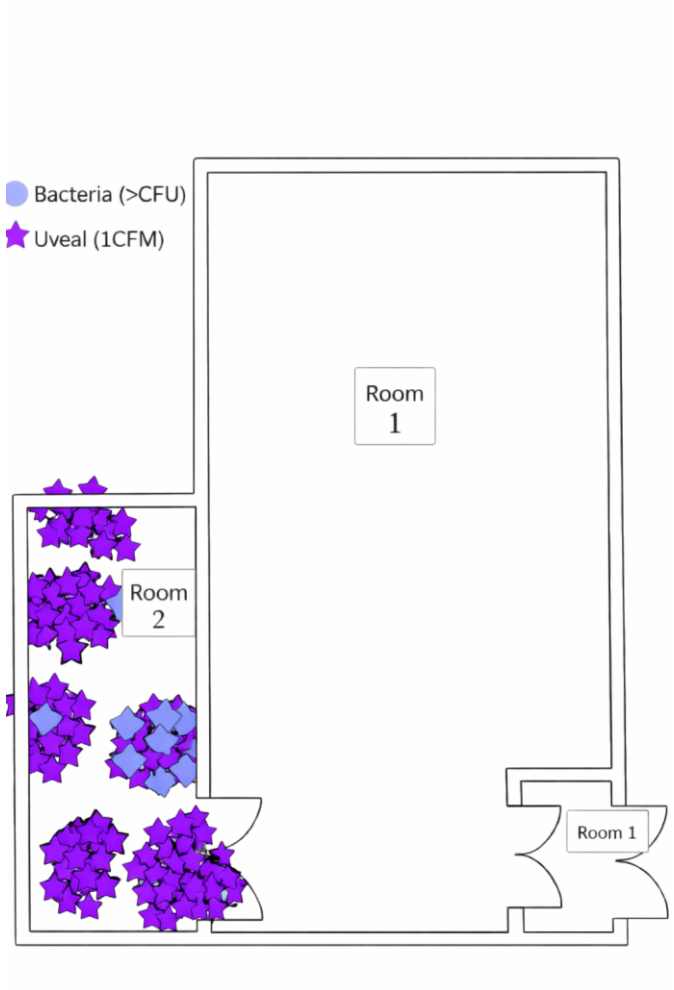
Without accurate microbial identification, investigations lack value



Data-Driven Source Identification

Aureobasidium melanogenum	**Dematiaceae / Deuteromycota**	**NA**	**NA**
Aureobasidium pullulans (Anamorph)	Dematiaceae / Deuteromycota	Discosphaerina fulvida (Teleomorph)	Asexual and sexual reproduction
Botrytis cinerea (Anamorph)	Dematiaceae / Deuteromycota	Botryotinia fuckeliana (Teleomorph)	Asexual and sexual reproduction
Chaetomium globosum	Sphaeriales/Ascomycota	NA	Sexual reproduction
Chaetomium undulatulum	Sphaeriales/Ascomycota	NA	Sexual reproduction
Cladosporium halotolerans	Dematiaceae / Deuteromycota	NA	Asexual reproduction
Cladosporium species	Dematiaceae / Deuteromycota	NA	Asexual reproduction
Cladosporium sphaerospermum	Dematiaceae / Deuteromycota	NA	Asexual reproduction
Cordyceps memorabilis (Teleomorph)	Ascomycota	Isaria farinosa (Anamorph)	Asexual and sexual reproduction
Didymella pomorum	Ascomycota	NA	Sexual reproduction

Data speaks, ID snitches!



Investigations

- Spraying, surface treatments, and fogging alone are often ineffective for proper remediation.
- While these approaches may create a temporary sense of control, mold frequently reappears because the underlying cause has not been addressed.
- Effective remediation begins with understanding the organism through proper analysis.
- This includes identifying probable sources based on findings, mapping pathways of transport, and ensuring that conditions do not allow further proliferation.
- Smoke studies using neutrally buoyant tracer particles are an important investigative tool, helping to visualize airflow patterns and identify hidden routes of contamination spread.

Reducing the CCS to "just a document" overlooks its true purpose and value.

The CCS is actively used to drive continuous improvement. It is not the end point; it is the starting point.

While it documents how we work, more importantly, it enables how we improve.

Thank you for your attention!

Slovenian Clearoom Society
Dimičeva street 13 I 1504 Ljubljana
T: +386 1 5898 312 I F: +386 1 5898 317 I E: ptz@gzs.si
https://scs.gzs.si

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

The Importance of Understanding your Microbiota for an Effective Contamination Control Strategy (CCS)

Duncan Barlow – Charles River Accugenix

30 – 31 MARCH 2026
BRDO PRI KRANJU, SLOVENIA

Contamination Control Strategy – EU GMP Annex 1

Contamination Control Strategy – Annex 1

2.5 The development of the CCS requires **detailed technical and process knowledge…**

…Elements to be considered within a CCS should include (but are not limited to):

xiii. **Cleaning and disinfection**

xiv. **Monitoring systems** - including an assessment of the feasibility of the introduction of **scientifically sound, alternative methods** that optimize the detection of environmental contamination.

xv. **Prevention mechanisms – trend analysis, detailed investigation, root cause determination corrective and preventive actions (CAPA)** and the need for comprehensive investigational tools.

xvi. **Continuous improvement** based on information derived from the above

Prevention: Trending

9.11 Monitoring procedures should define the approach to trending.

Trends should include, but are not limited to:

i. Increasing numbers of excursions from action limits or alert levels

ii. Consecutive excursions from alert levels.

iii. Regular but isolated excursion from action limits that may have a common cause, (e.g.. single excursions that always follow planned preventative maintenance.

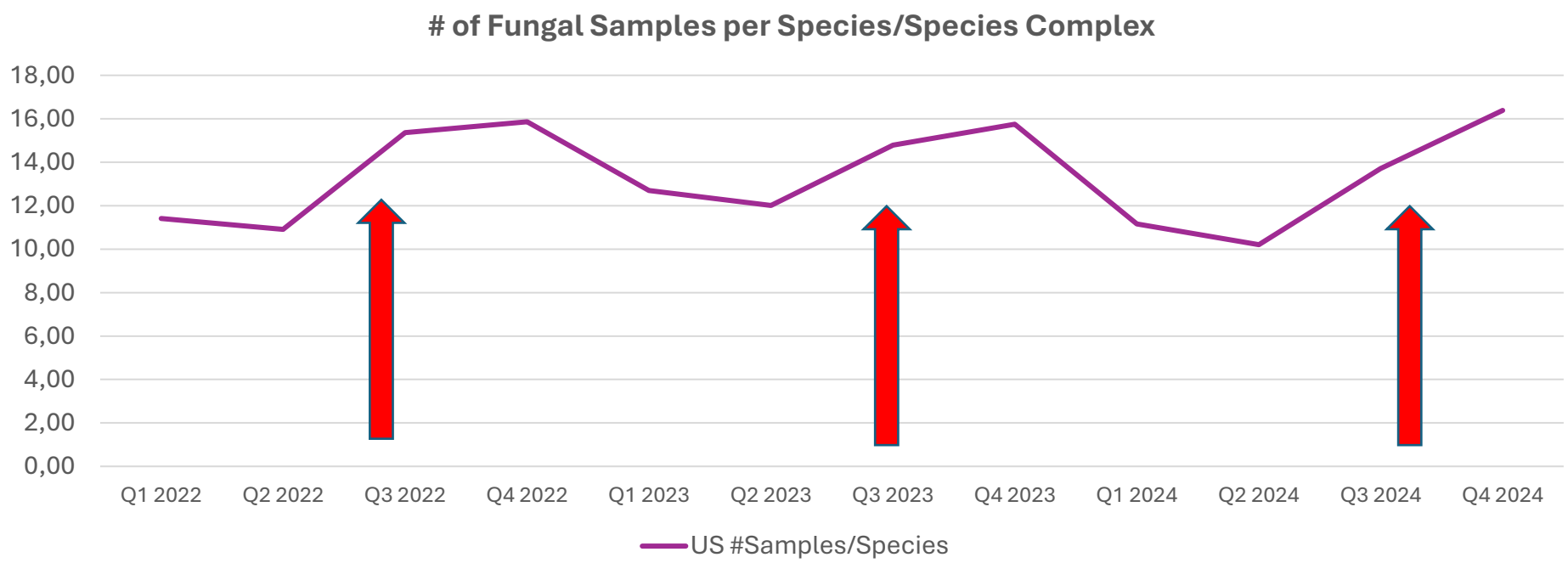
iv. **Changes in microbial flora type** and numbers and **predominance of specific organisms**. Particular attention should be given to organisms recovered that may be difficult to control such as **spore-forming microorganisms and moulds**

Prevention: Trending Example - Fungi

Oligotrophs

Spore formers

Metabolically diverse



Prevention: Investigations, Root Cause, CAPA

https://mhrainspectorate.blog.gov.uk/2018/03/02/out-of-specification-guidance/

PDA TR88: Microbial Data Deviation Investigations in the Pharmaceutical Industry

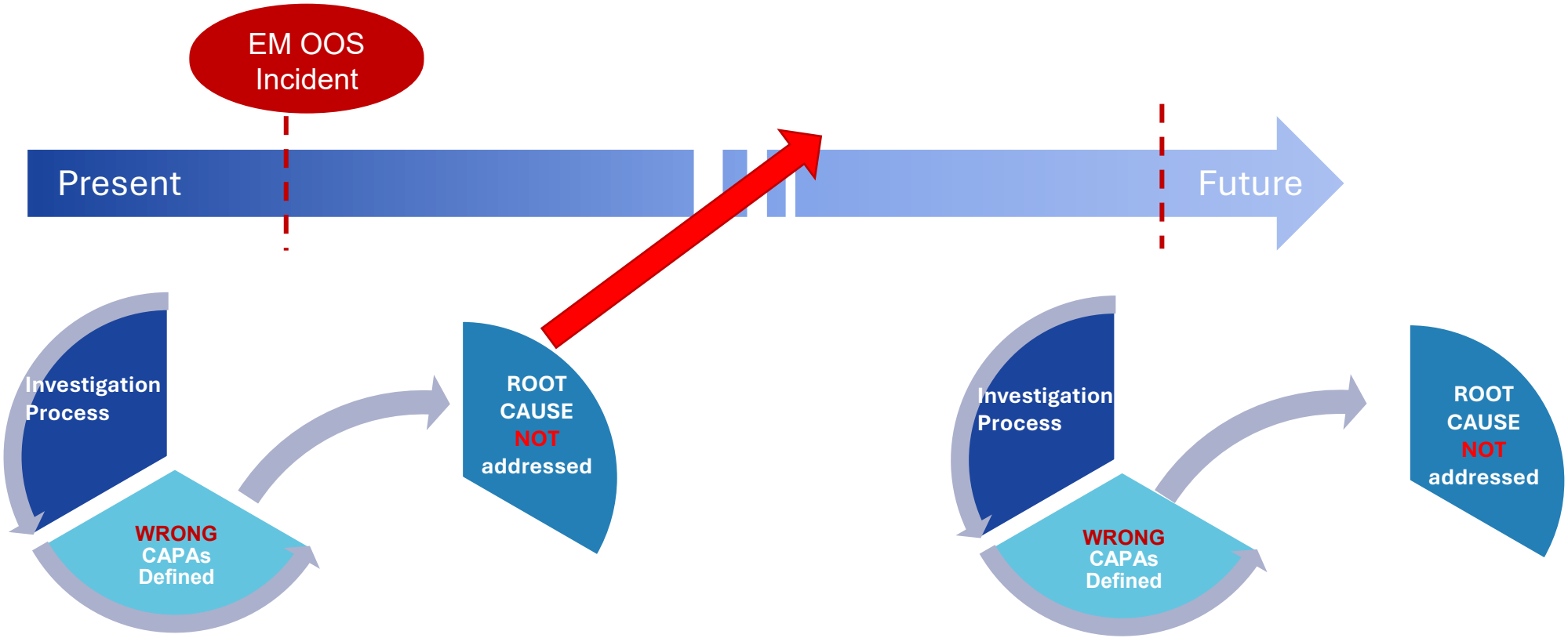
4.1 Microbial Identification

Identifying the microorganism(s) implicated in product failure is a critical step in a laboratory investigation and, subsequently, in the manufacturing investigation. **Knowledge of the source of the microorganism may lead to a possible route to determine which microorganism contaminated the product**

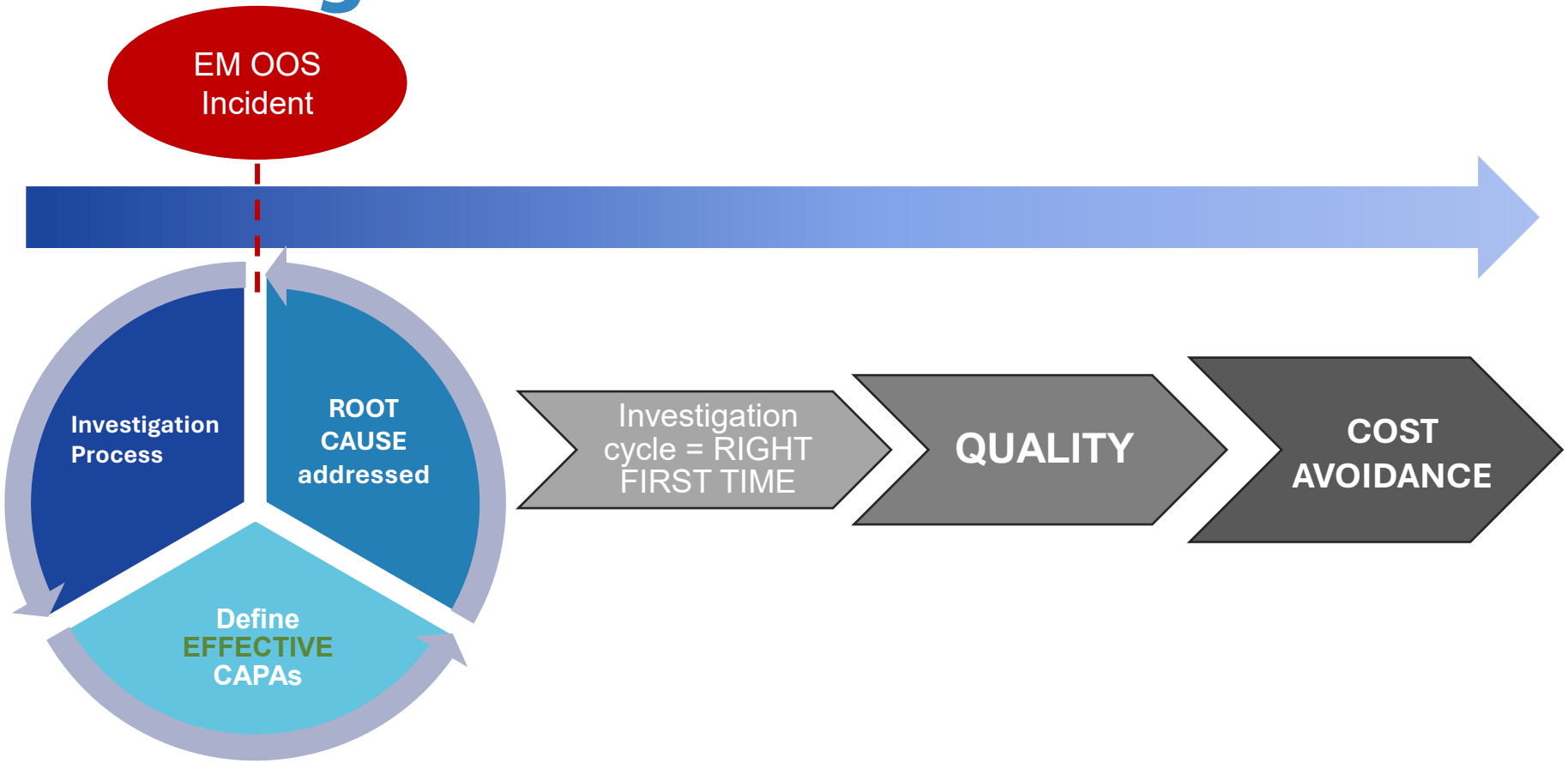
5.2.6.1 Historical Data Evaluation

Reviewing historical data for adverse trends can be helpful to the investigation. **Trending** will consider sample type, **organism type**, classified area, operational shift, or **seasonal pattern.**

Addressing Root Cause



Addressing Root Cause



The Importance of Understanding your Microbiota for an Effective Contamination Control Strategy (CCS)

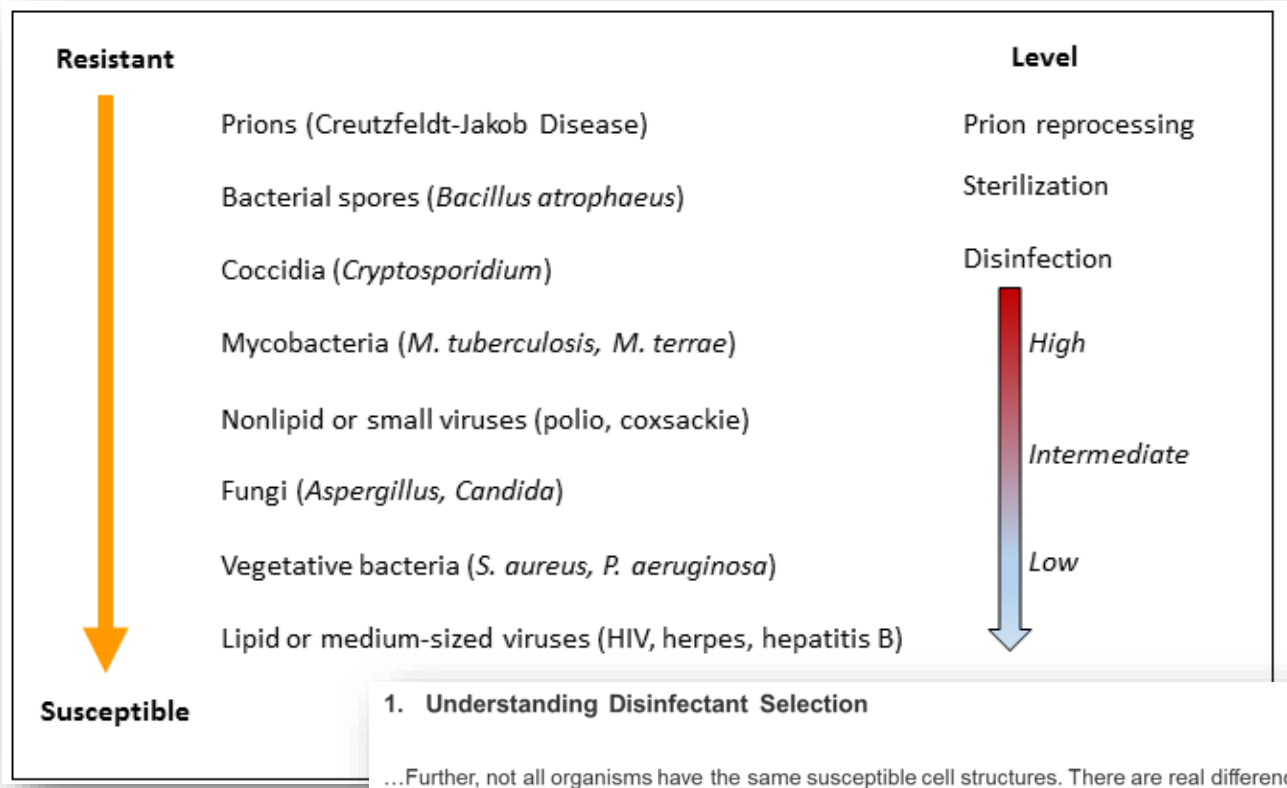
Duncan Barlow – Charles River Accugenix

Cleaning & Disinfection

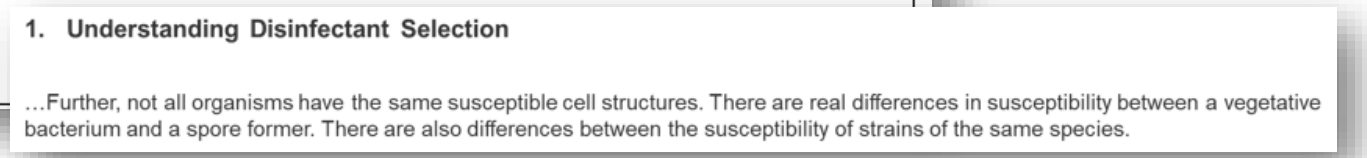
Disinfection

4.33 The disinfection of cleanrooms is particularly important. They should be cleaned and disinfected thoroughly in accordance with a written programme. For disinfection to be effective, prior cleaning to remove surface contamination should be performed. Cleaning programmes should effectively remove disinfectant residues. More than one type of disinfecting agent should be employed to ensure that where they have different modes of action and their combined usage is effective against bacteria and fungi. Disinfection should include the periodic use of a sporicidal agent. **Monitoring should be undertaken regularly in order to assess the effectiveness of the disinfection program and to detect changes in types of microbial flora (e.g.. organisms resistant to the disinfection regime currently in use).**

Resistance



https://www.cdc.gov/infection-control/hcp/disinfection-and-sterilization/resistance.html?CDC_AAref_Val=https://www.cdc.gov/infectioncontrol/guidelines/disinfection/tables/figure1.html



1. Understanding Disinfectant Selection

…Further, not all organisms have the same susceptible cell structures. There are real differences in susceptibility between a vegetative bacterium and a spore former. There are also differences between the susceptibility of strains of the same species.

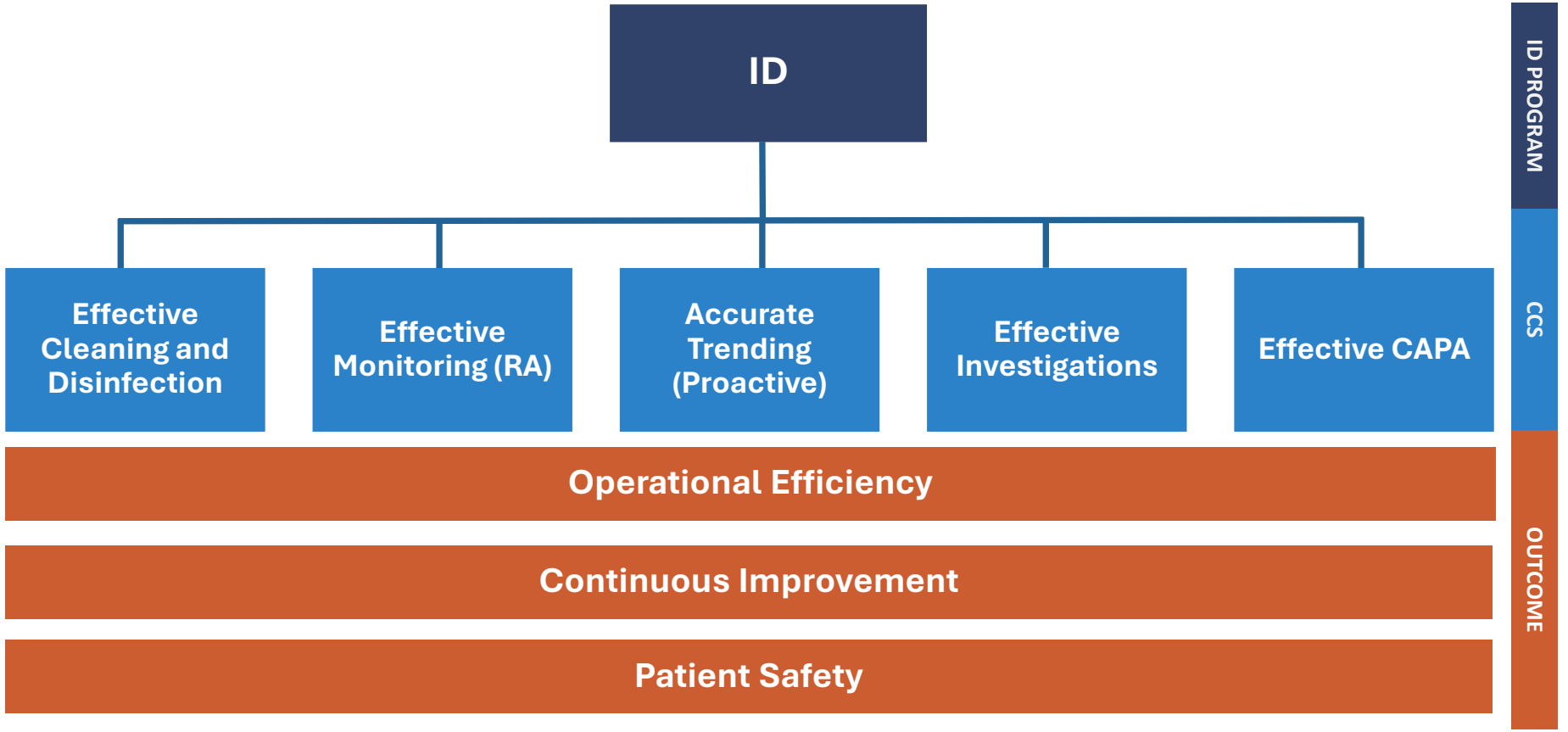
A3P La Vague 83: The Challenges of Floor Cleaning & Sanitization

Monitoring

Environmental and process monitoring
9.31
Microorganisms detected in a grade A or B areas should be identified to species level and the **potential impact of such microorganisms on product quality** (for each batch implicated) **and overall state of control should be evaluated.**

Consideration should also be given to the identification of microorganisms detected in Grade C and D areas (for example where action limits or alert levels are exceeded) or following the isolation of organisms that may be difficult to control such as spore-forming microorganisms and moulds and at a **sufficient frequency to maintain a current understanding of the typical flora of these areas.**

Importance of Microbial Identification



The Importance of Understanding your Microbiota for an Effective Contamination Control Strategy (CCS)

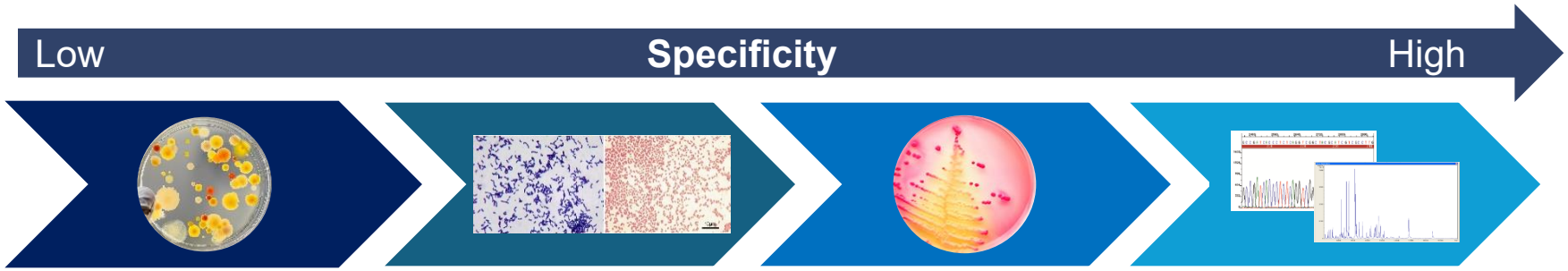
Duncan Barlow – Charles River Accugenix

Review of Microbial Identification Methods

The Importance of Understanding your Microbiota for an Effective Contamination Control Strategy (CCS)

Duncan Barlow – Charles River Accugenix

QC Microbiology: Identification Methods



Colony Morphology:
- Form
- Elevation
- Margin
- Size
- Colour

Cell Morphology & Staining:
- Rod/cocci/yeast
- Fungal structure
- Size & Aggregations
- Stains: Fungal, Gram, Spore

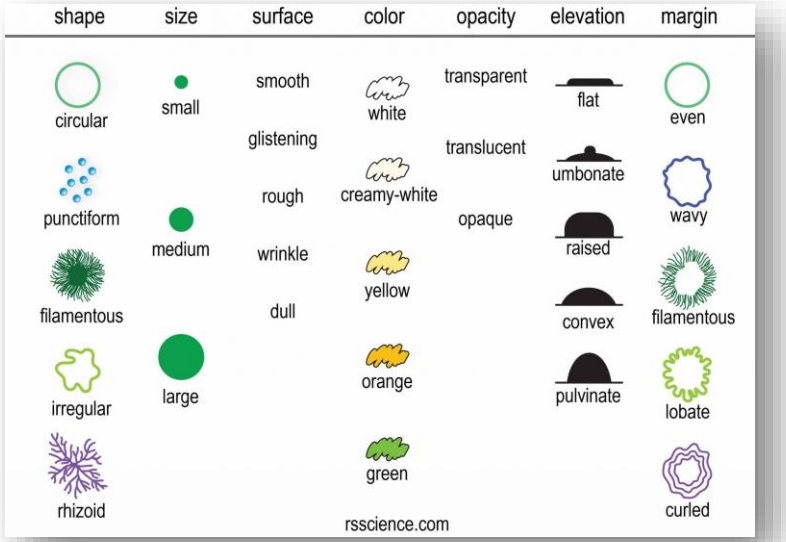
Selective Culture:
- Selective Media
- Incubation parameters

Genus/Species ID:
- Biochemical
- Proteotypic
- Genotypic

The Importance of Understanding your Microbiota for an Effective Contamination Control Strategy (CCS)

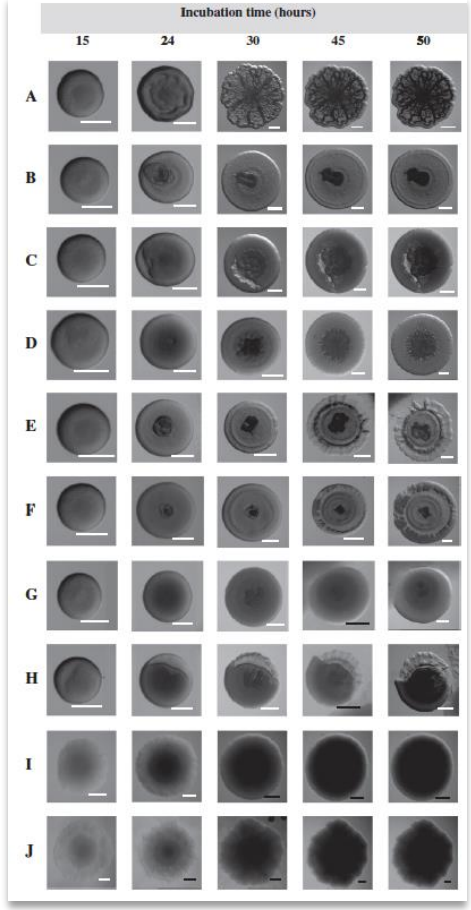
Duncan Barlow – Charles River Accugenix

Colony Morphology



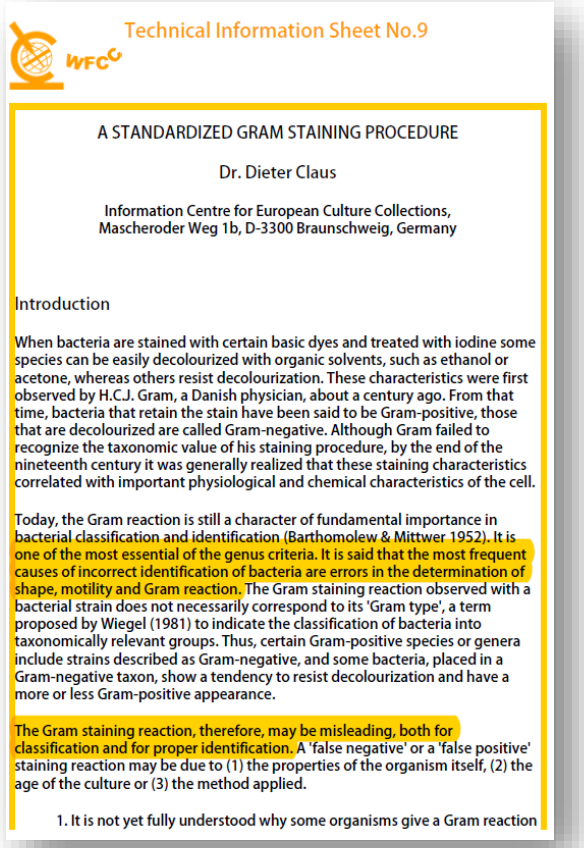
Comments	SEQ
Grey Raised	Mycobacterium llatzerense
Beige	Mycobacterium llatzerense
Beige	Mycobacterium llatzerense
Translucent Mucoid	Mycobacterium llatzerense
Red Flat	Mycobacterium llatzerense

Comments	SEQ
Beige Flat	Stenotrophomonas lactitubi
Beige Flat	Stenotrophomonas lactitubi
Beige Round Flat	Stenotrophomonas lactitubi
Peach Flat	Stenotrophomonas lactitubi
Translucent Mucoid	Stenotrophomonas lactitubi
Translucent Mucoid	Stenotrophomonas lactitubi
Translucent Mucoid	Stenotrophomonas lactitubi



Sousa AM, Machado I, Nicolau A, Pereira MO. Improvements on colony morphology identification towards bacterial profiling. J Microbiol Methods. 2013 Dec;95(3):327-35. doi: 10.1016/j.mimet.2013.09.020. Epub 2013 Oct 9. PMID: 24121049

Gram Stain – Case Study



Technical Information Sheet No.9

WFCC

A STANDARDIZED GRAM STAINING PROCEDURE

Dr. Dieter Claus

Information Centre for European Culture Collections,
Mascheroder Weg 1b, D-3300 Braunschweig, Germany

Introduction

When bacteria are stained with certain basic dyes and treated with iodine some species can be easily decolourized with organic solvents, such as ethanol or acetone, whereas others resist decolourization. These characteristics were first observed by H.C.J. Gram, a Danish physician, about a century ago. From that time, bacteria that retain the stain have been said to be Gram-positive, those that are decolourized are called Gram-negative. Although Gram failed to recognize the taxonomic value of his staining procedure, by the end of the nineteenth century it was generally realized that these staining characteristics correlated with important physiological and chemical characteristics of the cell.

Today, the Gram reaction is still a character of fundamental importance in bacterial classification and identification (Barthomolew & Mittwer 1952). It is one of the most essential of the genus criteria. It is said that the most frequent causes of incorrect identification of bacteria are errors in the determination of shape, motility and Gram reaction. The Gram staining reaction observed with a bacterial strain does not necessarily correspond to its 'Gram type', a term proposed by Wiegel (1981) to indicate the classification of bacteria into taxonomically relevant groups. Thus, certain Gram-positive species or genera include strains described as Gram-negative, and some bacteria, placed in a Gram-negative taxon, show a tendency to resist decolourization and have a more or less Gram-positive appearance.

The Gram staining reaction, therefore, may be misleading, both for classification and for proper identification. A 'false negative' or a 'false positive' staining reaction may be due to (1) the properties of the organism itself, (2) the age of the culture or (3) the method applied.

1. It is not yet fully understood why some organisms give a Gram reaction

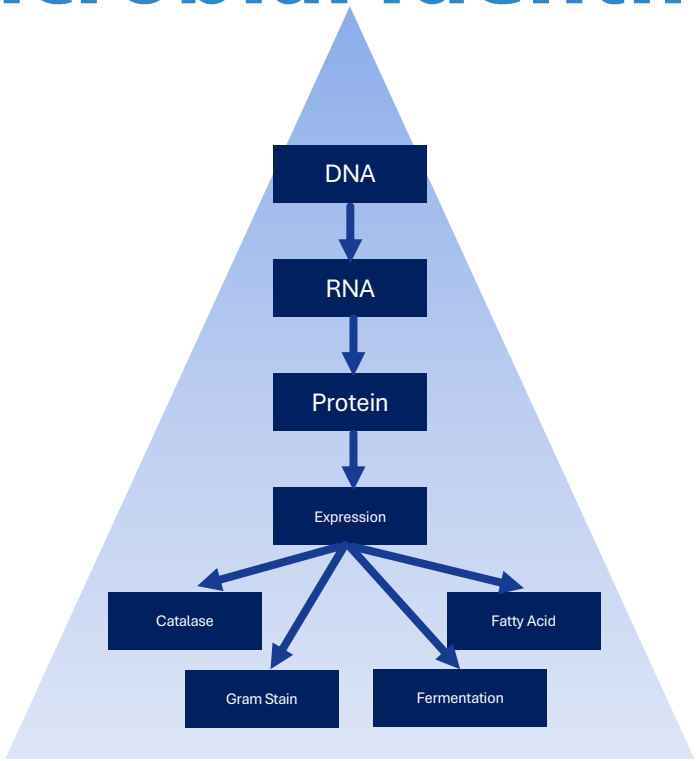
http://www.wfcc.info/tis/info9.pdf

Examples:

Genus	Total Samples	Gram Rxn	# Incorrect	% Incorrect
Pseudomonas	531	GN	136	**26**
Enterococcus	97	GP	3	**3**
Aerococcus	48	GP	0	**0**
Stenotrophomonas	38	GN	8	**21**
Bacillus	37	GP	3	**8**
Microbacterium	32	GP	12	**38**
Acinetobacter	29	GN	17	**59**
Mycobacterium	16	GP	6	**38**
Serratia	16	GN	3	**19**
Methylobacterium	15	GN	9	**60**
Psychrobacter	7	GN	6	**86**

Incorrect Gram Stains:
Customer study showed
331/1586 had wrong Gram stain
(21%)

Microbial Identification Methods



Genotypic
DNA sequencing of ribosomal RNA regions of bacteria and fungi

Proteotypic
MALDI-TOF mass spectrometry analysis of highly-abundant proteins such as ribosomal proteins

Phenotypic
Analysis of biochemical reactions, acid and salt tolerance, metabolism and fermentation, etc.

Accuracy and Reproducibility

Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic techniques. These methods are especially valuable for investigations into failures (e.g.., sterility test; media fill contamination).
FDA Guidance for Industry. Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice. (2004)

Duncan Barlow – Charles River Accugenix

Availability & Accuracy of Results

Technology	Library	Result
Genotypic Proteotypic Phenotypic	Presence/Absence Diversity Reliability	Available Accurate

The Importance of Understanding your Microbiota for an Effective Contamination Control Strategy (CCS)

Duncan Barlow – Charles River Accugenix

Importance of Libraries

USP <1113>

Introduction:

If a microorganism is not included in the database it will not be identified, so manufacturers should review the breadth of the database of the identification system they plan to use and its applicability to their needs.

EP 5.1.6

1-3 Identification Tests

Databases are part of the systems and are included in the primary validation. **As identification methods depend on the use of databases, the extent of coverage of the database with respect to the range of micro-organisms of interest** must be taken into account during validation

USP<1113> Microbial Characterization, Identification and Strain typing

The Importance of Understanding your Microbiota for an Effective Contamination Control Strategy (CCS)

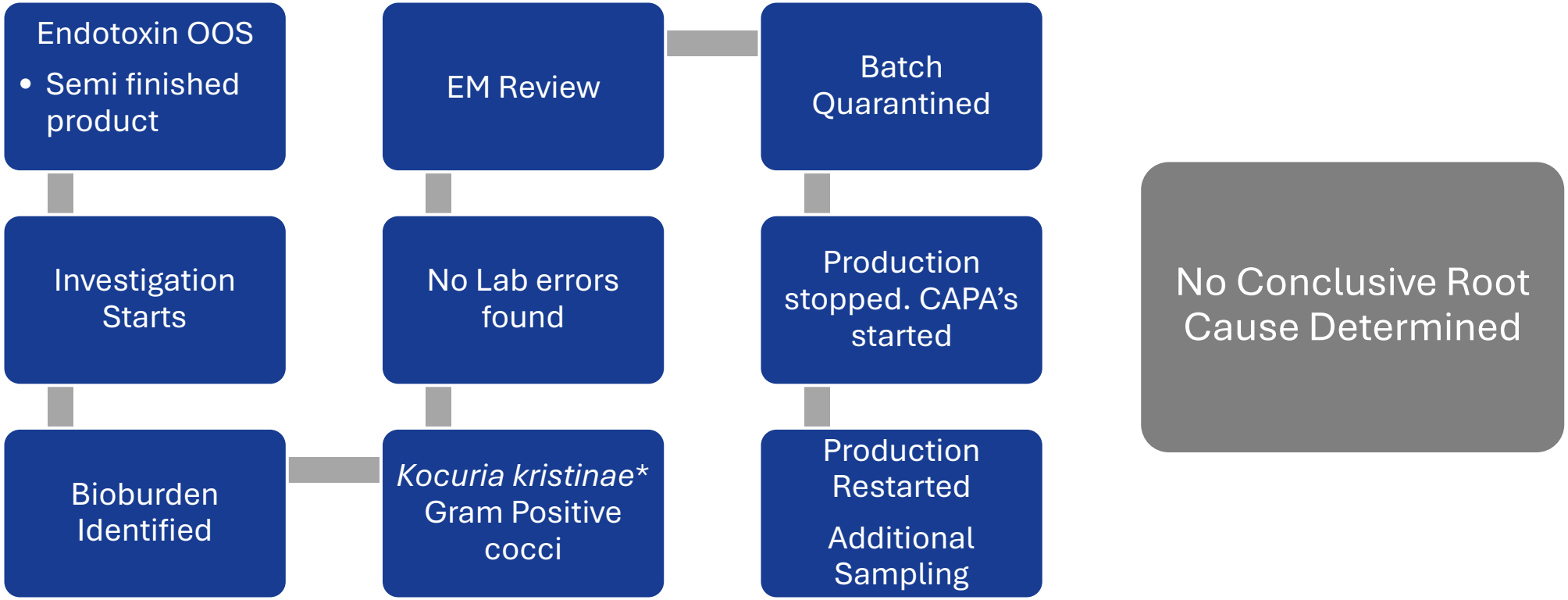
Duncan Barlow – Charles River Accugenix

Case Study

The Importance of Understanding your Microbiota for an Effective Contamination Control Strategy (CCS)

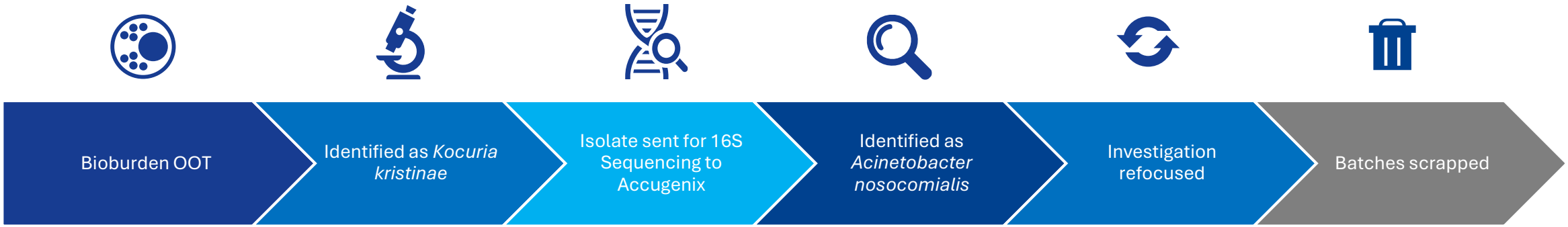
Duncan Barlow – Charles River Accugenix

Case Study



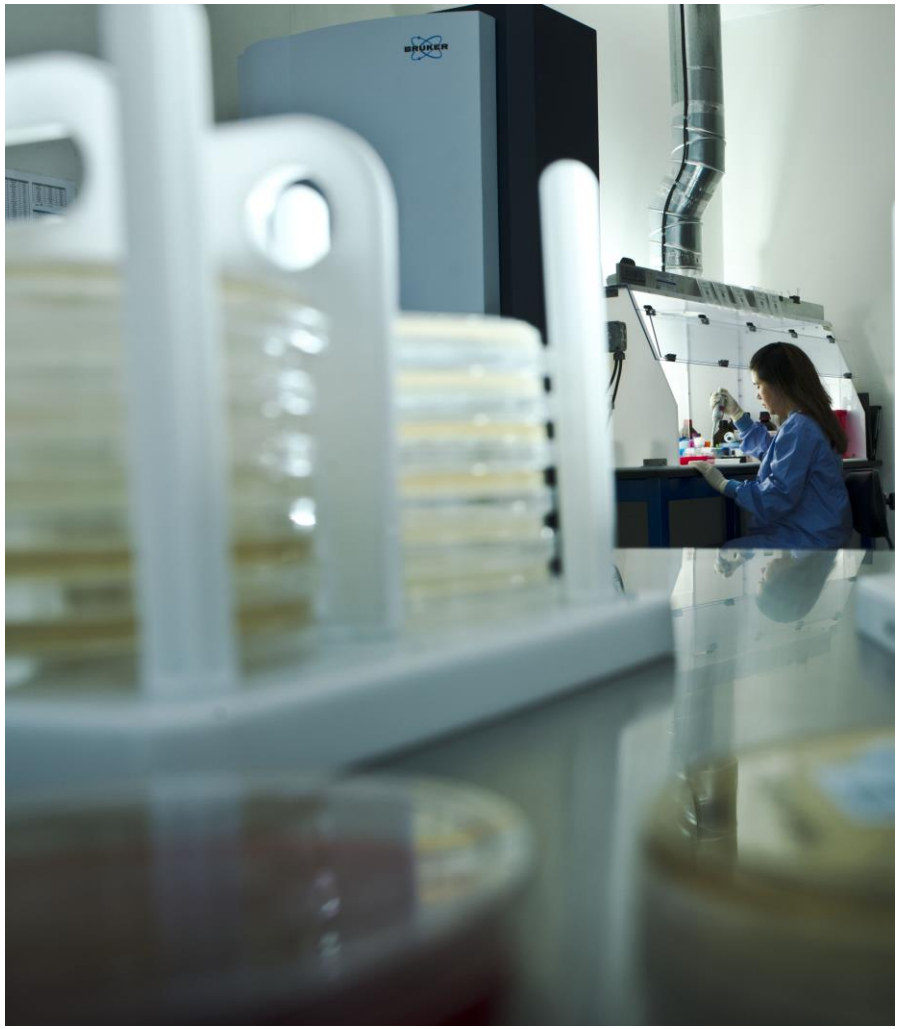
* *Kocuria kristinae* reclassified as *Rothia kristinae* as of 2018

Case Study – 2 Months Later



Cost Impact

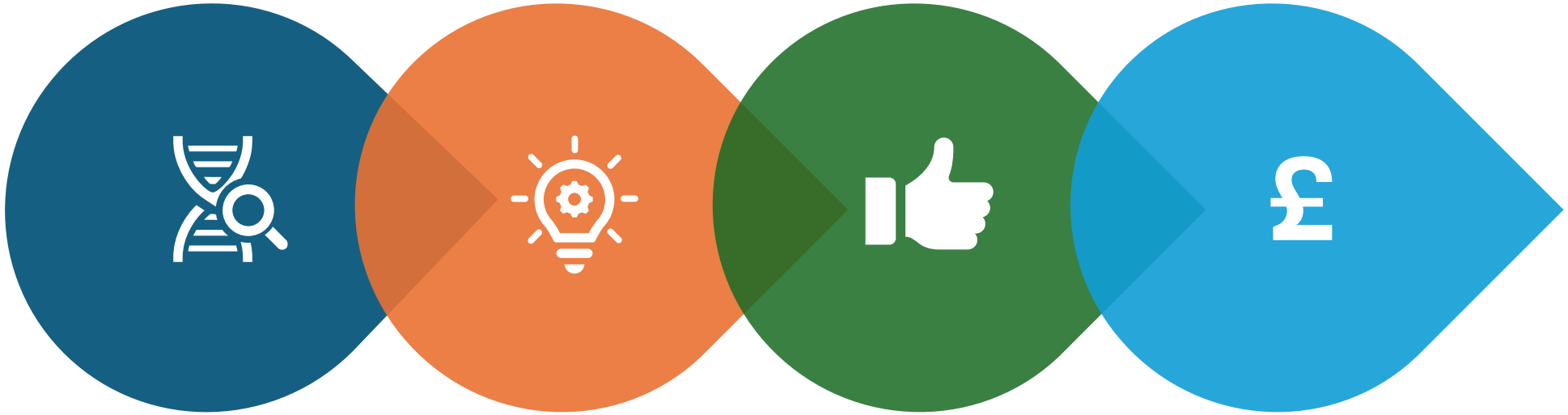
Summary



The Importance of Understanding your Microbiota for an Effective Contamination Control Strategy (CCS)

Duncan Barlow – Charles River Accugenix

Summary



ID Program

- Accurate technologies
- Comprehensive relevant Library

Knowledge

- Isolates
- Microbiology

Quality

- Investigations
- CAPA
- Trending
- Cleaning & Disinfection

Cost Avoidance

- Efficiencies
- Avoid hidden costs

Thank you for your attention!

Slovenian Clearoom Society
Dimičeva street 13 I 1504 Ljubljana
T: +386 1 5898 312 I F: +386 1 5898 317 I E: ptz@gzs.si
https://scs.gzs.si

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Round Panel Discussion in Contamination Control Strategies & Challenges

Moderator: Ziva Abraham, PhD, Founder and CEO, Microrite, Inc. USA

Govorci

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

30 – 31 MARCH 2026
BRDO PRI KRANJU, SLOVENIA

Bio – Conor Murray

Conor Murray, Principal Consultant
3dimension Cleanrooms
CQV Consultant, Cleanroom & Biosafety Designer, Energy Management

conor@3dimension.ie

ASHRAE®
ICS Irish Cleanroom Society

Conor Murray is a Process Engineer by profession, with 4 decades of experience in the design and construction of cleanrooms and biosafety labs; and since 2007 as an independent CQV Consultant. Conor represents Ireland as Head of Delegation for NSAI (national standards body) and is a SME in working groups on cleanroom standardisation in ISO/TC 209, since the early 2000s.
Conor is Chair of the Irish Cleanrooms Society (ICS), and a past Chair of the International Confederation of Contamination Control Societies, (ICCCS).
Conor is a past President of the ASHRAE Ireland chapter and currently Regional Vice Chair for the European Region on Technology Transfer, a member of the global EHC (Environmental Health Committee) and RAC (Research Administration Committee). He is a member of various TCs, including 2.4 Air Cleaning Devices, 2.9 Far UV-C and 9.11 Clean Spaces. Conor lectures internationally and gives cleanroom training and education courses on Annex 1 applications as well as ISO Cleanroom standards.

ISO Standards Are Key to Sustainability and a Circular Economy

Achieving a truly circular economy, where resources are kept in use for as long as possible, is paramount for sustainable development. ISO standards provide the essential framework, guiding organisations in optimising resource efficiency, minimising waste, and fostering environmental responsibility throughout their operations. They offer clear pathways for designing more sustainable products, processes, and systems – all aligned to the UN's 17 SDGs.

The 3 R's of a Circular economy are

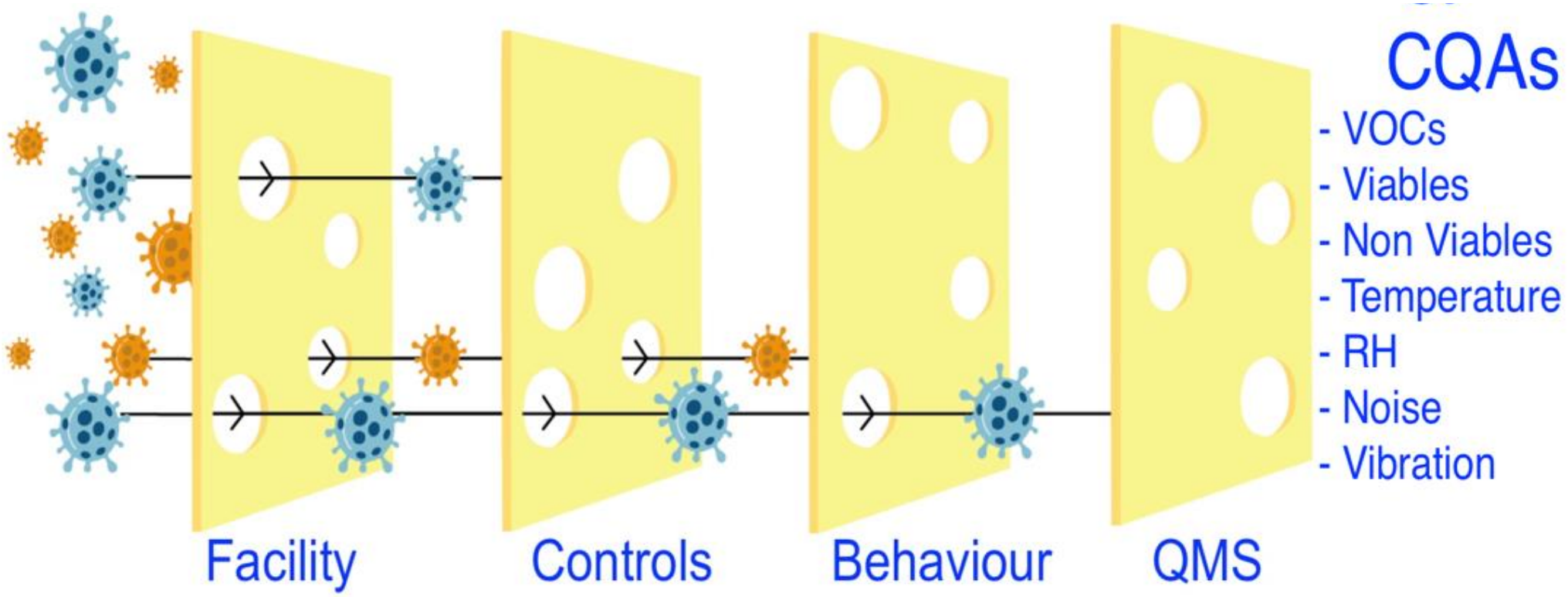
ReUse, RePurpose and ReCycle

The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

Cleanrooms "Swiss Cheese" Approach - Collected Effectiveness

Engineering + Operational Controls



The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

ISO Standards Are Key to a Circular Economy

Good Engineering Practice (GEP) design is fundamentally based on comprehensive knowledge of three critical elements: the manufacturing process itself, the risks (defined as IMPACT—the potential to cause harm), and the need and demand established through a robust Contamination Control Strategy (CCS).

Classification & Monitoring

ISO 14644-1 and -2 establish classification requirements and continuous monitoring protocols for Cleanrooms

Testing & Metrology

ISO 14644-3 defines testing methodologies and measurement techniques for Cleanroom performance verification

Design & Energy Efficiency

ISO 14644-4 and -16 provide frameworks for optimal design and energy management strategies

Operations & Associated

ISO 14644-5 addresses operational req'ts but refers to other standards in the family of ISO 14664 inc. particle deposition, separative devices, chemical control, & material suitability

The ISO 14644 family of standards covers the complete lifecycle of cleanroom facilities, from engineering controls (i.e. design) and operational controls, inc. specialised topics such as particle deposition (-17), separative devices like RABS & Isolators (-7), air and surface chemical contamination (-8 & -10), nanoparticles (-12), and materials and equipment suitability (ISO 14644-13, -14, -15, -18).

The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

Road to a Circular Economy – Leverage ISO 14644-4:2022

Table of Air Change Rates is GONE!

The 2022 revision of ISO 14644-4 represents a paradigm shift in cleanroom design methodology. The prescriptive table of air change rates has been eliminated, replaced by performance-based metrics that enable engineers to optimize facility design based on actual contamination removal requirements rather than arbitrary ventilation rates.

1

Air Change Effectiveness (ACE)

Quantifies how efficiently supply air reaches critical zones & removes contaminants based on local air volume flow rates

2

Contamination Removal Effectiveness (CRE)

Measures actual particle removal performance at critical control points using airborne particle count data

This approach aligns cleanroom HVAC design with the fundamental principles of risk management and contamination control, enabling facilities to achieve superior performance with reduced energy consumption. The shift from prescriptive to performance-based standards represents a significant advancement toward sustainable cleanroom operations.

The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

Air Change Effectiveness (ACE)

Air Change Effectiveness (ACE) is a dimensionless index that quantifies the efficiency of air distribution within a cleanroom. Unlike traditional air change rates that simply measure volumetric flow, ACE evaluates how effectively supply air reaches critical areas and facilitates contaminant removal.

01

Measure Local Air Velocity
Conduct detailed velocity measurements at critical control points throughout the cleanroom

02

Calculate Volume Flow Rates
Determine local air volume flow rates based on measured velocities and cross-sectional areas

03

Determine ACE Index

Compare local air change rates to nominal room air change rates to establish effectiveness

04

Optimize Distribution

Adjust supply and exhaust locations to maximize ACE at critical process zones

Air Change Effectiveness (ACE)

Air Change Effectiveness (ACE) is a dimensionless index that quantifies the efficiency of air distribution within a cleanroom. Unlike traditional air change rates that simply measure volumetric flow, ACE evaluates how effectively supply air reaches critical areas and facilitates contaminant removal.

ACE values greater than 1.0 indicate superior air distribution efficiency compared to perfect mixing, while values below 1.0 suggest areas of poor air circulation that may require design modifications. This metric enables engineers to optimize airflow patterns for both contamination control and energy efficiency.

Contamination Removal Effectiveness (CRE)

Contamination Removal Effectiveness (CRE) provides direct measurement of cleanroom performance by quantifying how efficiently particulate contamination is removed from critical areas. This performance-based metric uses actual airborne particle count data collected at strategic locations within the cleanroom environment.

CRE Measurement Protocol

Interpreting CRE Data

Contamination Removal Effectiveness (CRE)

CRE Measurement Protocol

CRE is determined by introducing a controlled particle challenge and measuring the time required to achieve specified particle count reductions at critical control points. The recovery curve provides insight into contamination removal dynamics and reveals potential dead zones or areas of poor air circulation.

Measurements are typically conducted using calibrated optical particle counters positioned at locations representing worst-case scenarios for contamination accumulation, such as near personnel activity zones or equipment that generates particles.

Interpreting CRE Data

CRE indexes enable direct comparison between different locations within a facility or between facilities. Values are calculated by comparing local particle decay rates to theoretical ideal mixing conditions.

Higher CRE values indicate more effective contamination removal, while lower values identify areas requiring airflow optimization or process modifications. This data-driven approach supports science-based decision making for HVAC system tuning and contamination control strategy refinement.

The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

Road to a Circular Economy – Add ISO 14644-16

"**Design your cleanroom with energy efficiency in mind, then use your collected performance data to 'tune' cleanroom HVAC to the real need — science-based INTELLIGENT decision making!**"

Adopt New Technology

Implement advanced monitoring and measurement (ARMM) technologies aligned with ISO 14644 standards for comprehensive contamination control strategies

Apply QRM Principles

Energy optimization emerges as a natural consequence of quality risk management when you thoroughly understand process requirements, contamination risks, and actual impact

Optimize Performance

Leverage ISO 14644-16:2019 Energy Management in Cleanrooms to systematically reduce energy consumption while maintaining product quality

The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

Road to a Circular Economy – Add ISO 14644-16

"**Design your cleanroom with energy efficiency in mind, then use your collected performance data to 'tune' cleanroom HVAC to the real need — science-based INTELLIGENT decision making!**"

- The integration of ISO 14644-16 with existing cleanroom standards creates a comprehensive framework for sustainable facility operations.
- By collecting performance data during commissioning and operational phases, facilities can make informed decisions about HVAC setpoints, airflow rates, and control strategies that balance energy efficiency with contamination control requirements.

Link to Annex 1: Best Practice GMP CCS & ISO Standards Design

Cleanroom Standards Design & Energy Management Best Practice

SMART Design and Digital Tools

Leverage Building Information Modeling (BIM), Virtual Reality (VR), Augmented Reality (AR), and Digital Twin technologies to optimize design decisions before construction begins and throughout the facility lifecycle

Modular and Offsite Approaches

Embrace modular construction and offsite fabrication to reduce installation time, improve quality control, and minimize on-site disruption during construction or upgrades

Early Engagement Strategy

Assemble experienced cross-functional teams early in the project lifecycle and maintain frequent communication throughout design, construction, and commissioning phases

Link to Annex 1: Best Practice GMP CCS & ISO Standards Design

Cleanroom Standards Design & Energy Management Best Practice

Detailed Planning with Change Control

Plan to the lowest achievable detail level while implementing robust change control processes to manage inevitable modifications without compromising project timelines or quality

Continuous Learning and Adaptive Control

Capture operational experience and implement adaptive control strategies based on actual need and demand, incorporating real-time detection capabilities using Bag-in-Filter Probe Counters (BFPC) as part of Pharma 5.0 evolution

Best Practice GMP CCS & ISO Standards Design

Optimising Cleanroom Footprint and Classification

Minimise Controlled Volumes

Keep cleanroom and clean zone areas as small as operationally feasible to reduce HVAC load, energy consumption, and maintenance requirements while maintaining product protection

Strategic Use of Separative Devices

Deploy isolators, Restricted Access Barrier Systems (RABS), mini-environments, and other separative devices with lower-grade classified areas as background environments to focus contamination control where it matters most

Right-Size Classification

Avoid over-designing by applying the appropriate ISO classification for each specific process step, eliminating unnecessary higher classifications that drive excessive energy consumption

Optimize Airflow Velocity

For unidirectional airflow (UDAF) applications, consider reducing air velocity below 0.35 m/s where appropriate, noting regulatory limitations in life sciences for ISO Class 5 areas

The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

Best Practice GMP CCS & ISO Standards Design

Optimising Cleanroom Footprint and Classification

- These design principles reduce both capital expenditure (CAPEX) and operational expenditure (OPEX) while maintaining or improving contamination control effectiveness.
- The strategic deployment of separative devices enables facilities to minimize the footprint of high-classification areas, concentrating resources where product exposure risk is greatest.

Best Practice GMP CCS & ISO Standards Design

System Optimization and Control Strategies

Turbulent Flow Optimization

Reduce air volume flow rates in turbulent flow cleanrooms by optimizing contamination removal effectiveness. Enhance perimeter airtightness to minimize pressure loss and eliminate wasted air through leakage

Extraction System Design

Accurately account for all extraction requirements including equipment exhaust, process ventilation, and room exhaust to prevent over-pressurization or under-extraction conditions

Personnel Training Focus

Invest in comprehensive personnel training programs emphasizing competence development and proper aseptic behavior, recognizing that human factors are often the primary contamination source

The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

Best Practice GMP CCS & ISO Standards Design

System Optimization and Control Strategies

Component Selection

Select equipment and design system components to operate at optimal efficiency points, incorporating features such as heat recovery, variable speed drives, and demand-based control

BMS/EMS Design

Implement sophisticated Building Management Systems (BMS) and Energy Management Systems (EMS) with appropriate control algorithms, setpoint schedules, and alarm management capabilities

The importance and role of ISO (14644) and CEN (EN 17141) standards in the application of GMP Annex 1

Conor Murray, 3dimension Cleanrooms

GMP CCS Optimization Guidance for Existing Cleanrooms

Aligning Contamination Control Strategy with Engineering Controls

For existing facilities, optimization opportunities exist to align the Contamination Control Strategy (CCS) required by Annex 1 with the engineered HVAC systems that provide physical contamination control. This alignment process requires systematic evaluation of current performance and strategic modifications to improve effectiveness.

Align CCS with HVAC

Tune Airflow Patterns

Apply ISO Best Practices

GMP CCS Optimization Guidance for Existing Cleanrooms

Aligning Contamination Control Strategy with Engineering Controls

Align CCS with HVAC

Systematically align the Contamination Control Strategy defined in Annex 1 with the Engineering Controls implemented in the cleanroom HVAC system

Tune Airflow Patterns

Adjust and fine-tune airflow patterns around process equipment and personnel workflows to optimize contamination removal effectiveness at critical control points

Apply ISO Best Practices

Develop recommendations based on ISO 14644-4 (Design) and ISO 14644-16 (Energy Management) as well as ISO 14644-5 Operations, applicable to both existing facilities and new construction projects

GMP CCS Optimization Guidance for Existing Cleanrooms

Aligning Contamination Control Strategy with Engineering Controls

- This optimization process leverages existing infrastructure while implementing strategic modifications that enhance both contamination control effectiveness and energy efficiency.
- The approach is particularly valuable for legacy facilities that were designed using outdated air change rate tables rather than performance-based metrics.

GMP CCS Optimisation Guidance for Existing Cleanrooms

Performance-Based Tuning Methodology

1 Focus on Effectiveness Metrics

Prioritize Contamination Removal Effectiveness (CRE) and Air Change Effectiveness (ACE) as the primary metrics for assessing and optimizing airflow patterns

2 Deploy Real-Time Monitoring

Utilize airflow visualisation techniques and real-time measurements of total airborne particle counts and viable organisms (CFU) to understand current

3 Measure Before and After

Establish baseline ACE and CRE measurements at critical control points, then re-measure after implementing HVAC modifications to quantify improvements

4 Evaluate and Optimise

Analyze results to systematically optimise airflow patterns, contamination removal effectiveness, energy efficiency, and overall operational costs

Poor CCS Design Leads to Trust Issues with Quality

Common Mistakes During Project Execution

Poor project execution and handover processes create lasting trust deficits between engineering and quality/regulatory affairs functions. These issues often stem from inadequate planning and documentation during the design and construction phases.

Missing User Requirements

Absence of comprehensive User Requirements Specifications (URS) leads to unclear expectations regarding energy management and performance qualification, violating Annex 15

Inadequate Planning and Budgeting

Insufficient allocation of resources for design changes and refinements during construction creates pressure to accept suboptimal solutions that compromise

Incomplete Design Specifications

Too many design unknowns coupled with poor change control processes result in systems that don't meet operational needs or regulatory expectations

Poor CCS Design Leads to Trust Issues with Quality

Common Mistakes During Project Execution

Poor project execution and handover processes create lasting trust deficits between engineering and quality/regulatory affairs functions. These issues often stem from inadequate planning and documentation during the design and construction phases.

Poor Technical Coordination

Inadequate technical coordination and commissioning (Cx) planning for performance verification leads to systems that cannot demonstrate compliance with design intent

No Post-Commissioning Review

Failure to conduct design reviews after commissioning to establish final operating setpoints for startup leaves facilities operating at suboptimal conditions

Poor CCS Design Leads to Trust Issues with Quality

Handover and Documentation Failures

- **Insufficient Learning Period**

  No gap period after collecting commissioning performance data prevents learning and proper alignment of HVAC system requirements to actual operational needs of end users

- **No Business Resilience Demonstration**

  Failure to demonstrate business resilience during handover, including startup and shutdown procedures, alarm responses, and cause-and-effect relationships as designed

- **Poor Handover Documentation**

  Inadequate training, incomplete as-built documentation, and deficient Operations & Maintenance (O&M) manuals leave facility teams unprepared to operate and maintain systems effectively

- **Missing Digital Tools**

  Lack of Building Information Modeling (BIM), Augmented Reality (AR), and Digital Twin capabilities for ongoing maintenance and lifecycle sustainability management

Poor CCS Design Leads to Trust Issues with Quality

Handover and Documentation Failures

- Insufficient Learning Period
- No Business Resilience Demonstration
- Poor Handover Documentation
- Missing Digital Tools

- Handover deficiencies create operational challenges that persist throughout the facility lifecycle.
- Quality and regulatory affairs teams naturally develop skepticism about system capabilities when documentation is incomplete and performance has not been adequately demonstrated.
- Establishing trust requires comprehensive handover processes including thorough training, complete documentation, + demonstrated system resilience under various operating scenarios.

Annex 1 – The Future HVAC as part of a GMP CCS

- ISO cleanroom standards are leading the transformation toward energy-efficient, compliant pharmaceutical manufacturing facilities as integral components of the circular economy.
- The future of GMP cleanroom HVAC systems will be characterized by intelligent, adaptive control strategies that respond to actual contamination control needs rather than operating at constant maximum capacity.

Annex 1 – The Future HVAC as part of a GMP CCS

Optimization

ACE and CRE-based tuning

Demand-Based Control

Real-time contamination monitoring

Setback Modes

Reduced operation when not in production

Adaptive Control

Product quality need and demand responsive

Annex 1 – The Future HVAC as part of a GMP CCS

- This evolution represents a fundamental shift from static, prescriptive HVAC operation to dynamic, performance-based contamination control.
- Advanced monitoring technologies enable real-time assessment of contamination levels, allowing HVAC systems to adjust airflow rates and operating modes based on actual needs.
- Setback modes during non-production periods provide significant energy savings while maintaining appropriate environmental conditions.
- The integration of these strategies supports both sustainability objectives and regulatory compliance requirements outlined in Annex 1.

Thank you for your attention!

Slovenian Clearoom Society
Dimičeva street 13 I 1504 Ljubljana
T: +386 1 5898 312 I F: +386 1 5898 317 I E: ptz@gzs.si
https://scs.gzs.si

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

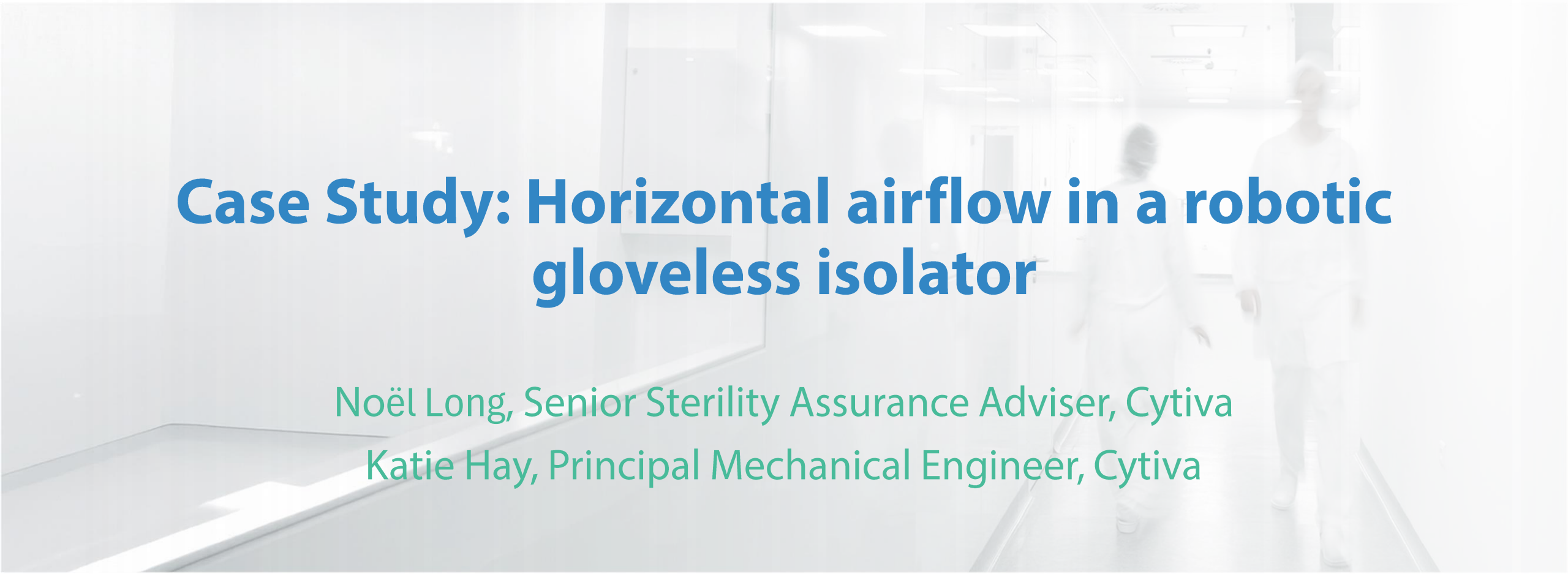
BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:
INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia



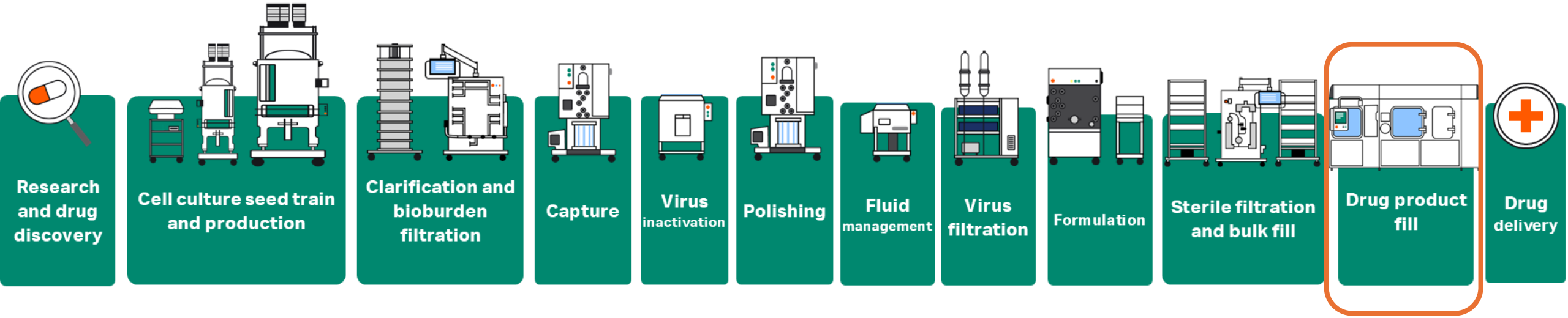
Case Study: Horizontal airflow in a robotic gloveless isolator

Noël Long, Senior Sterility Assurance Adviser, Cytiva
Katie Hay, Principal Mechanical Engineer, Cytiva

30 – 31 MARCH 2026
BRDO PRI KRANJU, SLOVENIA

Agenda

- Robotic gloveless isolators
- Horizontal airflow
  - Airflow visualization studies
  - Computational fluid dynamics
- Biofluorescent particle counters: baseline and interference studies



Microcell™ vial filler

Vial filling for personalized medicines and early-stage clinical trials (1.2K units per batch capacity)

SA25 aseptic filling workcell

Vial, syringe, and cartridge filling for commercial and clinical drug products (20K units per batch capacity)

Setup

- Install change tooling
- Install pre-sterilized flowpath, swab and bottle
- Calibrate scales
- Leak testing

VPHP decontamination

- Full system VPHP decontamination
- DSI loading of RTU components
- DSI VPHP decontamination

Operation

- Open fill needle and calibrate pump
- Material transfer from DSI to FI
- Filling and stoppering
- Material returned to DSI
- DSI carousel reload and re-decontaminate

Post-fill activities

- DSI unload
- Flowpath uninstalled
- Cleaning of non-product contact surfaces.
- Post-fill visual inspection

Personnel gowning, facility material transfer and cleaning are used for risk mitigation

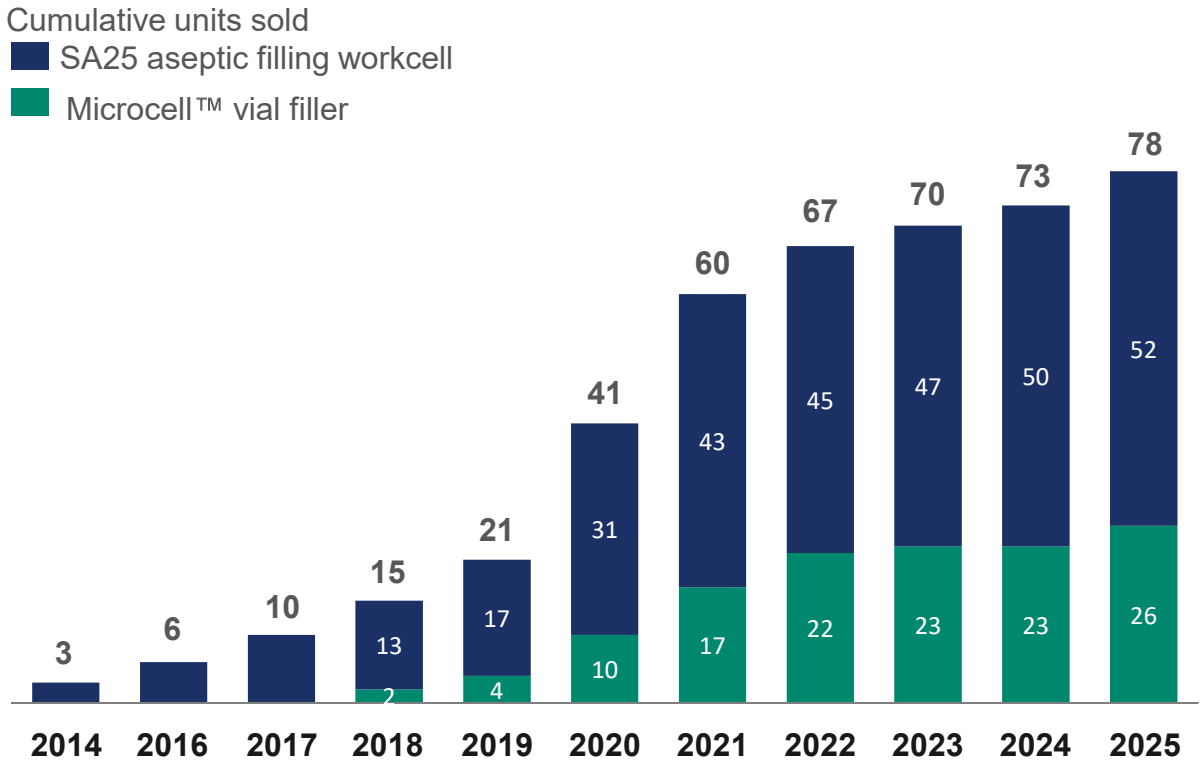
After leak testing and VPHP decontamination, the operator cannot access filling / stoppering activities.

Personnel gowning, facility material transfer and cleaning are used for risk mitigation

VPHP = vapor-phase hydrogen peroxide. DSI = decontamination and staging isolator.
RTU = ready to use. FI = fill isolator.

SA25 workcell: Removing interventions and indirect product contact parts

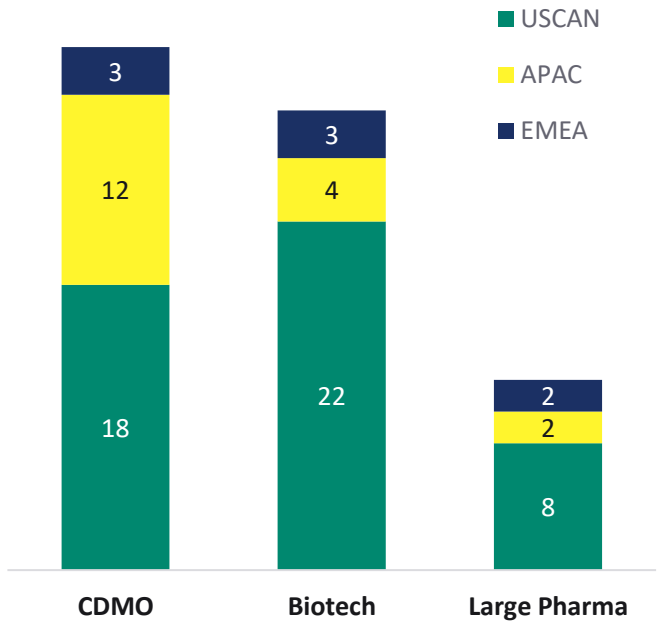
Our global user group



70+ workcells sold for GMP production across USCAN, EMEA, India, China, Taiwan, Korea, and Australia since inception.

Blue-chip customer base and presence across key segments.

More than 50 unique customers by type and region



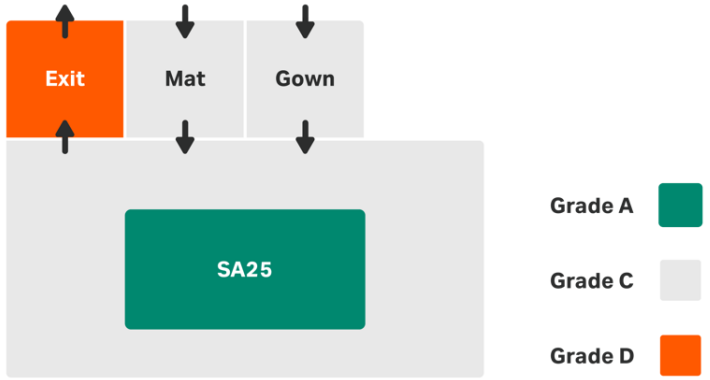
CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge — Chamber of Small Business and Trade

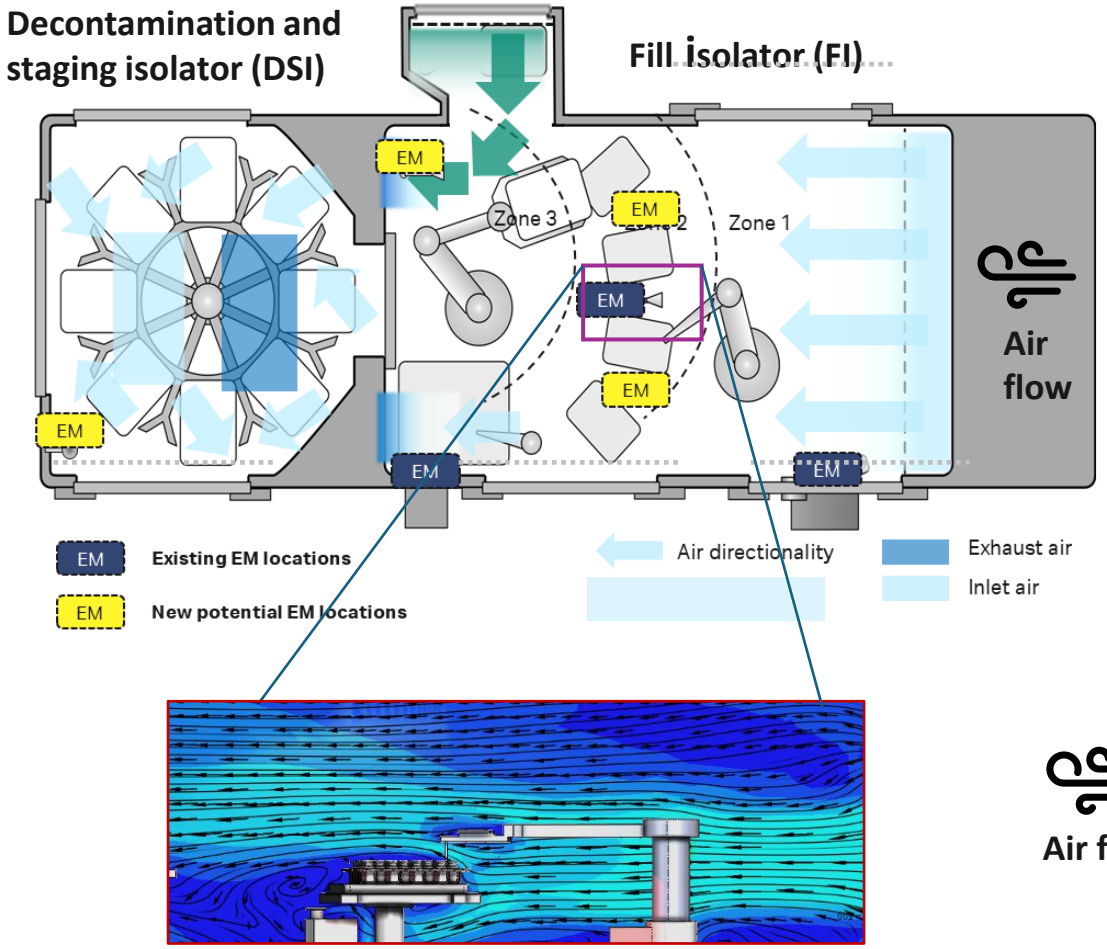
Gospodarska zbornica Slovenije — Chamber of Commerce and Industry of Slovenia

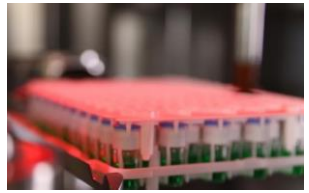


52% Less area vs conventional isolator

66% Less area vs restricted access barrier system (RABS)

Significant reduction in Grade A space (lower cost and carbon footprint)





Air flow

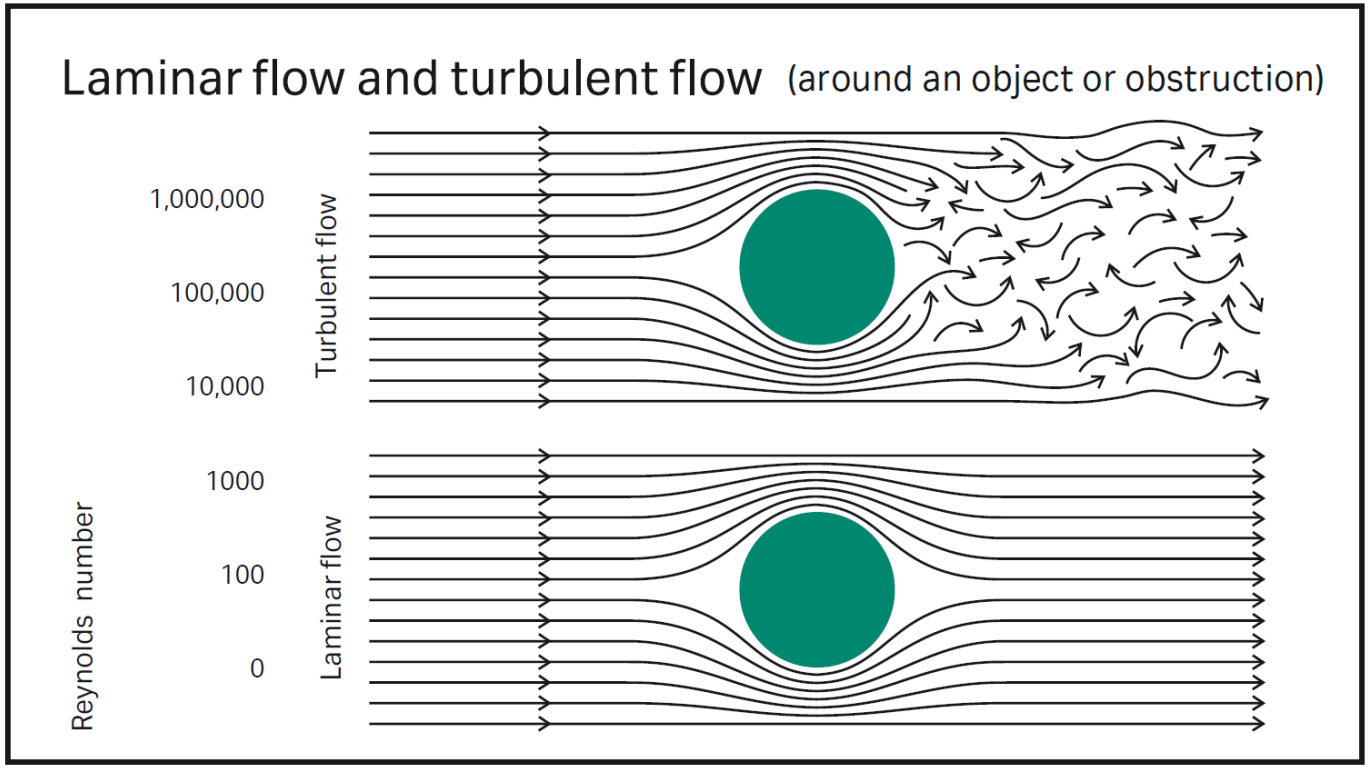
Side view CFD particle paths (filling operation)

CFD = computational fluid dynamics.

Design	Annex 1: Manufacture of sterile medicinal products
HEPA filtered HEPA = high-efficiency particulate air.	4.14 …filtered air supply that maintains a positive pressure
Differential pressure	4.14… Adjacent rooms of different grades should have an air pressure difference of a minimum of 10 Pascals (guidance value). 4.20 Airflow pattern studies should be performed at the interfaces of open isolators to demonstrate the absence of air ingress. 8.12 Where an isolator is used, the background should be in accordance with paragraph 4.20.
Air changes	4.14 …filtered air supply that …should flush the area effectively…
Air speed/velocity	4.30 …Unidirectional airflow systems should provide a homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance value) …
Air direction	4.4, 4.15, 4.19, 4.23; Unidirectional airflow – An airflow moving in a single direction, in a robust and uniform manner, and at sufficient speed, to reproducibly sweep particles away from the critical processing or testing area.
Temperature and humidity	9.6 Other characteristics, such as temperature and relative humidity, should be controlled within ranges that align with product/processing/personnel requirements and support maintenance of defined cleanliness standards (e.g. grade A or B).

EudraLex - Volume 4 - Good Manufacturing Practice (GMP) guidelines; Annex 1 – Manufacture of Sterile Medicinal Products. European Commission. August 22, 2022. Accessed March 19, 2026. https://www.gmp-compliance.org/files/guidemgr/20220825_gmp-an1_en_0.pdf

Airflow design for isolators – turbulence



Example calculation:

$$Re = \frac{V \cdot D \cdot \rho}{\mu} = \frac{0.2 \cdot 0.9 \cdot 1.204}{1.825 \times 10^{-5}} = 11{,}875$$

Adapted from: Turbulent Flow. Nuclear Power. https://www.nuclear-power.com/nuclear-engineering/fluid-dynamics/turbulent-flow/. Accessed March 19, 2026.

Airflow in isolators – velocity and turbulence

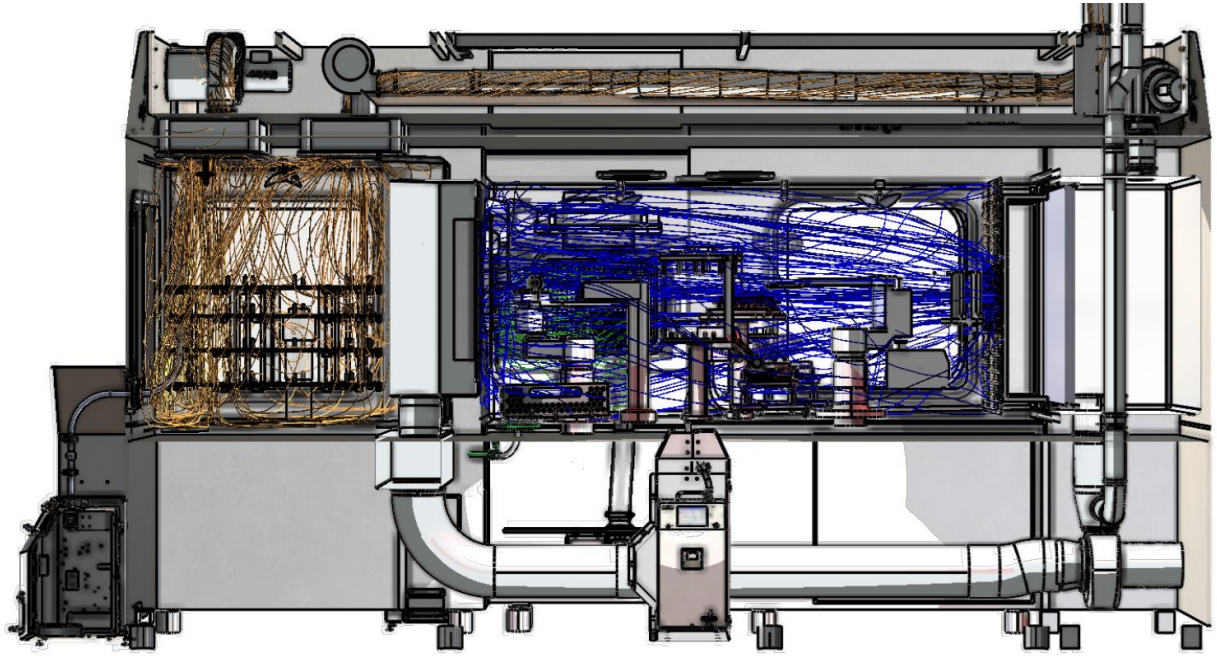
- Turbulence is often said to be the "**last unsolved problem in classical mathematical physics.**"
- The main tool available for turbulence analysis is CFD analysis.
- Velocity: slower flow will have less turbulence based on the lower Reynolds number.
- Isolator airflow speeds rarely lift settled particles off of surfaces.

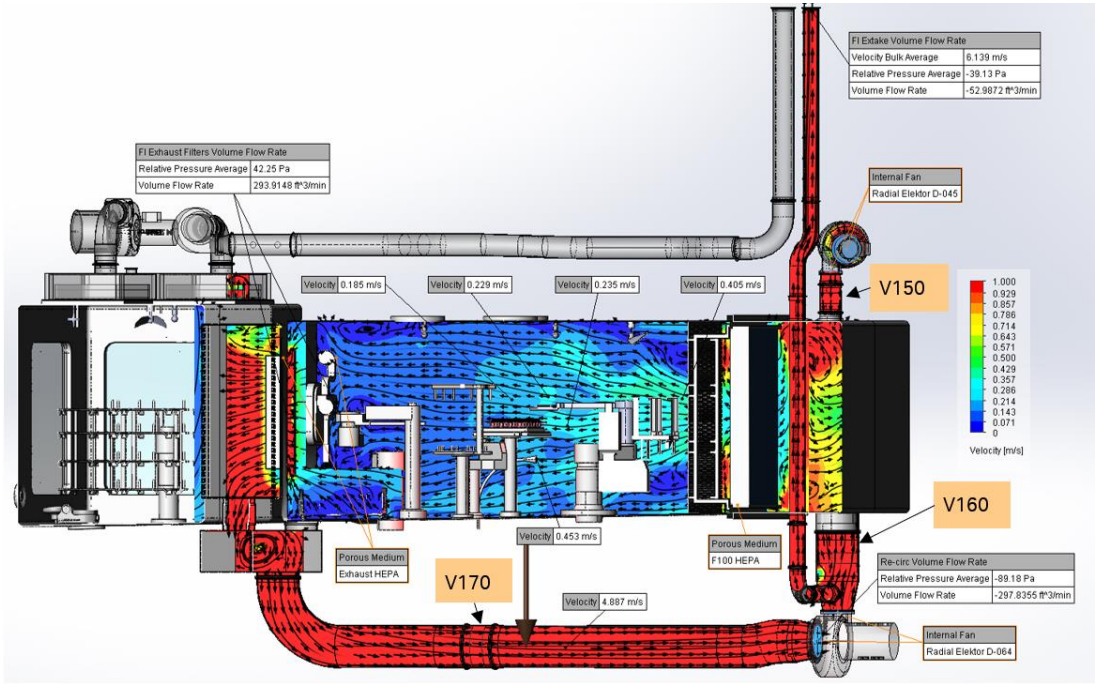
Airflow design – airflow visualization

DSI smoke – no leak to FI

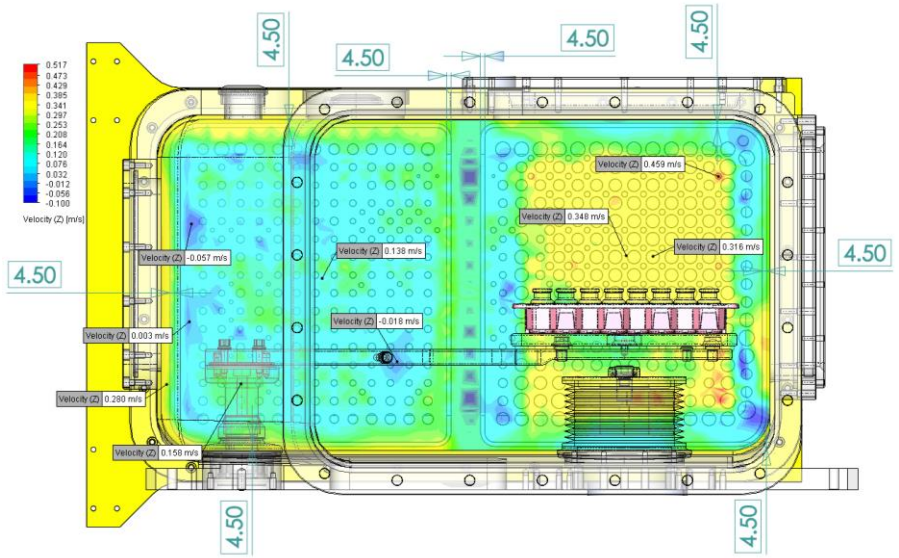
- DSI chamber filled with smoke
- Isolator pressures tuned to eliminate smoke ingress into FI.
- Smoke (and particles) that are touching the DSI door get carried back into the DSI within a few seconds
- Active flow continues towards the DSI while door remains open
- Tub that is transferred into the FI pauses in transfer tunnel to wash over with unidirectional FI airflow

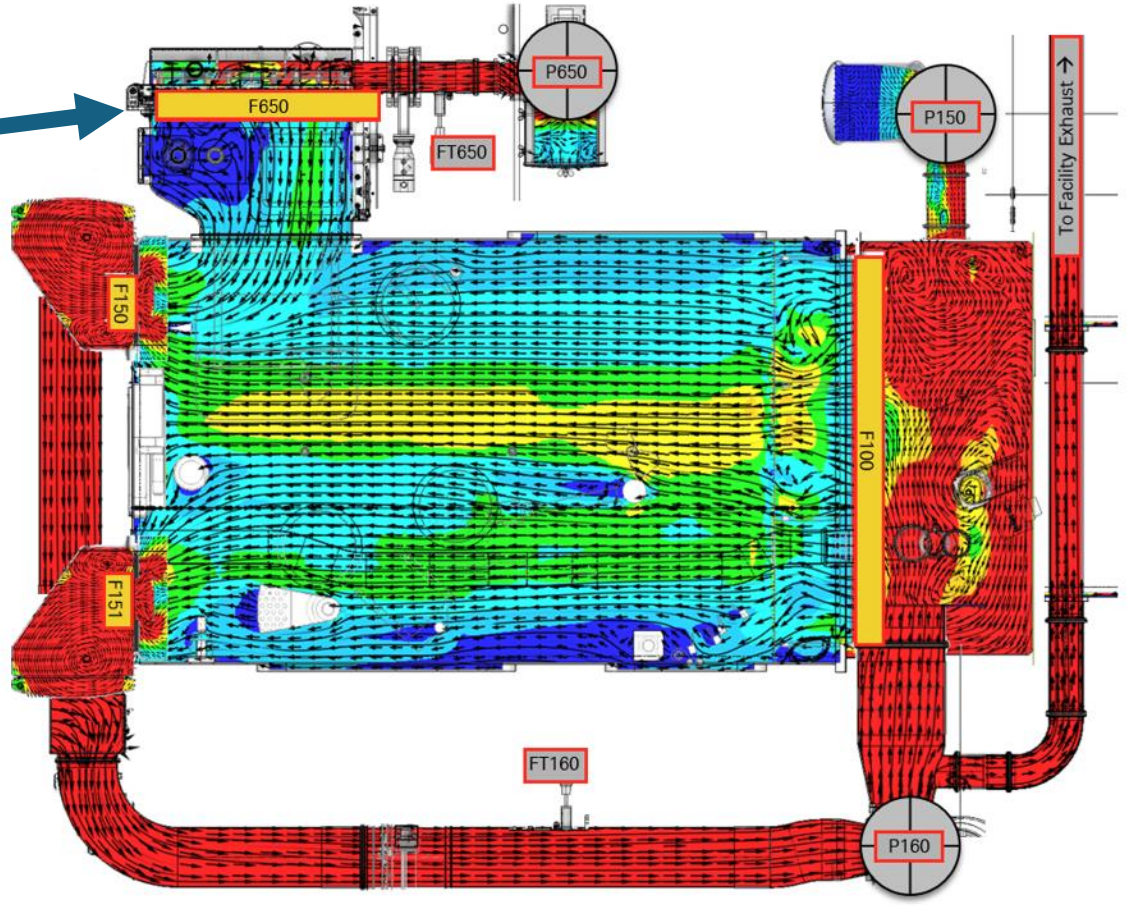
SA25 Workcell Documented Airflow Design

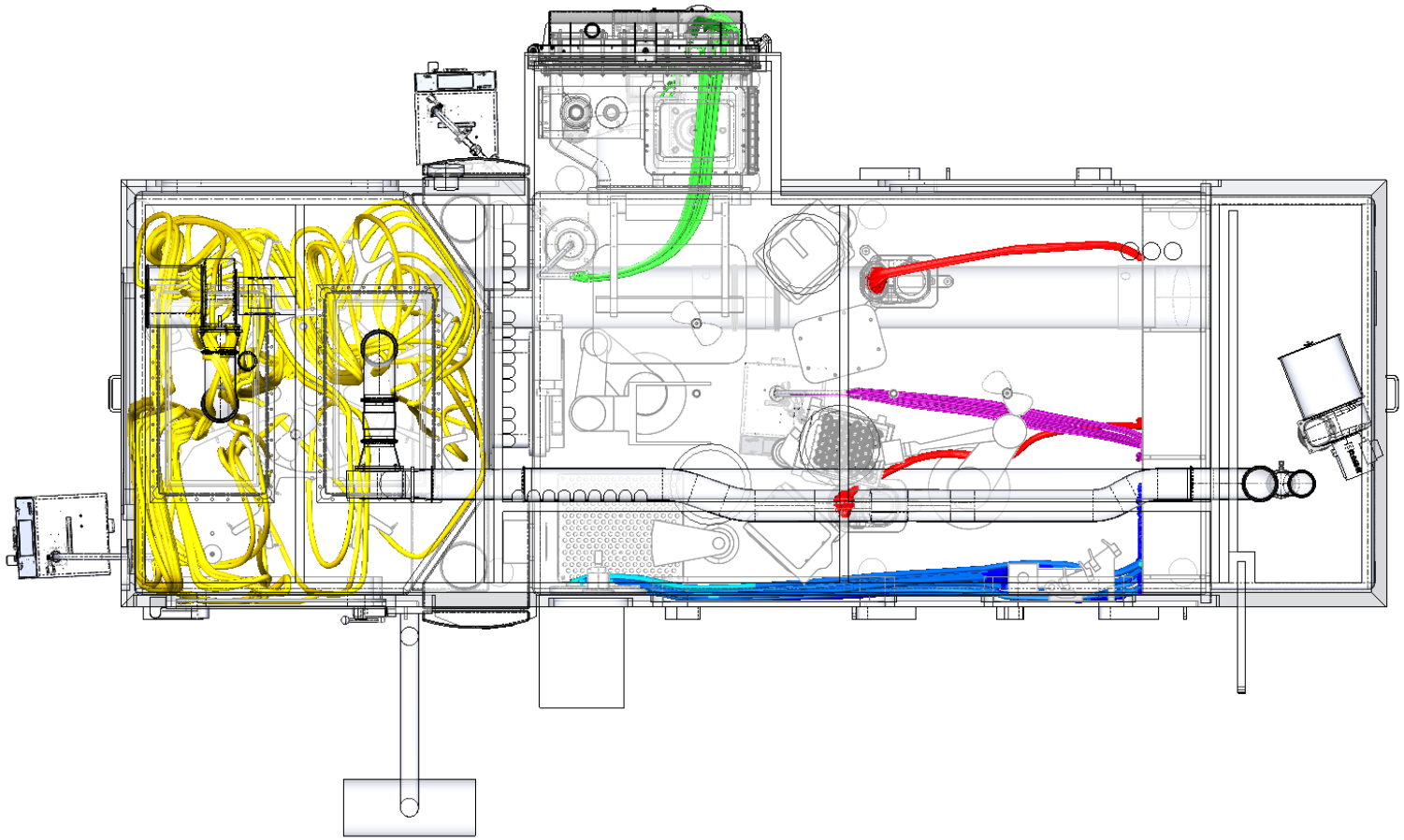




CFD tuned diffuser for optimal performance



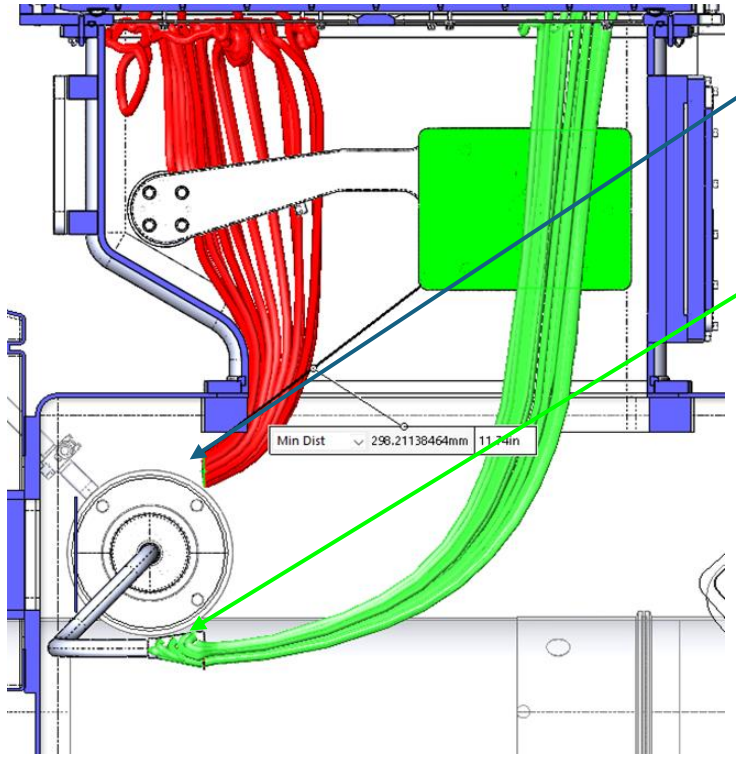




- EM is included in CFD
- EM options can be analytically justified, compared and optimized for your personal risk profile

EM = environmental monitoring

Airflow visualization CFD and AFV

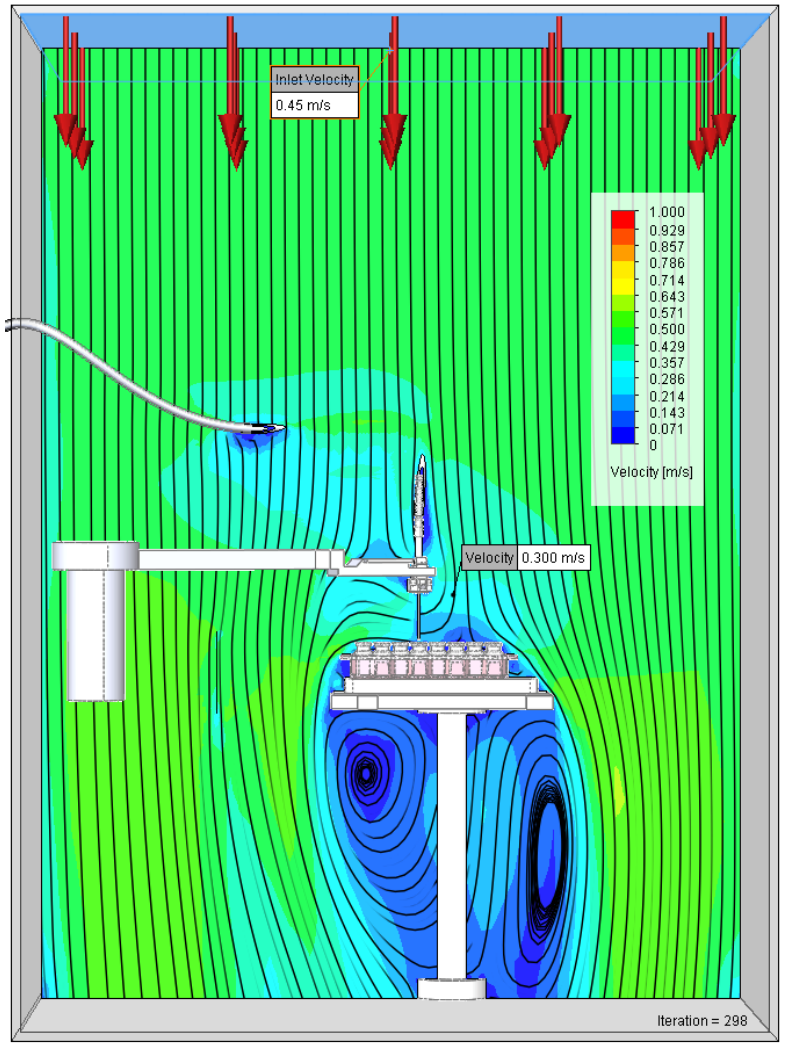


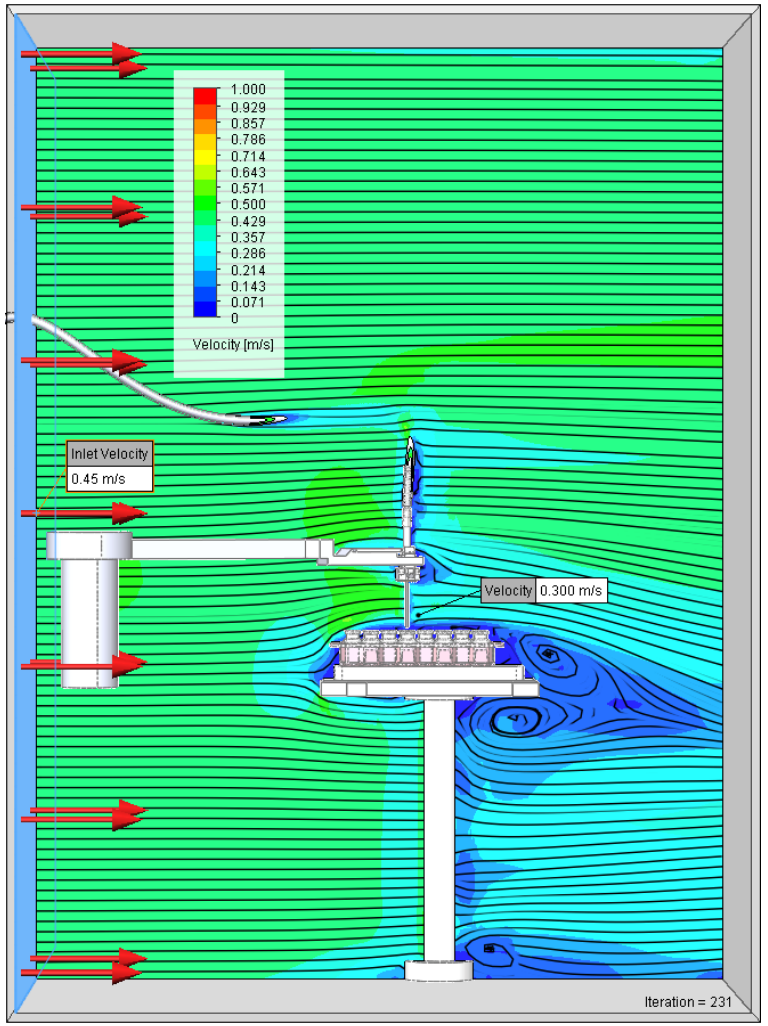
Previously evaluated PC location

Final optimized PC location.

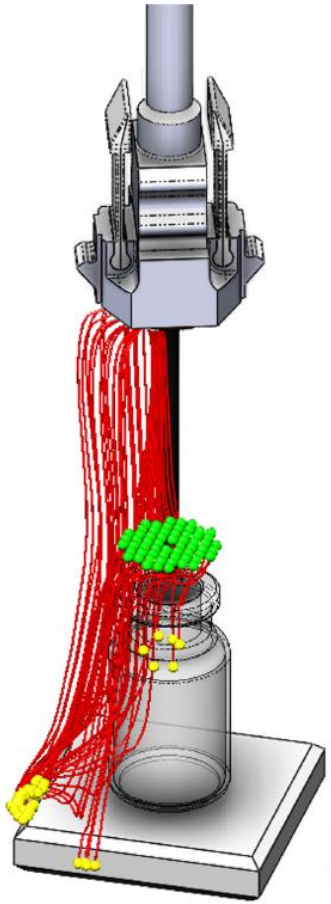
AFV = airflow visualization
PC = particle counter

Airflow design CFD

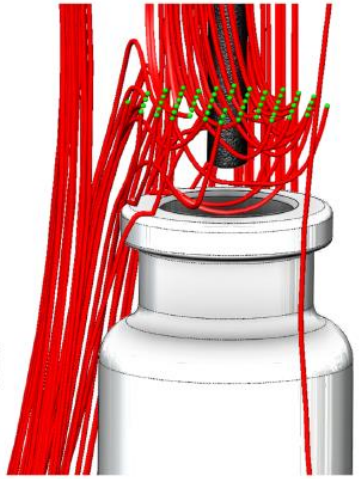




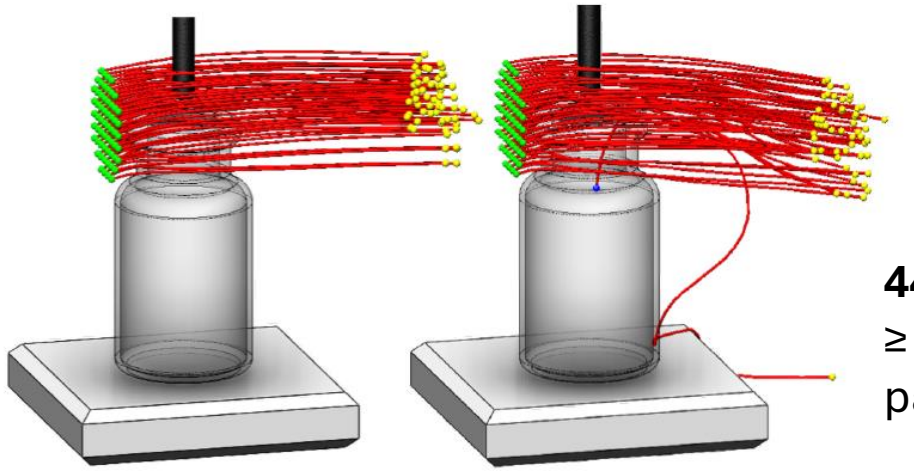
Particle simulation: vertical and horizontal airflow



13 µm shown
≤ 13 µm particle skip over vial



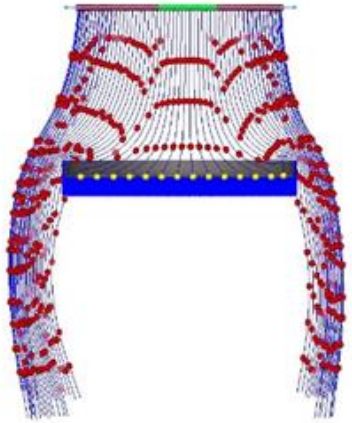
13 µm shown
≤ 43 µm particle skip over vial

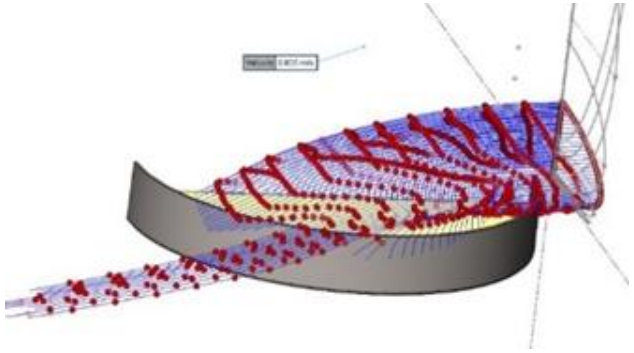


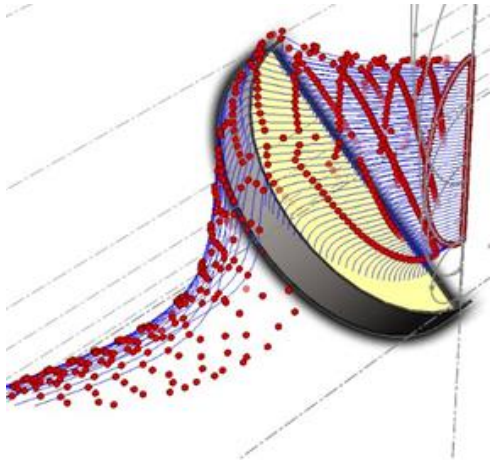
25 µm shown
≥ 14 µm particle ingress

44 µm shown
≥ 44 µm particle ingress

Settle plate particle loss study







Phyical Collection Efficency - Vertical Airflow					
Airspeed	0.5 m/s	0.4 m/s	0.3 m/s	0.2 m/s	0.1m/s
Particle					
5 µm	0%	0%	0%	0%	3%
10 µm	1%	3%	4%	6%	9%
15 µm	6%	7%	9%	11%	14%
20 µm	9%	10%	11%	14%	19%
30 µm	12%	14%	16%	20%	26%
40 µm	18%	19%	22%	26%	33%
50 µm	24%	25%	27%	32%	38%

Phyical Collection Efficency - Horizontal Airflow					
Airspeed	0.5 m/s	0.4 m/s	0.3 m/s	0.2 m/s	0.1m/s
Particle					
5 µm	0%	0%	0%	0%	0%
10 µm	0%	0%	0%	0%	2%
15 µm	0%	0%	1%	2%	4%
20 µm	1%	2%	2%	4%	9%
30 µm	3%	4%	6%	10%	23%
40 µm	6%	9%	12%	21%	42%
50 µm	10%	14%	22%	33%	65%

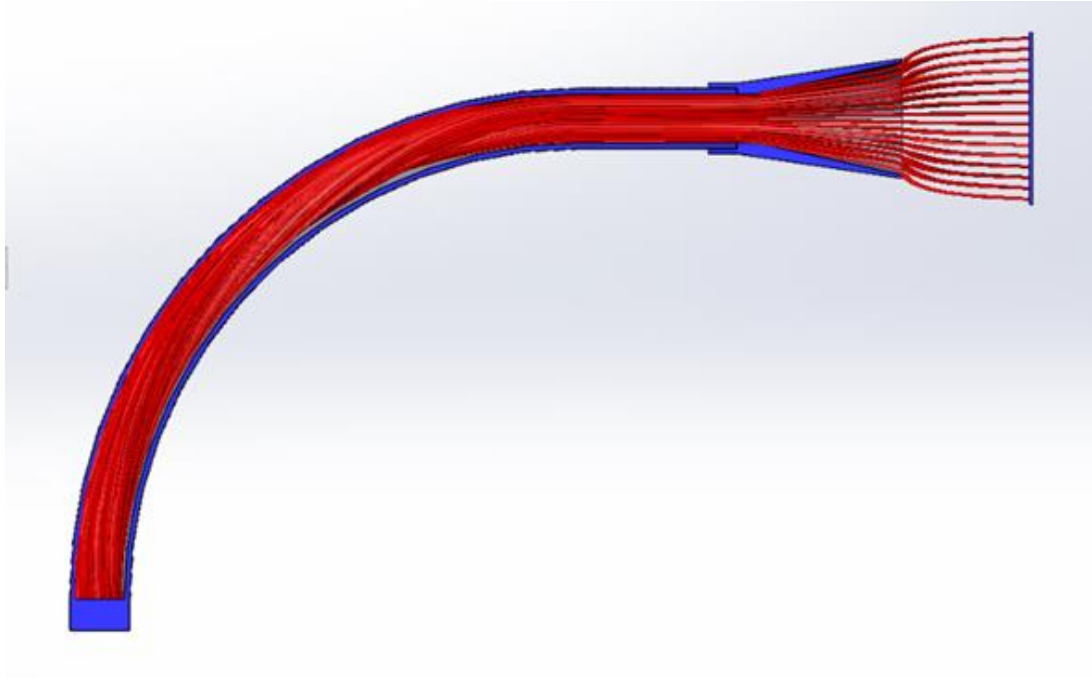
Phyical Collection Efficency - Horizontal Airflow					
Airspeed	0.5 m/s	0.4 m/s	0.3 m/s	0.2 m/s	0.1m/s
Particle					
5 µm	0%	0%	0%	0%	1%
10 µm	0%	0%	0%	3%	3%
15 µm	1%	2%	4%	5%	9%
20 µm	5%	7%	9%	11%	11%
30 µm	10%	12%	15%	19%	26%
40 µm	16%	18%	21%	26%	38%
50 µm	20%	23%	28%	35%	53%

Average size of microbe carrying particles is about 12 µm, with 1% below 1 µm, 25% below 7 µm, 25% above 24 µm, and 5% above 50 µm

(Whyte W, Hejab M. Particle and microbial airborne dispersion from people. *European Journal of Parenteral and Pharmaceutical Sciences*. 2007;12(2):39-46. https://eprints.gla.ac.uk/84357/)

Evaluating the probe in 0.2 m/s flow

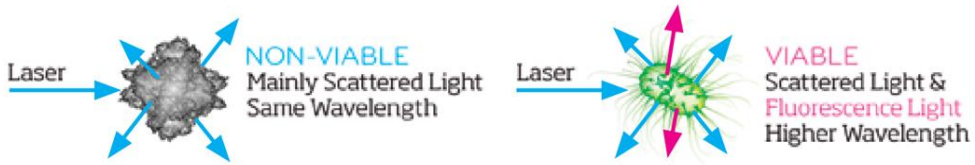
- All particle diameters up to 20 µm do not break free and are fully counted (100% capability).



Biofluorescent particle counters (BFPCs)

BFPCs directly detect presence of biofluorescent particles and then use multiple wavelength detectors and algorithms to predict if particle is an organism.

- Instruments use multiple wavelength detectors and particle sizes to reduce false positives without increasing false negatives.
- Some particles like glass can interfere with results.
- Organisms' biofluorescent signature has a range.



Source: BioTrak FAQ_A4_5001727_WEB

Real world example on SA25 workcell

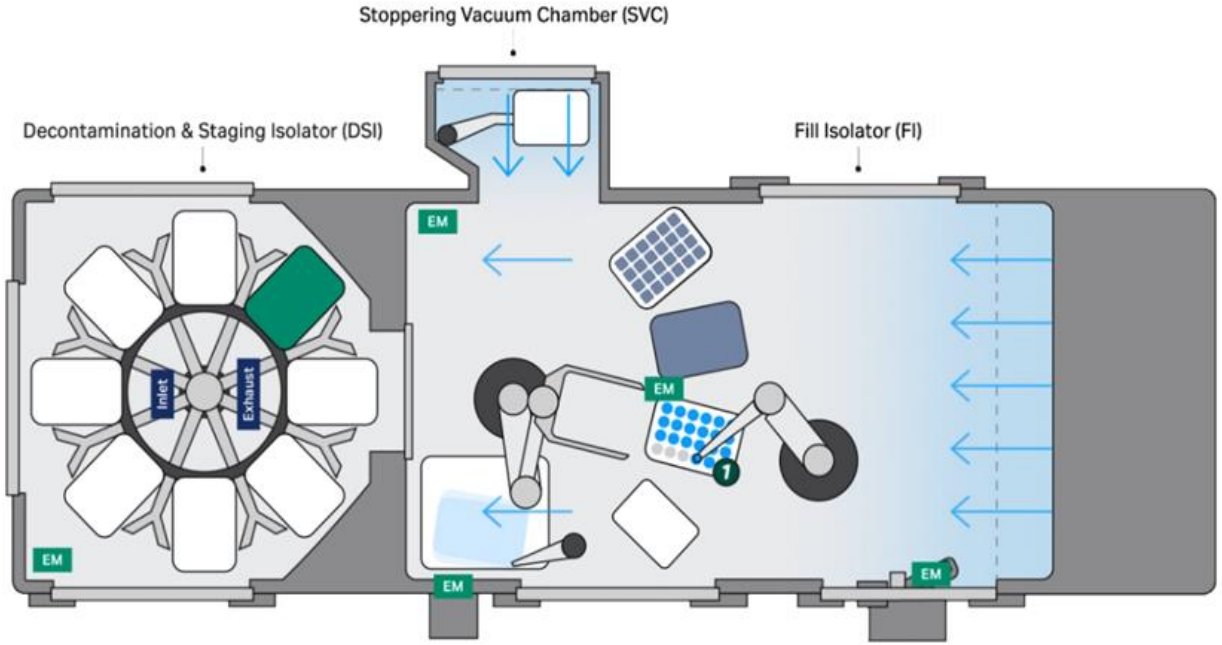
Potential interferent materials:

- Glass
- Cleaning materials
- Liquid drug product
- Material processing
  - Tyvek peeling
  - Stoppering
  - Flowpath movements

Particle source designed out of our workcells

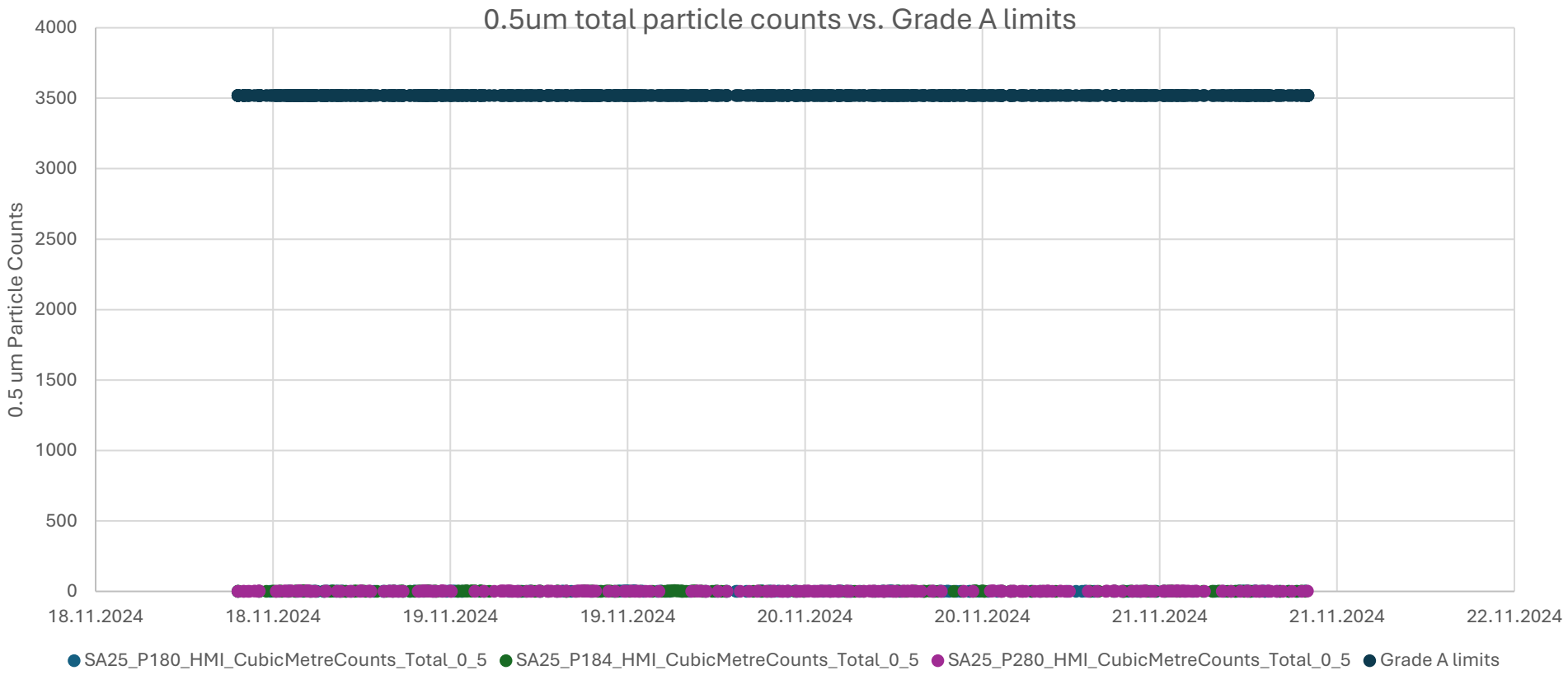
Requires customer testing as materials vary

Focus of study



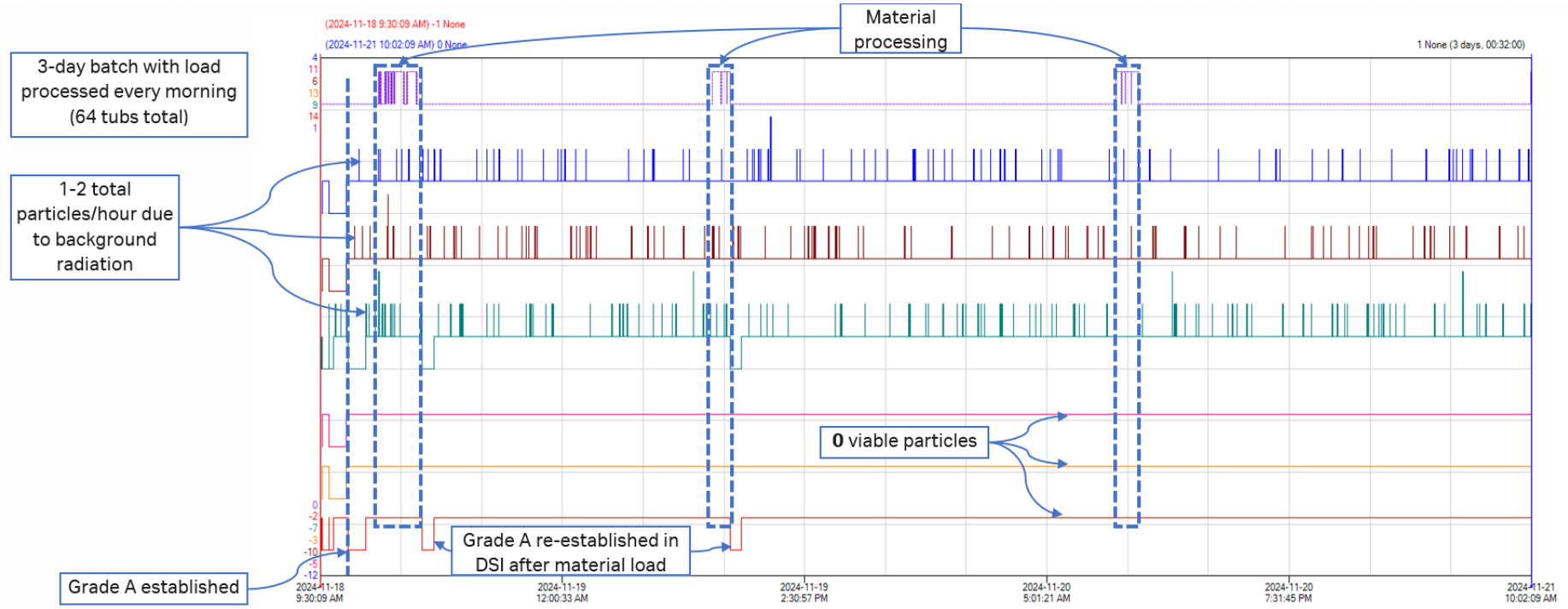
SA25 workcell top view graphic.

Baseline and interference study particle chart – ISO limits



ISO = International organization for standardization

Baseline and interference study particle chart – zoomed in



No viables over 3-day cycle under static and dynamic conditions

Baseline and interference study outcome

Outcome:

1. We identified the sources of particles
2. Each source was isolated and studied for probability of viable particles given total particles
3. Tests executed to determine probability of any particles reaching critical zones
4. Interference study conducted in uncontrolled warehouse to confirm the hypothesis
5. Data from study confirms the hypothesis of no particles due to material processing and machine operations

- Understanding and creating a baseline converts data into actionable information

Thank you for your attention!

Slovenian Clearoom Society
Dimičeva street 13 I 1504 Ljubljana
T: +386 1 5898 312 I F: +386 1 5898 317 I E: ptz@gzs.si
https://scs.gzs.si

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:
INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

From Traditional Monitoring to Advanced BFPC & Airflow Visualization

Practical Insights from Cleanroom Environments

Nataša Štirn, Klimer d.n.o

Agnes Krmelj, Lonstroff d.o.o.

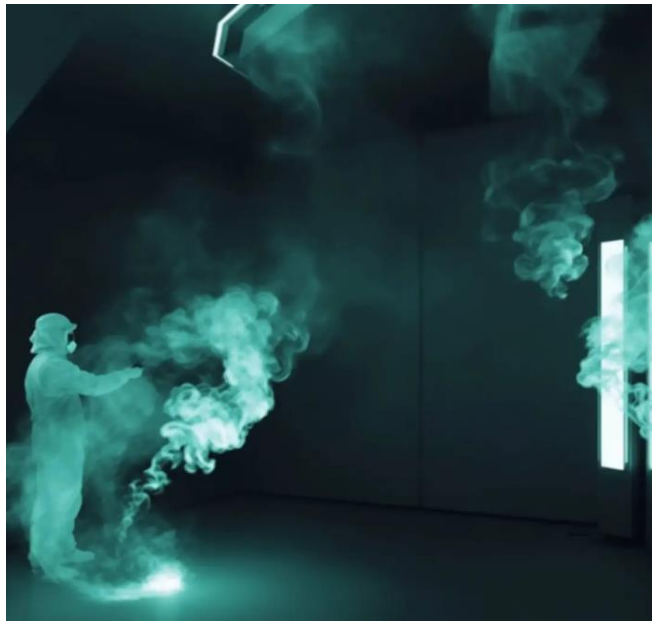
Dražana Radonjić, Labena d.o.o.

30 – 31 MARCH 2026
BRDO PRI KRANJU, SLOVENIA

Today's Journey

- **Introduction & Regulatory Framework**
  Nataša Štirn
- **Case Study 1: Airflow Visualization & BFPC**
  Agnes Krmelj
  → ISO 5 → GMP Grade B
- **Case Study 2: Real-Time Microbial Monitoring (BFPC)**
  Dražana Radonjić
  → BioTrak in Grades B & A

Airflow Visualization – smoke studies
Introduction



Nataša Štirn, Klimer d.n.o.

Importance of Smoke Studies

What are smoke studies?

- A **vital technique** for evaluating and optimizing **HVAC performance**
- Use of **neutral-buoyancy tracer smoke** to **visualize and assess airflow patterns**
- Commonly applied in **critical clean environments**, such as:
  - **Operating theatres**
  - **Cleanrooms**
  - **Pharmaceutical production areas**

Why Are They Important?

✅ Detect:
- Air turbulence
- Dead zones
- Poor air circulation

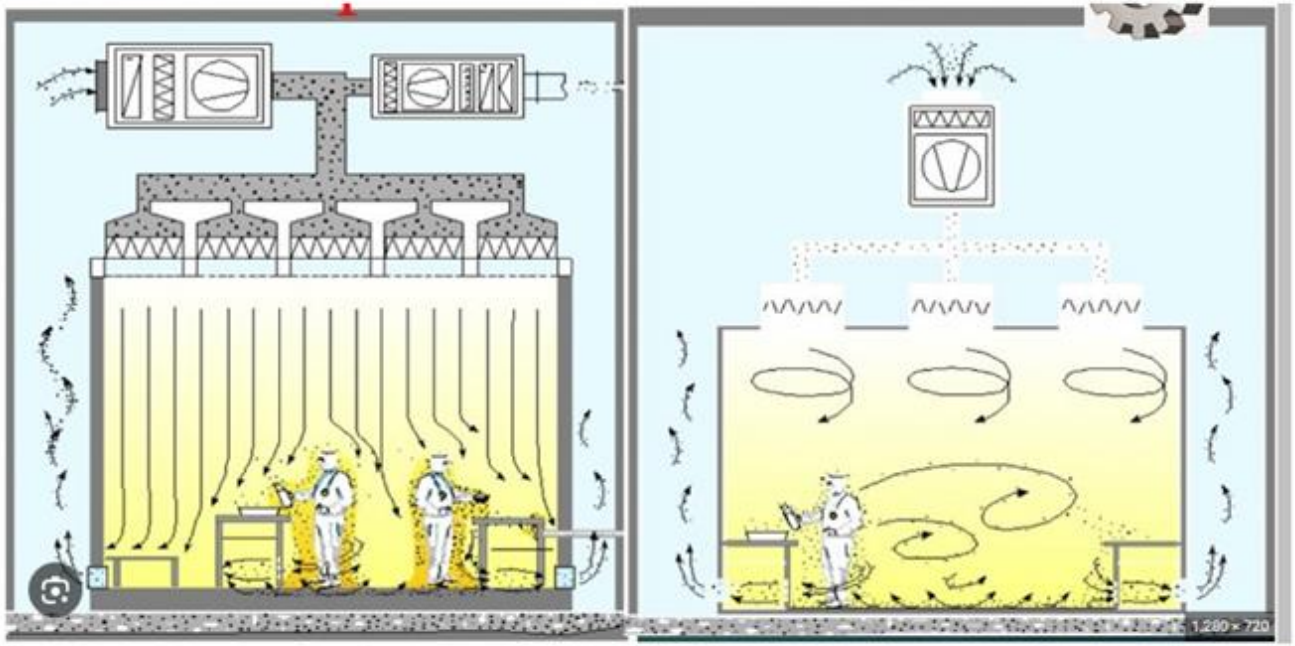
✅ Enable:
- System adjustments (e.g., repositioning diffusers, adding filters)
- Reduced contamination risk
- Improved compliance with regulatory standards

Regulatory Requirements and Industry Guidance

- **ISO 14644-3:2019**
  - Airflow visualization listed under “supportive tests”
  - Recognized as one of the **core cleanroom qualification tests**
- **EU GMP Annex 1 (2022)**
  - Highlights airflow visualization in:
    - **Clause 4.15** – Qualification
    - **Clause 4.31** – Environmental Monitoring locations
    - **Clause 7.18** – Operator training
- **FDA Aseptic Processing Guidance (2004)**
  - Emphasizes need to evaluate airflow for **turbulence or eddy currents**
  - Requires **in situ** air pattern analysis to verify **unidirectional airflow** and **sweeping action** under **dynamic conditions**
- **ISO 14644-4:2022**
  - Provides updated international guidance on airflow visualization methods
- **Additional Industry Guidance**
  - **USP <1116>**, **ISPE**, **PDA**, and **CETA**
  - CETA emphasizes using a **visible medium that is as close to neutrally buoyant as possible**
  - **ASTM E3379-25** (2025) New standard reinforcing airflow visualization

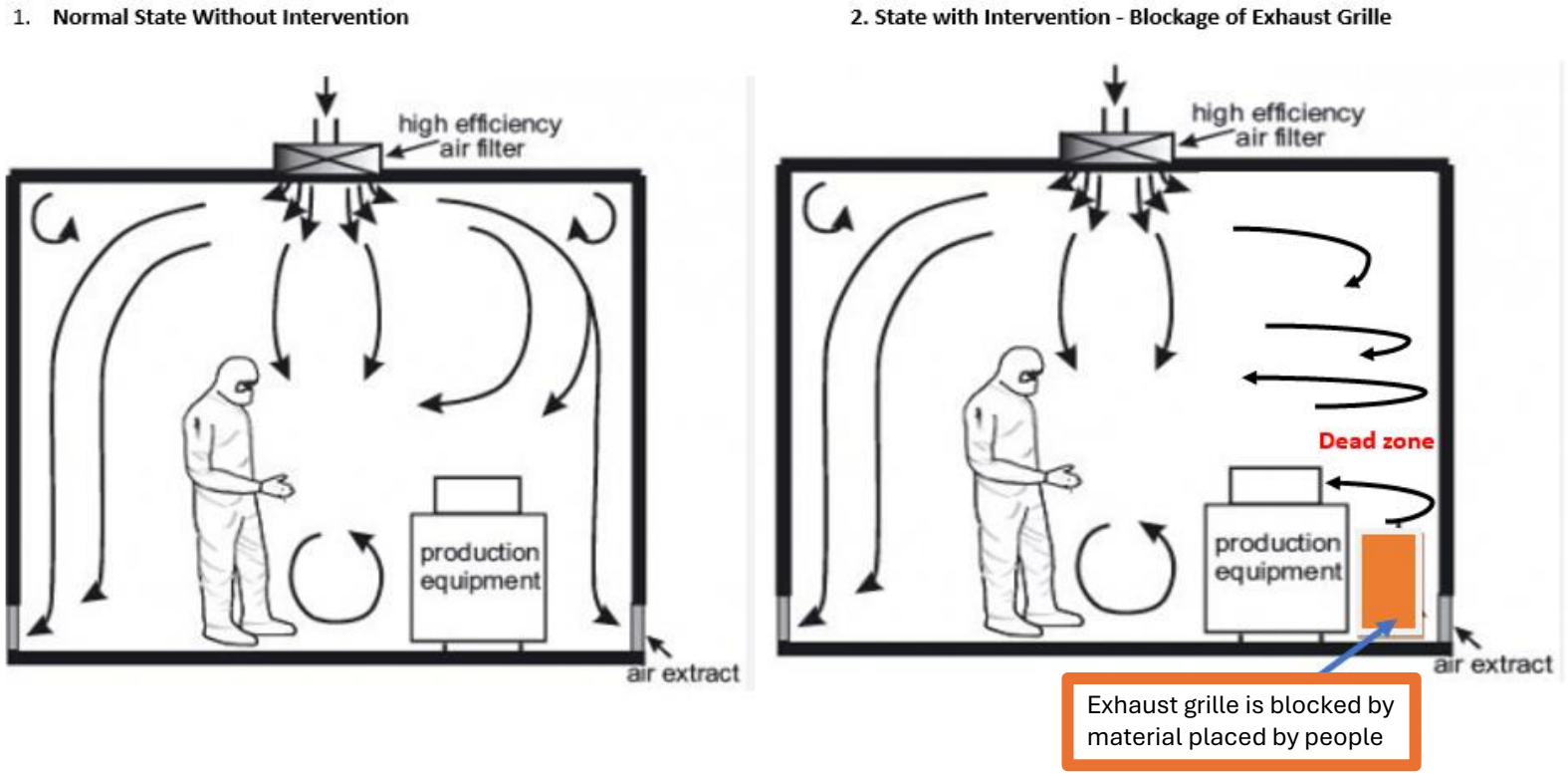
Airflow Visualization Studies in Cleanrooms

- It is necessary to distinguish:



- **Unidirectional Airflow:** Airflow that moves in one direction, robustly and uniformly, and with sufficient velocity to constantly flush particles away from the critical area.
- **Non-Unidirectional / Turbulent Airflow:** Airflow patterns are designed to be turbulent to ensure dilution and mixing of clean and particulate-contaminated airflows. Airflow flushes the entire space by following the turbulent airflow pattern and spreading throughout the space, diluting and exhausting the pollution created

Schematic Representation of the Impact of Personnel Intervention in a Non-Unidirectional Air Flow Room



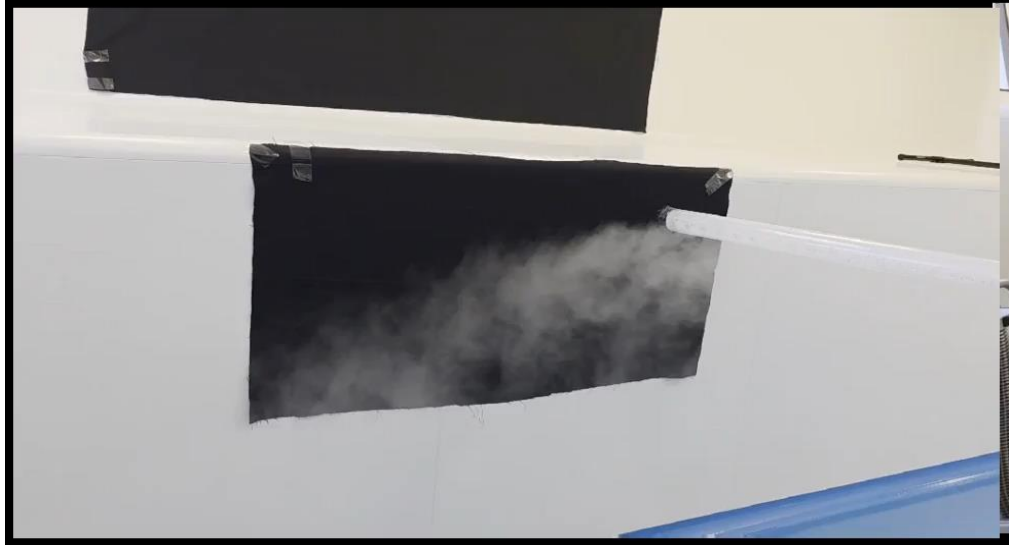
AIRFLOW VISUALIZATION METHODS

ASTM E3379-25 (2025)

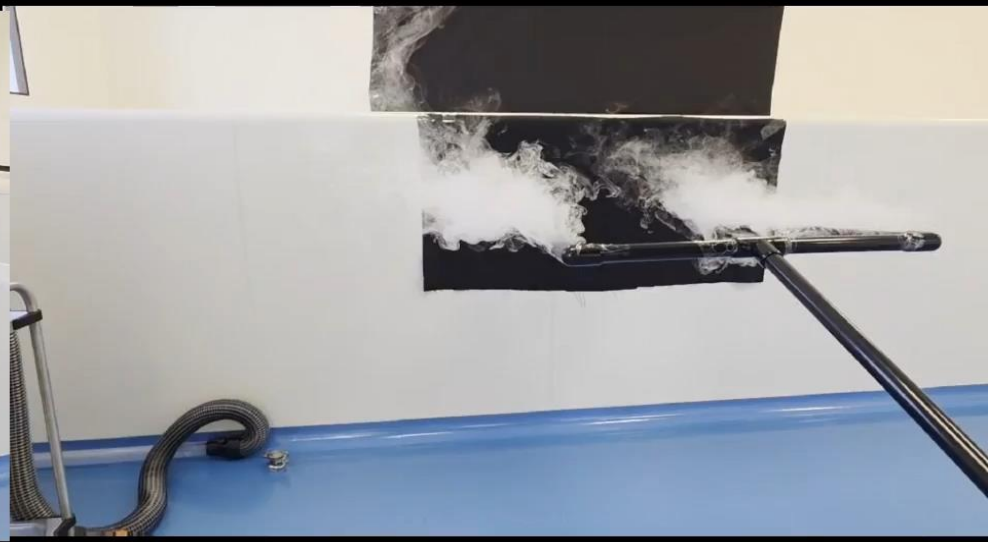
Method	Particle Size	Buoyancy	Key Limitation
Watter-based smoke	>5 µm	❌ Heavier than air	Misleading airflow
Glycol-based smoke	<1 µm	✅ Neutral	Most realistic
Oil-based smoke	0.2–0.3 µm	✅ Neutral	Not pharma standard
CO_2 fog	10–100 µm	❌ Heavier	Short duration
Nitrogen fog	2–10 µm	⚠ Variable	Unstable
Tracer gas	Atomic	✅ Ideal	Complex setup

Tracer particles must be:
“as close to neutrally buoyant as possible”
📌 CETA Guidance

Not all smoke behaves the same!



Watter-based smoke



Glycol-based smoke

Using the Right Tool & Substance as a Tracer Particle

Why Tracer Particle Choice Matters

- Tracer particles must **accurately follow air movement**, not gravity.
- Using **non-buoyant or oversized particles** leads to false interpretation — they **settle downward** instead of floating with airflow.

📌 **Common Mistake**

- Many systems use **inappropriate chemicals or substances**.
- These particles tend to move **straight down due to gravity**, not air currents.

📌 **How to Verify Your Setup**

- Run your airflow visualization **in a static room (no ventilation active)**.
- If particles **fall directly down**, you're seeing **gravitational movement**, not true airflow.

CASE STUDY – LONSTROFF d.o.o.

New method allows to better test

Airflow Patterns & Particle Behavior during Stopper Processing

Agnes Krmelj, Lonstroff d.o.o.

Objective

- To perform **an in-operation** assessment of UDAF performance and contamination control.

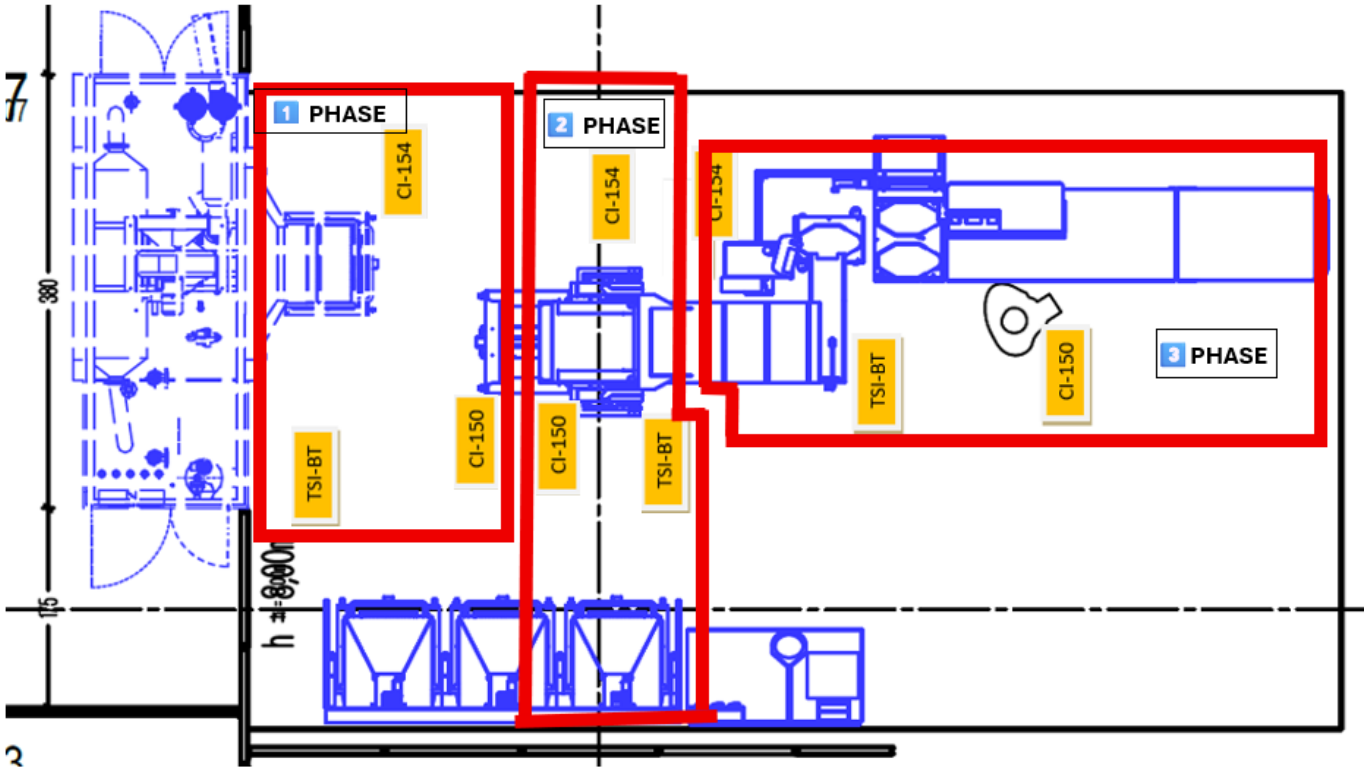
Goal

- To determine whether the existing **ISO 5 at rest** UDAF system meets **GMP Grade B** requirements **in operation**.

Focus of the Study

- Airflow visualization (UDAF performance)
- Particle monitoring
  - Total particles
  - Viable (microbiological) particles

Process Phases (Dynamic Conditions)



1. Emptying washer drum
2. Transfer to dispenser
3. Packing into bags

UDAF unit: Area = 50,0 m^2 / HEPA filters = 33 pcs

Measurement setup

- **UDAF unit:** FFU9555-2 (S/N **1334**)

Equipment Airflow Visualization (Smoke Studies)

- **Smoke generator:** Microrite CRV-M1
- **Smoke medium/solution:** Microrite (Neutral Buoyancy)

(selected to faithfully follow airflow streamlines and avoid gravity-bias of water fogs)

- **Dispersion hardware:** spray tubes / nozzles (as per generator kit)
- **Video documentation:** GoPro cameras (dual-angle capture)

Method note: Airflow visualization recorded as two video clips aligned to routine operations (Phases 1–3).

Instruments used (total & viable particles)

- **TSI BioTrak (LAPC&BFPC)**
- **Climet CI-150t (LAPC)**
- **Climet CI-154 (LAPC)**

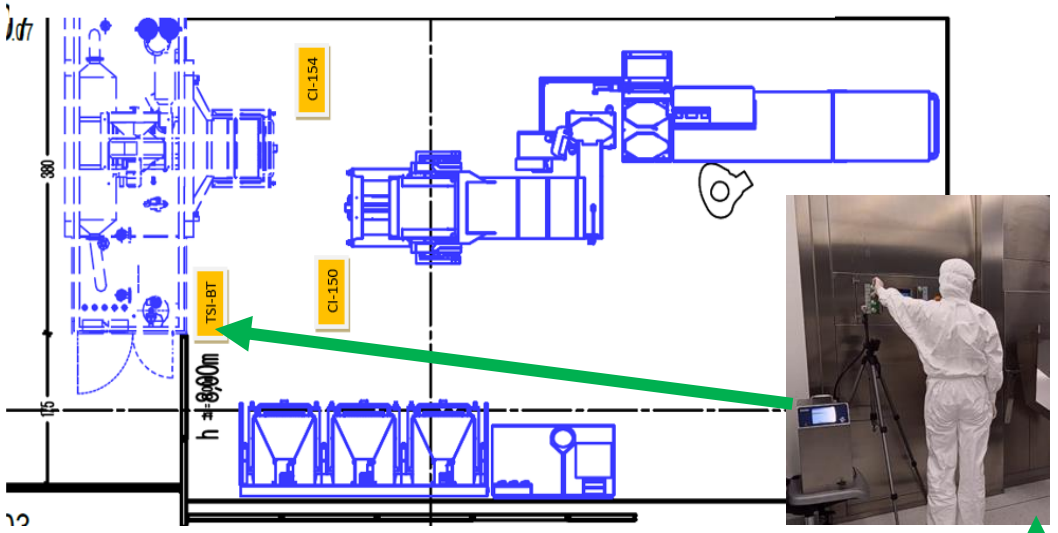
Note on data units: 1-minute samples at 1 CFM equal 0.0283 m³ of air. Convert to particles/m³ by dividing raw counts by 0.0283 (or × 35.34).

Phase 1 – Drum emptying (transfer from washer drum to bin)

Smoke studies - Key Observations

- **Breakdown of unidirectional flow (UDF)**
  → transition to turbulent airflow
- **Airflow deflection to the right**
  → formation of cross-flows and eddies (in front of washer)
- **Directional plume observed**
  → consistent with higher particle counts (BioTrak data)
- **Smoke spread beyond critical zone**
  → transport toward all particle counter locations
- **Process-induced disturbance identified**
  → drum tipping generates airflow disruption
- **Impact on measurements**
  → short, transient particle spikes across sampling points

Locations of particle counters during Phase 1

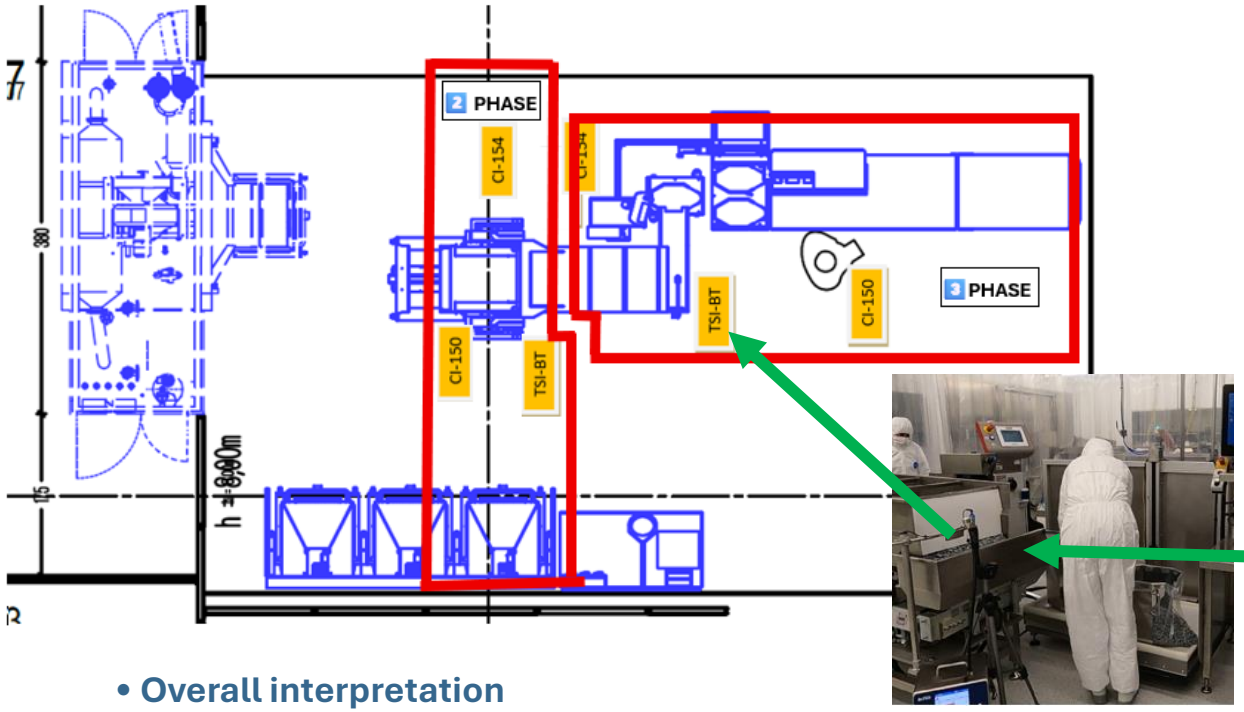


- A clear **transient peak** at the start of handling (09:52–09:53) on both Climet positions; counts **decay within ~1–2 min**, indicating effective dilution/directional flow in the UDAF area.
- BioTrak shows a concurrent rise in **total 0.5 µm** and a small rise in **viables** (max 4), consistent with mechanical agitation of stoppers and operator presence.
- **≥5.0 µm** particles remain at **zero or very low**, consistent with good protection against coarse particles.

Case Study 1: Airflow Visualization & BFPC
Agnes Krmlej, Lonstroff d.o.o.

Phases 2 & 3 (combined): Transfer from bin to dispenser and bagging of stoppers

Locations of particle counters during Phase 2&3



Particle Monitoring – Observations & Interpretation

- **Transient particle release during transfer (CI-154)**
  → short sub-micron spike with rapid return to baseline

- **Bagging phase – localized total particle spikes (CI-150t)**
  → ≤9 particles/ft^3 (≥0.5 µm), single isolated ≥5.0 µm event

- **Position-dependent exposure**
  → CI-154 remained unaffected (shielded location)

- **BioTrak response**
  → elevated non-viable counts near handling (≈72k–96k/m^3)
  → low viable counts (≤10/ft^3)

- **Coarse particles (≥5.0 µm)**
  → negligible or absent across all measurements

- **Overall interpretation**
  → mechanical handling generates localized particle release
  → limited microbiological impact
  → no sustained contamination observed

Phases 2 & 3 (combined): Transfer from bin to dispenser and bagging of stoppers

Smoke studies - Key Observations

- **Airflow Visualization (Smoke Studies)**
→ non-uniform airflow from HEPA filter to critical zone (UDAF not maintained during operation)
→ horizontal air movement toward room exhaust grilles
→ particles not consistently removed downward (Loss of vertical sweeping effect)
→ sideways transport of particles from operator/work zone (Impact on contamination control)

- **Clear evidence from smoke visualization**
→ confirmed in both camera angles and video recordings

- **Correlation with measurements**
→ localized increase in particle counts near dispenser

Key Findings & Interpretation - Total particles

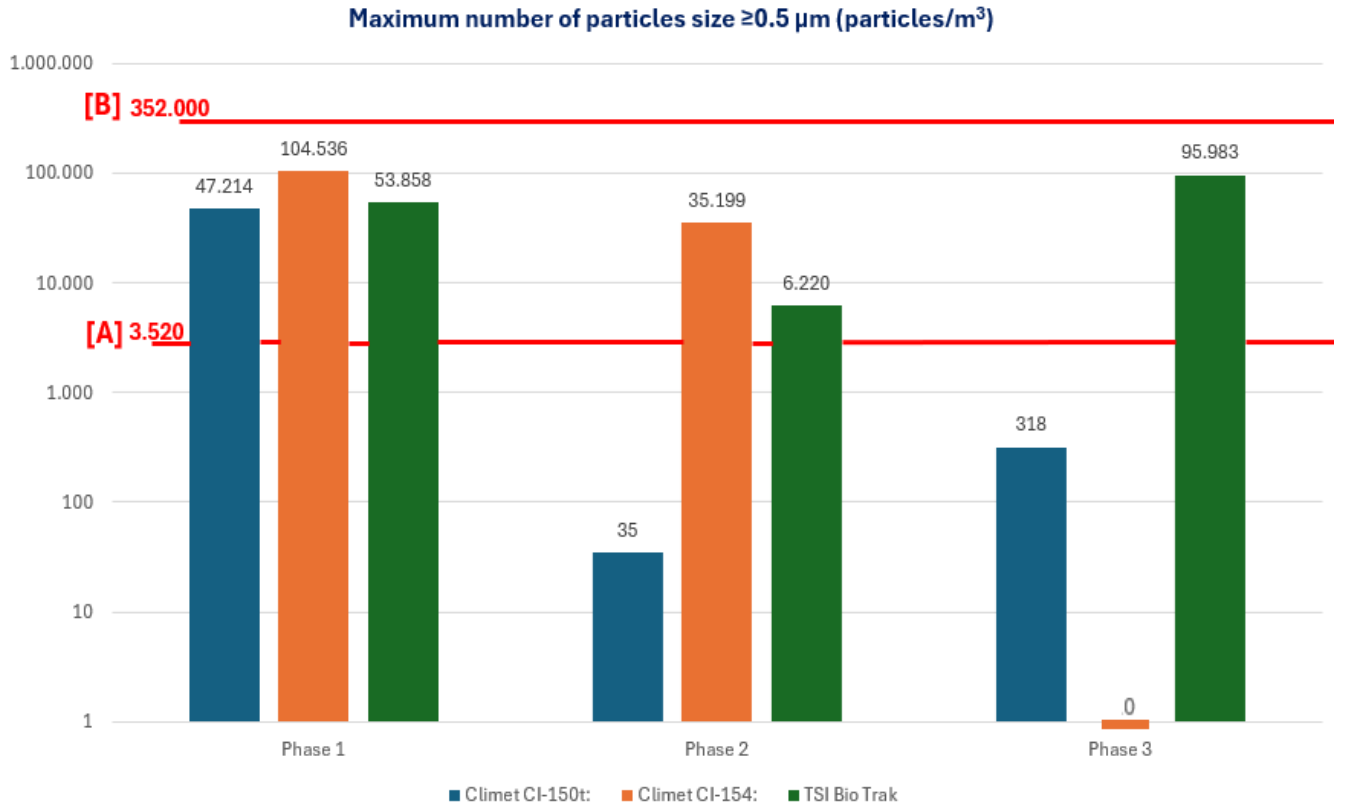
- **Highest particle release in Phase 1**
  → unloading from barrel → elevated counts
  → within the Grade B at all locations

- **Moderate increase in Phase 2**
  → peak at CI-154 and BioTrak (< Grade B)
  → CI-150t remained at background levels

- **Localized impact in Phase 3 (bagging)**
  → elevated counts at BioTrak position (< Grade B)
  → Climet positions remained low → strong positional effect

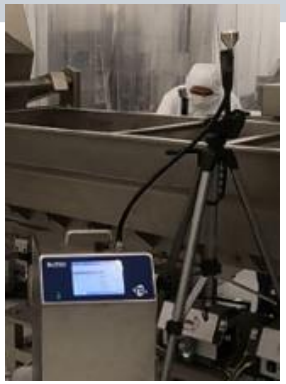
- **Multiple exceedances of Grade A limits**

- **Strong spatial dependency**
  → particle peaks are localized
  → measurement results depend on instrument position vs operator/activity



Key Findings & Interpretation - Viable Particles (BioTrak)

GMP Requirement viable particles (≥0.5 µm) AFU/ft³	Observed Results (BioTrak) viable particles (≥0.5 µm) AFU/ft³		
	Phase 1 – Unloading	Phase 2 – Transfer	Phase 3 – Bagging
Grade A (0 AFU/ft³)	0 → 4 AFU/ft³ Transient detections present	0 → 1 AFU/ft³ Minimal but not zero	5 → 10 AFU/ft³ Highest viable activity (handling impact)
Grade B	COMPLAINT	COMPLAINT	COMPLAINT



Low viable detections = compliance with Grade B

👉 Real-time detection reveals events that traditional methods may miss
👉 By using the better Airflow Patterns & Particle Behavior method during Stopper Processing, our clean room is confirmed Class B

Case Study 1: Airflow Visualization & BFPC
Agnes Krmlej, Lonstroff d.o.o.

Annex 1 GMP - a "Call to Action"

Real-Time Microbial Monitoring

Introduction

Nataša Štirn, Klimer d.n.o.

Annex 1 (2008) vs Annex 1 (2022)

Shift towards Continuous Monitoring

Annex 1 (2008)
(16 pages)

- ✓ limited scope
- ✓ no dedicated monitoring chapter
- ✓ overlap between classification and monitoring

👉 ***Monitoring not clearly defined***

Annex 1 (2022)
(58 pages)

- ✓ structured approach to classification (Chapter 4)
- ✓ **NEW: Continuous Monitoring (Chapter 9)**
  - → detection of transient / impulsive events
  - → real-time response capability
  - → focus on ≥5.0 µm particles
  - → continuous viable monitoring

👉 ***Monitoring becomes a core control strategy***

"The focus has shifted from static compliance to continuous process understanding and control."

CLASSIFICATION vs MONITORING

ISO 14644-1


INTERNATIONAL STANDARD ISO 14644-1

Second edition 2015-12-15

Cleanrooms and associated controlled environments —

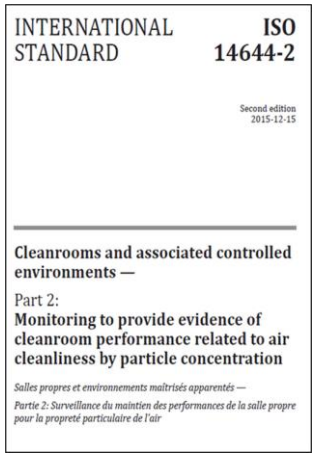
Part 1:
Classification of air cleanliness by particle concentration

Salles propres et environnements maîtrisés apparentés —
Partie 1: Classification de la propreté particulaire de l'air

Classification = ***verification of cleanroom conditions (establishing control)***

Monitoring = ***continuous understanding of cleanroom behaviour (demonstrating control)***

ISO 14644-2


INTERNATIONAL STANDARD ISO 14644-2

Second edition 2015-12-15

Cleanrooms and associated controlled environments —

Part 2:
Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration

Salles propres et environnements maîtrisés apparentés —
Partie 2: Surveillance du maintien des performances de la salle propre pour la propreté particulaire de l'air

Classification

- defined states (as-built / at-rest / operational)
- point-in-time assessment
- compliance-based

👉 **Defines cleanliness**

"A cleanroom is not defined by classification — but by how it performs in operation."

Monitoring

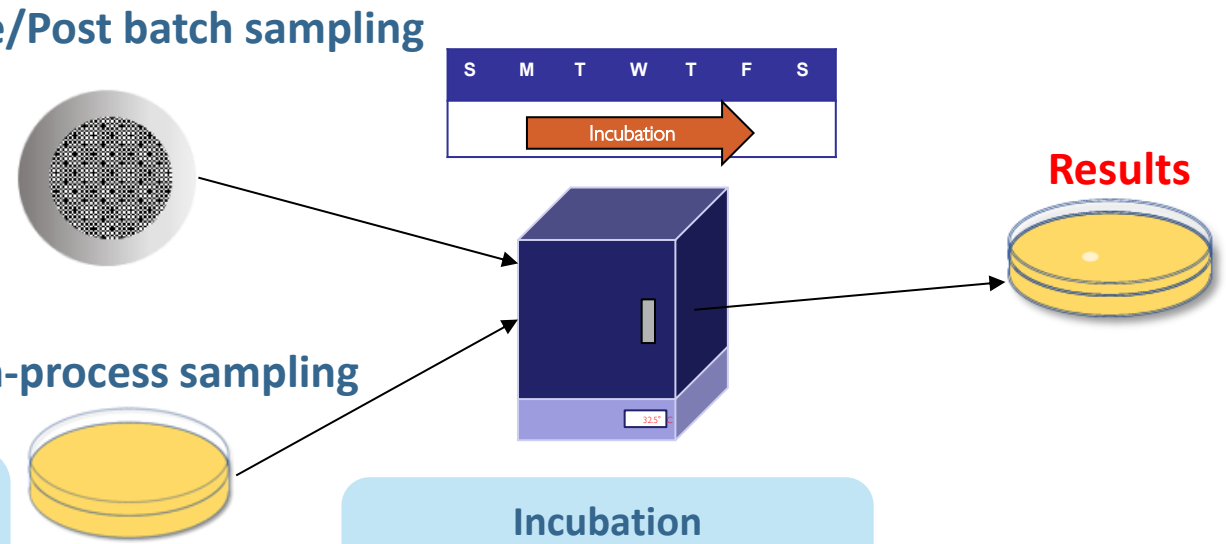
- continuous / real-time
- operational conditions
- dynamic environment

👉 **Reveals behaviour**

Traditional Viable Air Monitoring

Active Air (Volumetric) Sampling
- Air drawn through sampler
- Particles captured on media

Settle Plates (Passive Sampling)
- Open plate exposure (≤4 h)
- Passive particle deposition



Incubation
- 2-7 days incubation
- Single/dual temperature

Plate Count
- Replication until visibly countable
- Colony forming unit (CFU)

Current limitations:
→ Manual process
→ Results available only after incubation → delayed decision-making

EUROPEAN COMMISSION

Regulatory Perspective (EU GMP Annex 1)

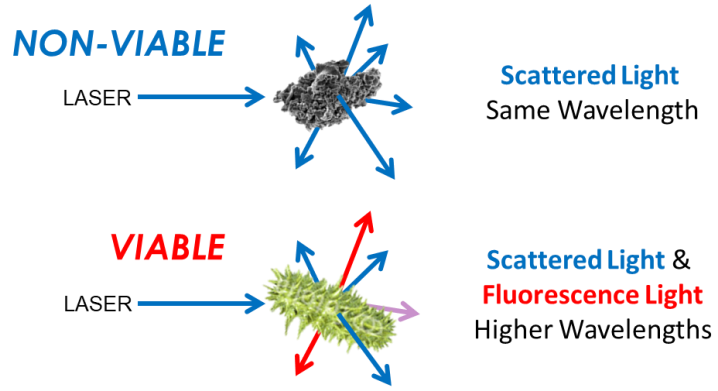
ARMM in Qualification (Clause 4.31 & Table 2) and Routine EM (Clause 9.28 & 9.30 Table 6)

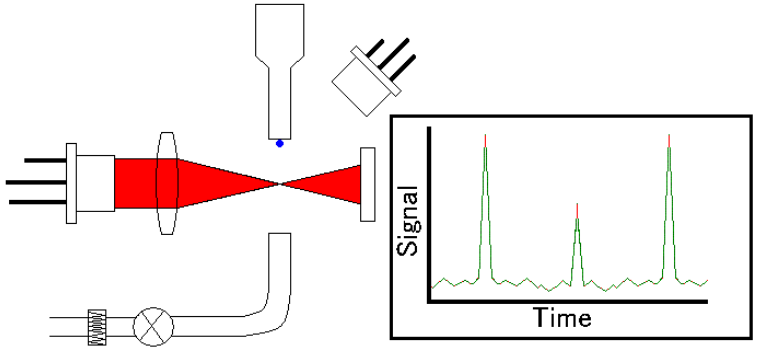
Rapid / alternative methods are encouraged

- ✓ Should be considered to reduce contamination risk
- ✓ Enable faster detection
- ✓ Can be used if validated (equivalent or better)

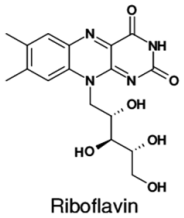
What is Biofluorescence Particle Counting (BFPC)?

- Alternative Microbiology Method
- Laser Induced Fluorescence (LIF)→ Not Growth Based





Microorganisms contain unique fluorescent molecules



The Challenge

But implementation is not straightforward!

Key challenges

- AFU ≠ CFU → **different sensitivity**
- Continuous monitoring → **more data / more noise**
- Difficult correlation with traditional methods

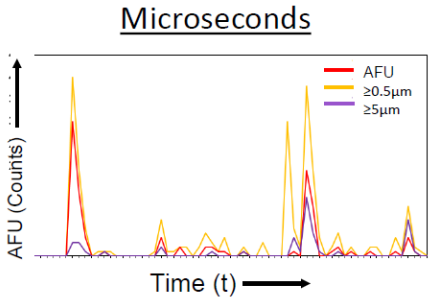
Same Analyte, Different "Signals"

Colony-forming unit (CFU) is a unit used to estimate the number of viable and culturable bacteria or fungal cells in a sample.

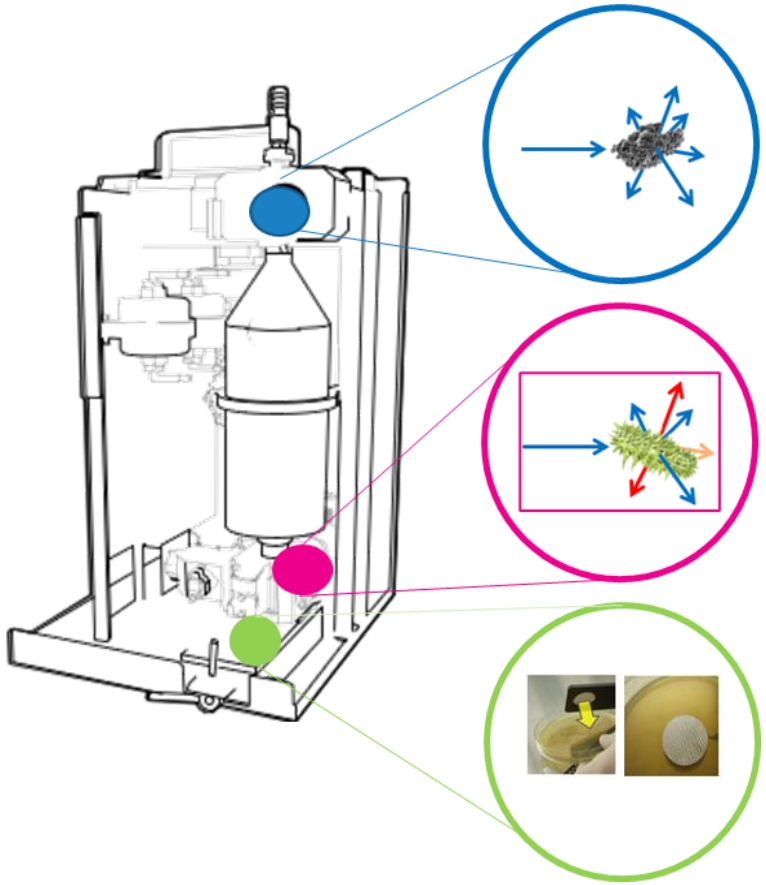
Auto-Fluorescent Unit (AFU) is a unit that reflects both size and fluorescence of the particle that can detect viable but non-culturable cells in a sample

New technology requires a new monitoring approach

CFU ≠ AFU



Alternative Monitoring System



Total Particle Counter

- Light Scatter
- ISO 21501-4 Compliant


or

Biofluorescent Particle Counter

- Detection by Laser Induced Fluorescence (LIF)
- Light Scatter and Fluorescence

Particle Collection

- Captures particles on gelatin filter
- Provides opportunity for identification

CASE STUDY – LABENA d.o.o. - Microbiology laboratory

Real-Time Microbial Monitoring (BFPC)

Dražana Radonjić, Labena d.o.o.

Why consider real-time viable monitoring?

Current challenges with traditional methods

- Manual process → risk of human error
- Delayed results (days) → reactive response
- Limited sampling → poor time resolution
- Viable data only → incomplete picture

What we wanted to achieve

- Real-time insight into viable contamination
- Continuous monitoring of cleanroom conditions
- Improved data integrity (automation)
- Better decision-making (proactive vs reactive)

Operator Training & Usability

Initial concern

- Handling of gelatin filters
- Risk of improper manipulation

Training approach

- 2-day hands-on training
- Operator performed all steps independently
- Based on standard operating procedure

Outcome

- No issues with filter handling or exchange
- Process quickly became routine
- High operator confidence

Sample processing

- Filters placed directly on solid media (standard method)

Key message:
BioTrak operation is practical and easily adoptable in routine work

Labena Case Study – Study Design

Environment

- Sterile area (Grade B, GMP)
- Laminar airflow cabinet (Grade A)

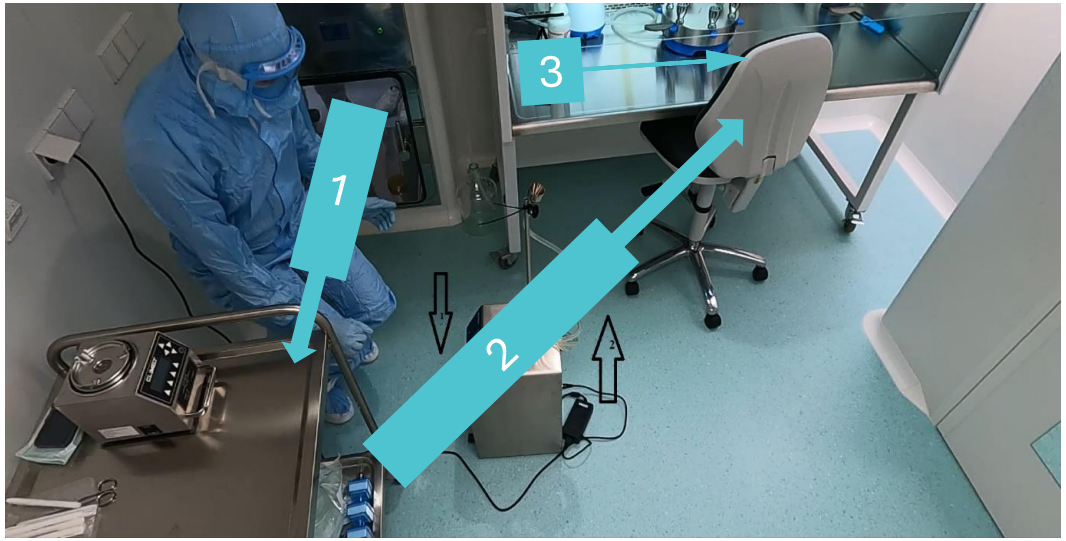
Monitoring locations (3 critical points)

1) Material transfer from pass-box → trolley
2) Transfer from trolley → laminar cabinet
3) Sterility testing inside laminar cabinet

Study approach

- Comparison of:
  - **Compliant (protocol-based) operations**
  - **Non-compliant / deviations**

- Parallel monitoring:
  - Traditional methods (CFU)
  - **BFPC real-time monitoring (BioTrak)**



Study Design & Monitoring Approach

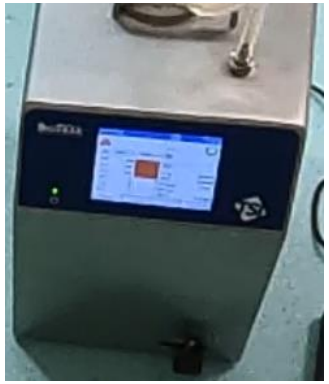
Parallel monitoring using traditional and real-time methods

2 days of monitoring:

10.03.2026 → compliant handling
11.03.2026 → non-compliant handling

Sampling methods (traditional)

- Active air sampling
- Passive sampling (settle plates)
- Surface imprints
- Glove / suit prints



Additional monitoring (BioTrak)

- Total particles (real-time)
- Viable particles (real-time, BFPC)
- Gelatin filter sampling (for CFU comparison)

Traditional Monitoring Results (Control Measurements)

Results

All measurements: 0 CFU

- Across all locations
- Across all sampling methods
- In both compliant and non-compliant scenarios

Key observation

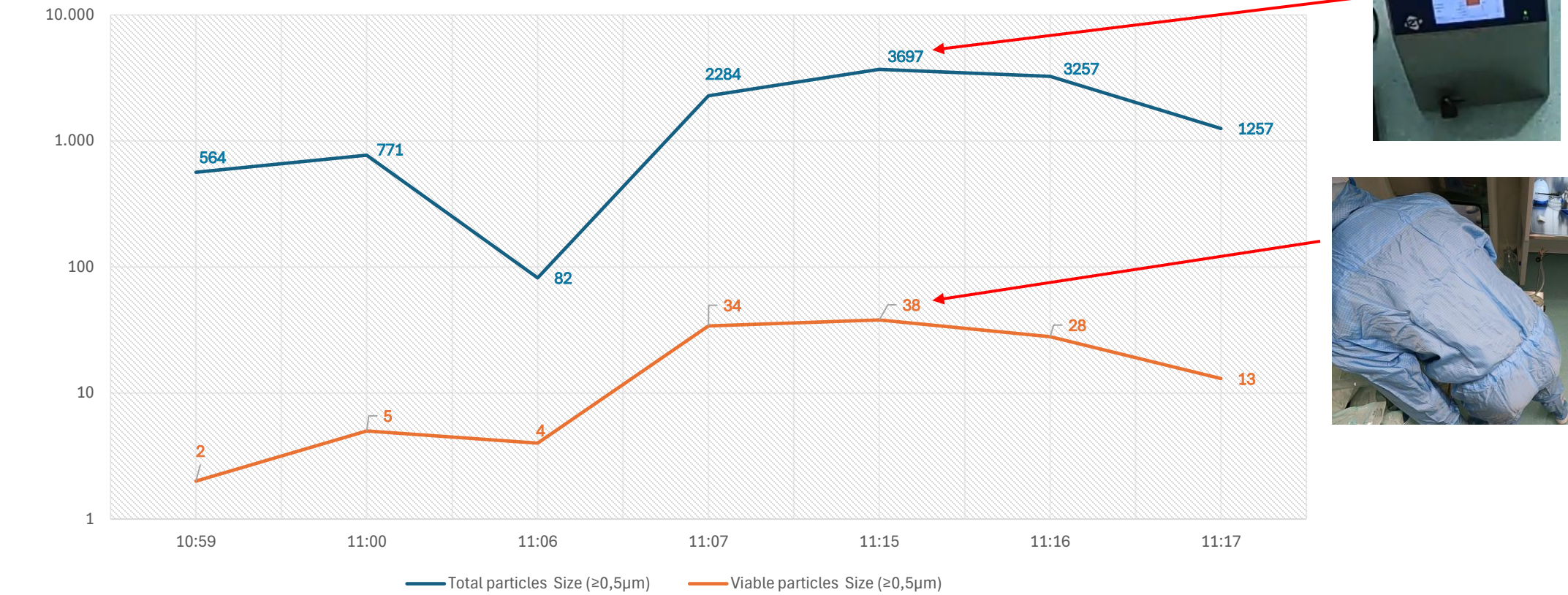
No difference detected between correct and incorrect handling

Implication

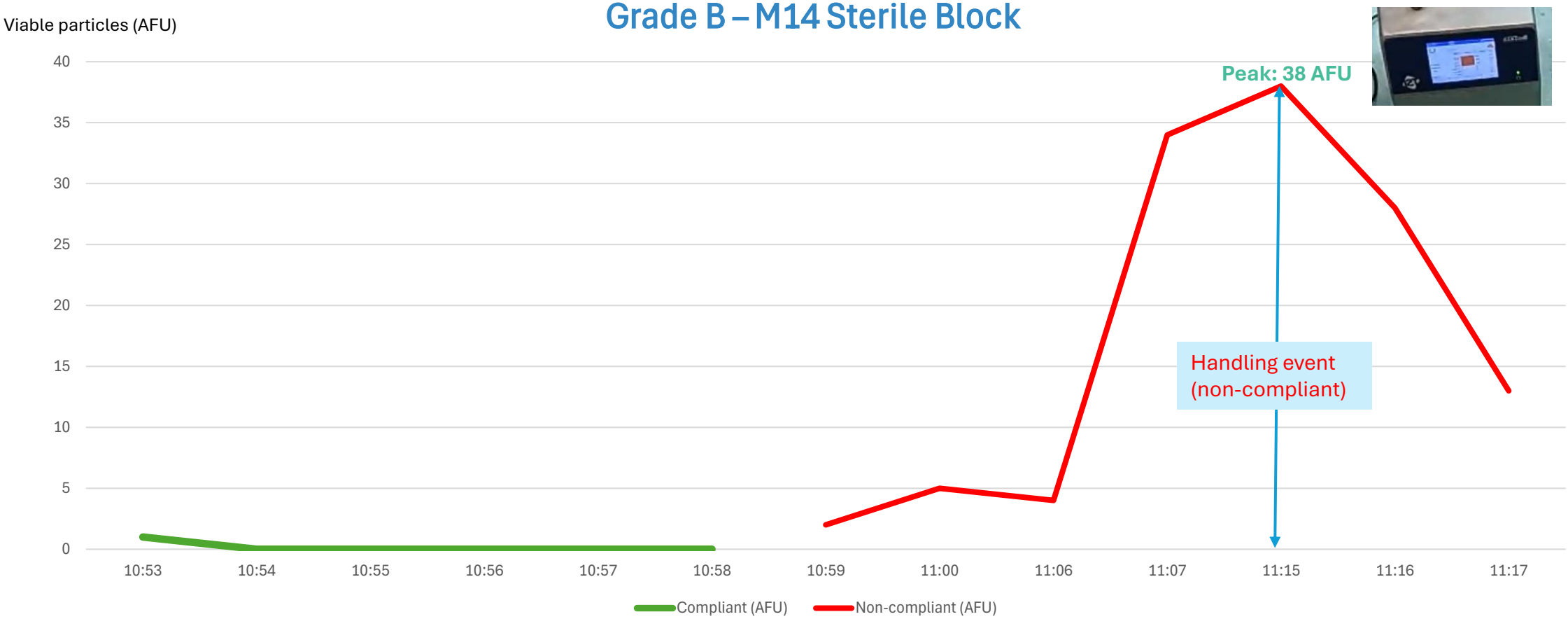
Traditional methods did not detect any contamination events

Real-Time Monitoring Reveals Viable Particle Events (Grade B, M14)

Non-compliant handling: transfer from trolley to laminar (11.03.2026)



Impact of Handling on Viable Particles (AFU)
Grade B – M14 Sterile Block



Traditional methods: 0 CFU → no signal
BioTrak: detects real-time excursions

From Evaluation to Implementation

Next Steps for BioTrak Adoption

What we learned

🟩 **Study Outcome**

- Traditional EM: **0 CFU detected**
- BioTrak: **real-time viable particle detection**
- Clear difference between:
  - ✓ compliant handling
  - ❌ non-compliant handling

🔬 **Key Insight**

- Contamination events **do occur**
- But are **not captured by growth-based methods**
- BioTrak provides **time-resolved visibility**

👉 **Real-time insight = better process understanding**

What we do next

🚀 **Next Steps**

1. Qualification

- AFU and CFU equivalence
- Side-by-side comparison
- Define acceptance criteria

2. Implementation Strategy

- Define monitoring locations
- Integrate into EM program
- Develop SOPs

3. Data & Limits

- Establish baseline
- Define alert/action levels
- Trend analysis

4. Operations

- Operator training
- Routine use procedures
- Maintenance & calibration

💡 **Final Message**

Shift from reactive → proactive contamination control

Thank you for your attention!

Slovenian Clearoom Society
Dimičeva street 13 I 1504 Ljubljana
T: +386 1 5898 312 I F: +386 1 5898 317 I E: ptz@gzs.si
https://scs.gzs.si

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

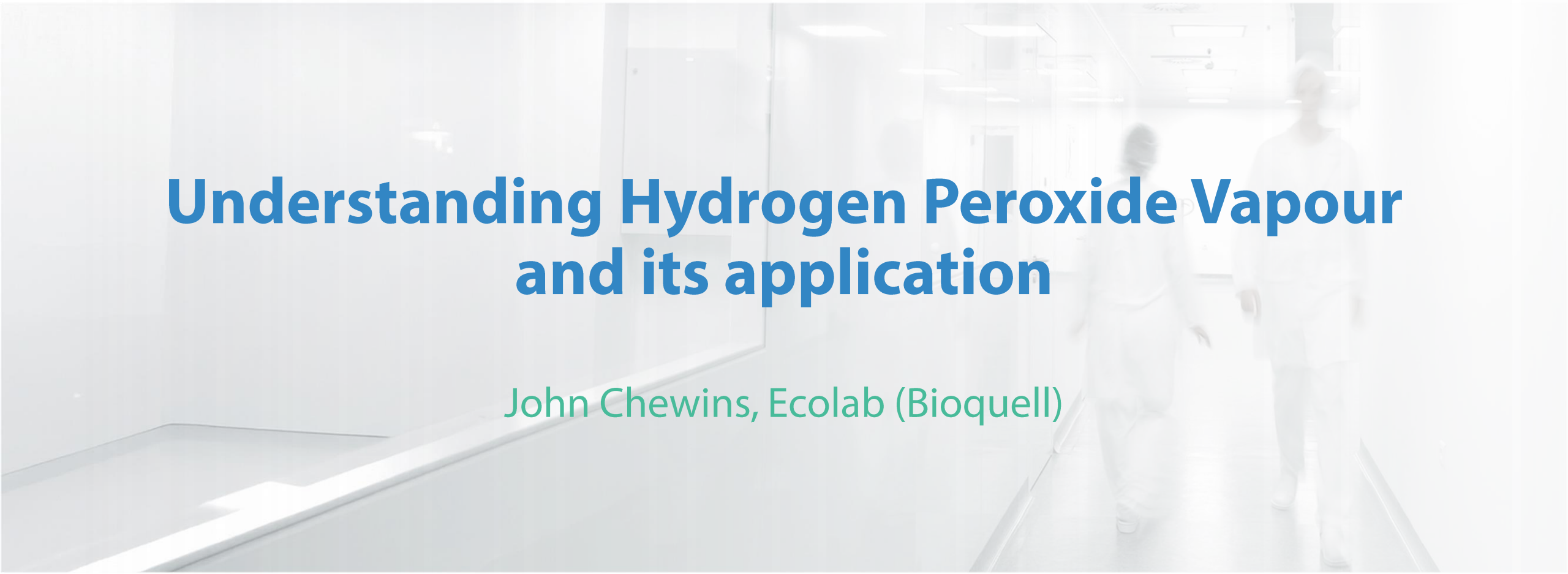
International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

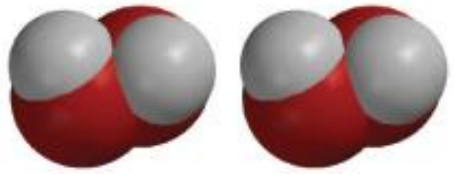
BRDO PRI KRANJU, SLOVENIA



Understanding Hydrogen Peroxide Vapour and its application

John Chewins, Ecolab (Bioquell)

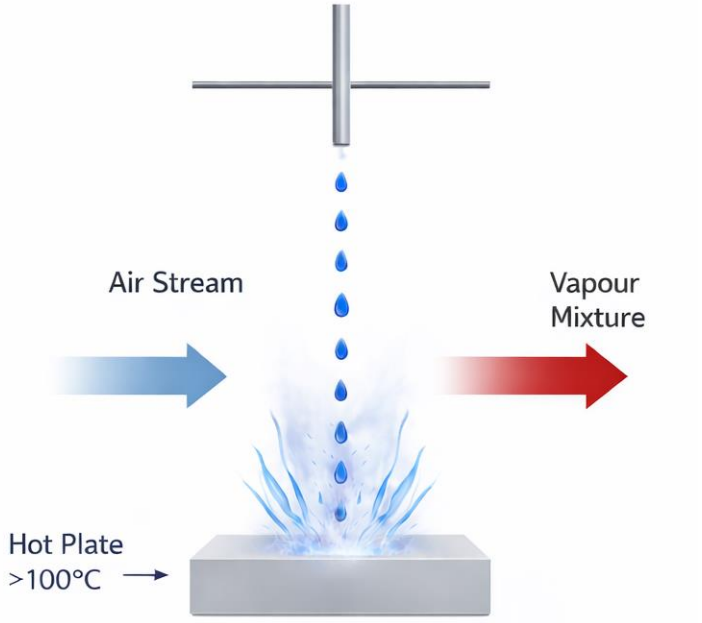
What is hydrogen peroxide vapour?



$2H_2O_2$

hydrogen peroxide

Usually 35% - disinfection / biodecontamination
Usually 50 - 59% - sterilisation



Vapour – delivered into a sealed enclosure

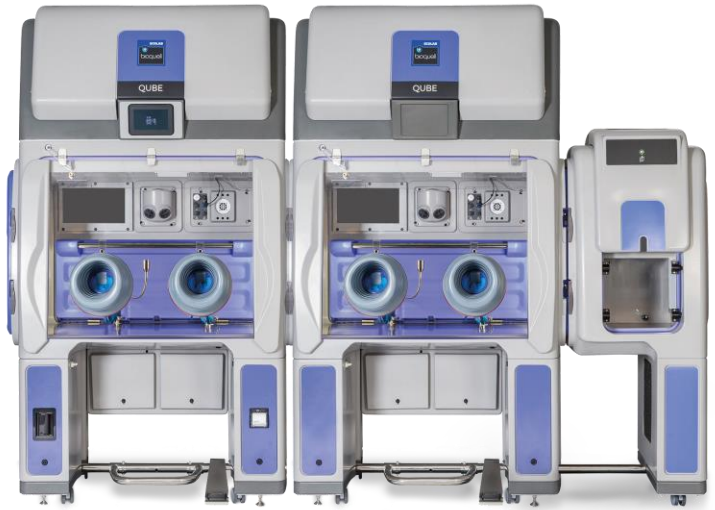
For past 25 years debate:

Microcondensation

VS.

Non-condensing

Sterilisation vs Disinfection / Biodecontamination



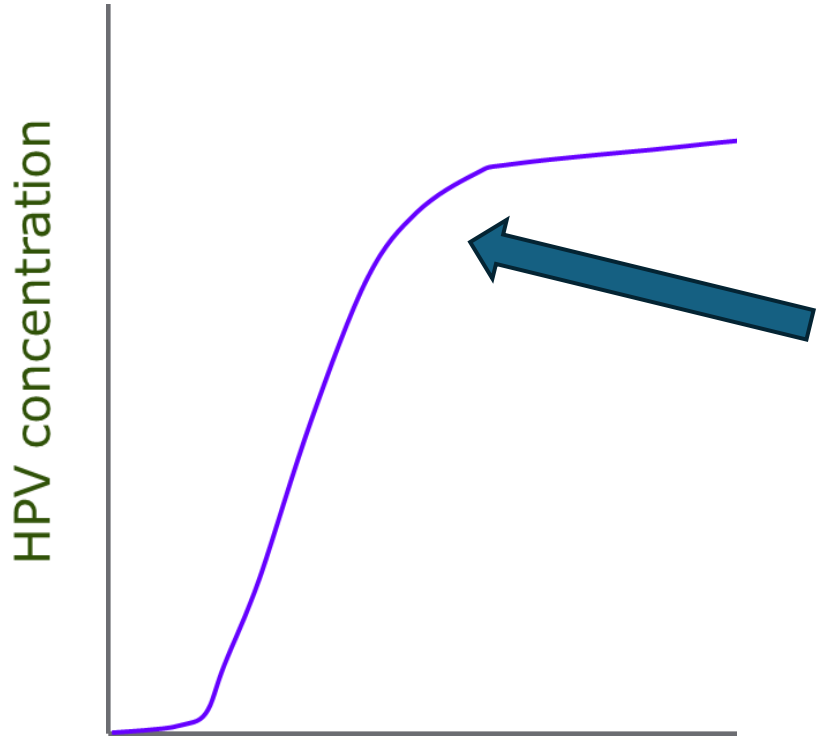
VH2O2 sterilisation – occurs under vacuum, with 50 – 60% peroxide and applies a SAL (sterility assurance level). Standardised under ISO 22441 and upcoming EN 17180

Hydrogen peroxide biodecontamination applies a sporicidal kill to exposed surfaces. It is a disinfection process, regulated in Europe under the Biocidal Products Regulation (BPR). Used to produce aseptic processing environments.

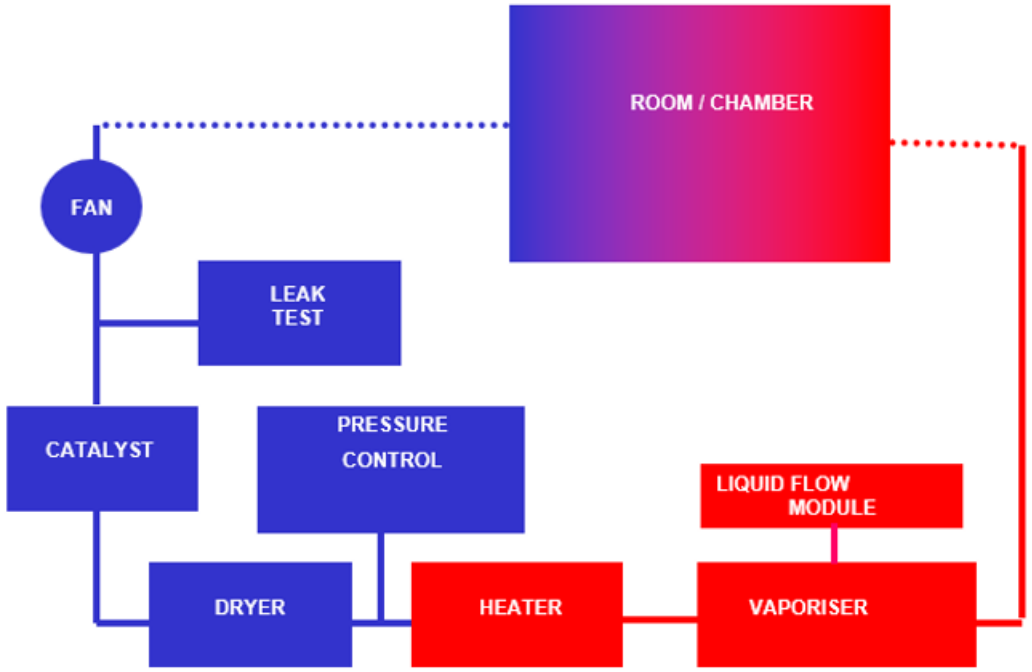
Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

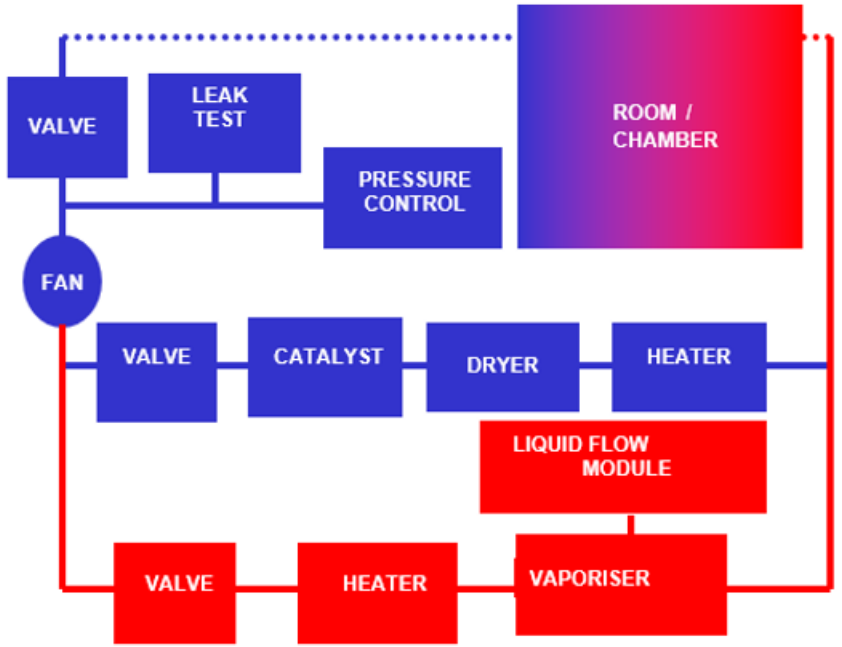
Microcondensation vs non-condensing – which is it?



Why does the peroxide concentration curve flatten?



"Non-condensing" process



Microcondensation process

Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

Don’t take my word for it -

J Pharm Innov (2008) 3:123–133
DOI 10.1007/s12247-008-9027-1

RESEARCH ARTICLE

The Influence of Humidity, Hydrogen Peroxide Concentration, and Condensation on the Inactivation of *Geobacillus stearothermophilus* Spores with Hydrogen Peroxide Vapor

Beatriz Unger-Bimczok · Volker Kottke · Christian Hertel · Johannes Rauschnabel

humidity. Subvisible condensation was found to be necessary for short inactivation times, but condensation in the visible range did not further enhance the sporicidal activity. The molecular deposition of water and hydrogen peroxide on the target surface represents the determining factor for microbial inactivation, whereas the hydrogen peroxide concentration in the gas phase is of secondary importance.

➡ Rapid hydrogen peroxide vapour biodecontamination is facilitated by microcondensation

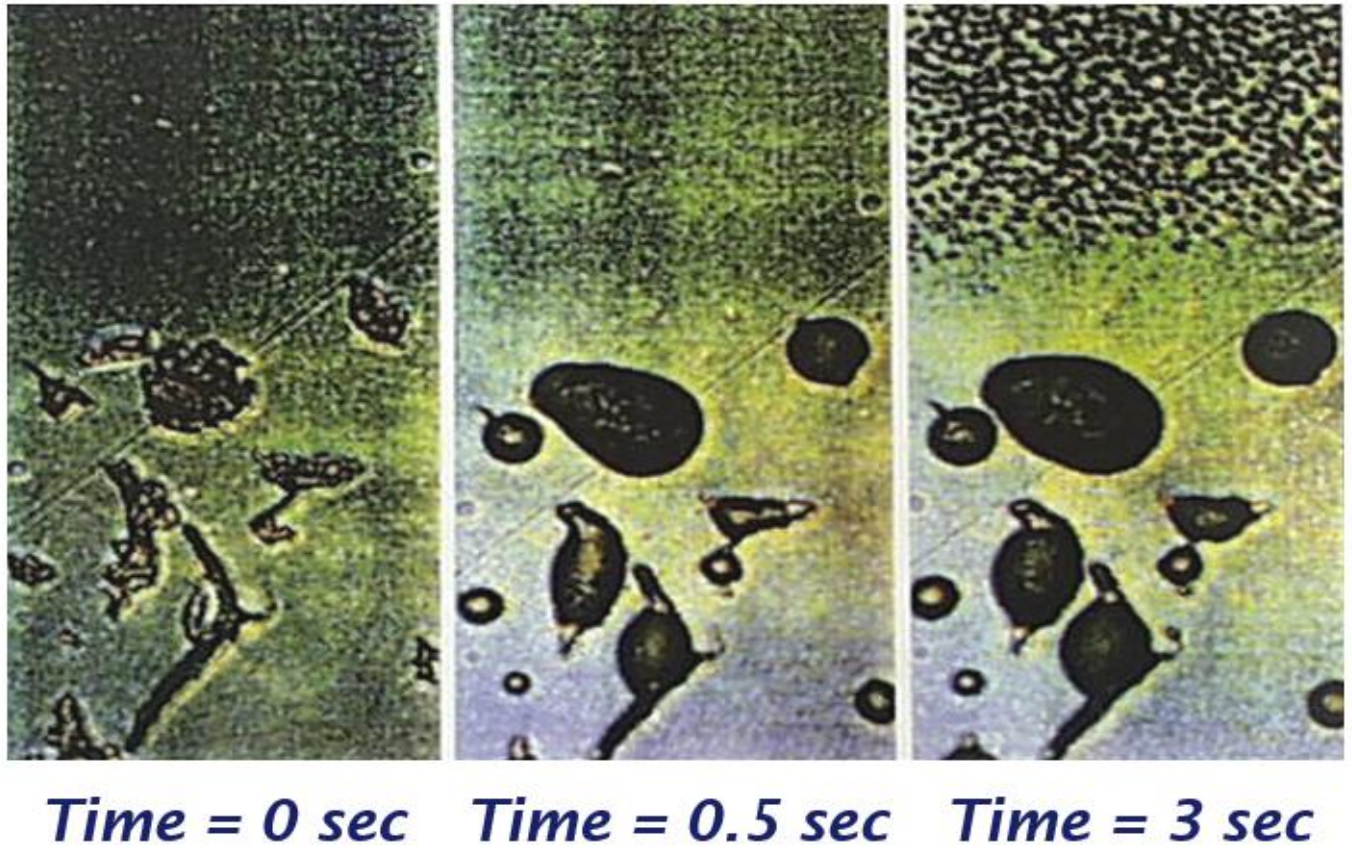
Understanding Hydrogen Peroxide Vapour (HPV) and its application
John Chewins Ecolab (Bioquell)

Microcondensation

Microcondensation forms on any exposed surface (need to be mindful of occlusion)

The microorganisms act as nucleation sites for the microcondensation – it forms on them and builds.

Image shows spores of *Bacillus marcesans* on glass



Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:
INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Hydrogen Peroxide Vapour Regulatory Compliance

30 – 31 MARCH 2026
BRDO PRI KRANJU, SLOVENIA

For HPV in EU Pharma aseptic manufacturing there are two key regulatory frameworks:

PIC/S PHARMACEUTICAL INSPECTION CONVENTION
PHARMACEUTICAL INSPECTION CO-OPERATION SCHEME

PS/INF 26/2022 (Rev. 1)
9 September 2022

REVISED ANNEX 1
(MANUFACTURE OF STERILE MEDICINAL PRODUCTS)
TO
GUIDE TO GOOD MANUFACTURING PRACTICE FOR MEDICINAL PRODUCTS

Revised Annex 1 will be incorporated into the PIC/S GMP Guide (PE 009) and enter into force on 25 August 2023, except for point 8.123 which is postponed until 25 August 2024

Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

Active substance manufacturer

ECHA list of approved actives

Product authorisation

H_2O_2

- Active substances can only come from manufacturers on the Article 95 list
- If active substances do not obtain approval, they cannot be used in biocidal products

Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

PT	Product Type	PT	Product Type
1	Human Hygiene	12	Slimicides
2	Disinfectants not intended for direct applications to humans or animals	13	Working & cutting fluid preservatives
3	Veterinary Hygiene	14	Rodenticides
4	Food & feed areas	15	Avicides
5	Drinking water	16	Molluscicides & vermicides
6	Preservatives for products during storage	17	Piscicides
7	Film preservatives	18	Insecticides & acaricides
8	Wood preservatives	19	Repellants / Attractants
9	Fibre, rubber, leather preservatives	20	Other vertebrates
10	Construction material preservatives	21	Anti fouling products
11	Preservatives for liquid cooling	22	Embalming / taxidermy

For example, Bioquell HPV-AQ has an authorisation for use in PTs 2, 3 & 4

Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

Slovenian Cleanroom Society

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

PT2

PT3

PT4

If a product is not authorised under a specific PT it cannot legally be used in that area

Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

Where is it to be used?

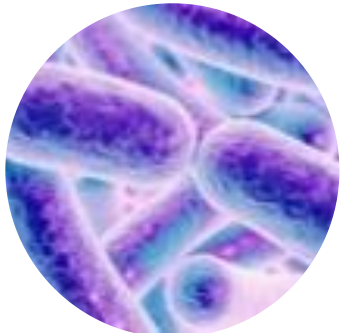
Union Authorisation – authorised for use across the entire EU

National Authorisation – authorised for use in the specific national territory ONLY

National Authorisation + Mutual recognition – authorised for use in the specific national territory + specific additional territories

- NA + MR – generally the same in which territory, but not always the case – can be significant differences in authorised use
- Bioquell HPV-AQ has a Union Authorisation – same requirements in all EU territories
- Brexit – UK HSE reviewing disinfectant products under GB BPR

What efficacy claims?

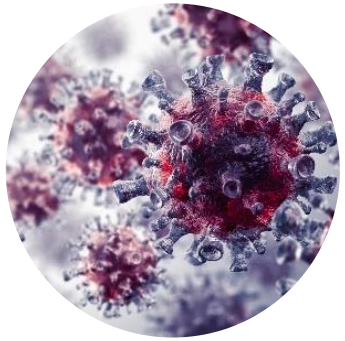


Bacteria

Mycobacteria

Spores

Fungi & Yeasts



Viruses

Bacteriophage

➡ Important considerations – cleanrooms (sporicidal), cell & gene therapy (virucidal)

Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

What enclosure volume?

Small enclosures? Large enclosures?
Whole facilities / buildings?

Authorised enclosure volume is detailed in the products SPC

Bioquell HPV-AQ authorised for use in small enclosures (0.25m^3 – 4m^3) and large enclosures (>4m^3) – substantial amount of efficacy data required to establish these authorised claims.

Enforcement example:

Official Journal of the European Union

EN
L series

2025/495

19.3.2025

COMMISSION IMPLEMENTING DECISION (EU) 2025/495

of 17 March 2025

concerning the extension of the action taken by the Belgian Federal Public Service Health, Food Chain Safety and Environment permitting the making available on the market and use of the biocidal product [redacted] in accordance with Regulation (EU) No 528/2012 of the European Parliament and of the Council

(Only the Dutch and French texts are authentic)

The biocidal product [redacted] contains hydrogen peroxide as an active substance and is applied by automated vaporisation for the disinfection of the internal surfaces of the isolators. A Union authorisation for [redacted] has been granted, as part of the biocidal product family [redacted] Biocidal Product Family 1' (2). However, the product is authorised for disinfection of enclosures of at least 15 m^3, while the volume of the isolators at UZ Ghent is 1,5 m^3. The temporary permit granted by the Belgian competent authority allows the use of the [redacted] under different conditions than those included in the authorisation.

Emergency Use Authorisations are temporary – this one expires on the 1st May

Product Authorisations are publicly available on ECHA website

https://echa.europa.eu/information-on-chemicals/biocidal-products

Trade name	Product-type	Active Substance	Market area	Authorisation type		Compare
Bioquell **HPV-AQ**	PT02 PT03 PT04	Hydrogen peroxide	Austria Belgium Bulgaria Croatia Cyprus 23 more entries	Union authorisation		

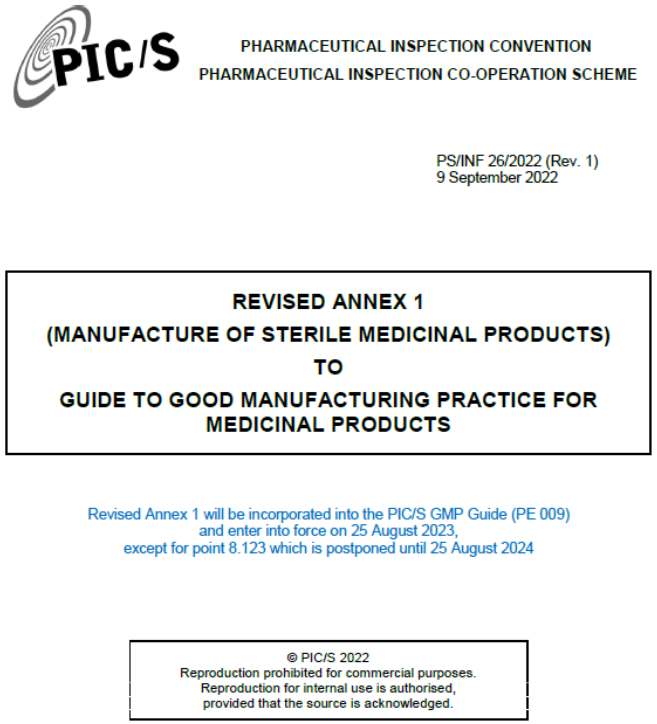
- Product Assessment Reports (PAR) and Summary of Product Characteristics (SPC) are freely available
- Speak to your biocide supplier if you have any questions or concerns (and get responses in writing)

GMP Annex 1

Annex 1 is actually a Guidance document – however, it is globally applied (via PICS) to regulate medicinal product manufacture

A fundamental principle of Annex 1 is moving manufacturing from open to closed system processing (i.e. remove humans as a contamination source)

"Any alternative approaches to the use of RABS or isolators should be justified"



PIC/S
PHARMACEUTICAL INSPECTION CONVENTION
PHARMACEUTICAL INSPECTION CO-OPERATION SCHEME

PS/INF 26/2022 (Rev. 1)
9 September 2022

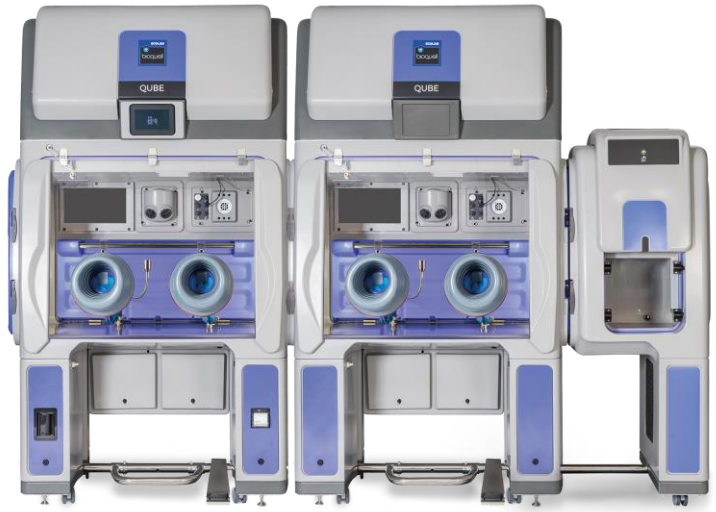
REVISED ANNEX 1
(MANUFACTURE OF STERILE MEDICINAL PRODUCTS)
TO
GUIDE TO GOOD MANUFACTURING PRACTICE FOR MEDICINAL PRODUCTS

Revised Annex 1 will be incorporated into the PIC/S GMP Guide (PE 009) and enter into force on 25 August 2023, except for point 8.123 which is postponed until 25 August 2024

Application of HPV within closed systems (Isolators)

Section 4.22 i) **Isolators**

*The bio-decontamination process of the interior should be **automated**, **validated** and controlled within **defined cycle parameters** and should include a **sporicidal** agent in a suitable form (e.g. **gaseous or vaporized** form). Methods used (cleaning and **sporicidal bio-decontamination**) should render the interior surfaces and critical zone of the isolator free from viable microorganisms*



Application of HPV within closed systems (Isolators)

*…should include a **sporicidal** agent in a suitable form (e.g. **gaseous or vaporized** form). Methods used (cleaning and **sporicidal bio-decontamination**)…*

Note “bio-decontamination” not sterilisation. What is a sporicidal agent?

Bio-decontamination – A process that eliminates viable bioburden via use of sporicidal chemical agents

*Sporicidal agent – An agent that destroys **bacterial and fungal spores** when used in sufficient concentration for a specified contact time. It is expected to kill all vegetative microorganisms*

Hydrogen peroxide example:

4.1. **Use description**

Table 1. Use # 1 – Surface disinfection by vaporized hydrogen peroxide (VHP) process

Product type	PT02 - Disinfectants and algaecides not intended for direct application to humans or animals (Disinfectants)
Where relevant, an exact description of the authorised use	-
Target organism(s) (including development stage)	Common name: Bacteria Development stage: - Common name: Yeasts Development stage: -
Field(s) of use	Indoor Disinfection of dry surfaces and equipment in hospital rooms, laboratories and other enclosed spaces, which do not come in contact with food and feed.
Application method(s)	Method: Vaporization Detailed description: Automated disinfection with Vaporized Hydrogen Peroxide, generated with aid of a VHP generator. Main specifications of a VHP generator

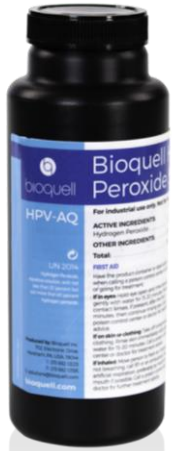
- 35% hydrogen peroxide – specific use for vaporised hydrogen peroxide surface disinfection
- Only authorised for use with bacteria and yeasts – not authorised for use with spores or fungi

Hydrogen peroxide example:

	Diffusion principle: vaporization, disinfection with gaseous hydrogen peroxide Room Volume: 30 - 150 m³ Product concentration: 3120 mg/m³. Relative humidity: 40 - 80% Temperature: room temperature
Application rate(s) and frequency	Application rate: The ready to use product should be applied in a hydrogen peroxide concentration of 1092 mg/m³ (780 ppm) by the VHP generator. Dilution (%): Number and timing of application: Contact time: ≥ 4 hours Frequency: daily /if required Max 3 times per day

- Authorisation limited to 30 – 150m3 – cannot be used in isolators, transfer chambers, etc.
- Validated contact time in standardised efficacy testing = min 4 hours

Bioquell HPV-AQ Union Authorisation number = EU-0027469-0000



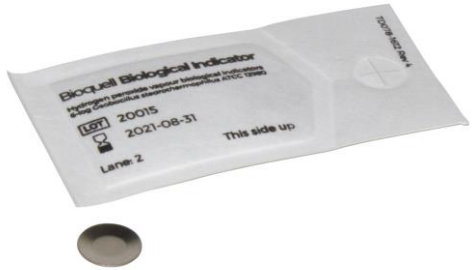
Bioquell HPV-AQ dossier submitted in Jan 2017 – **Took 5.5 years to obtain Union Authorisation**

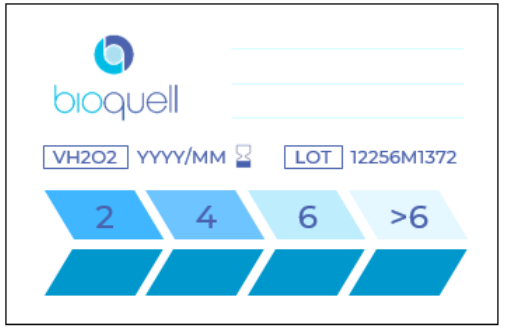
- Valid from 7th Aug 2022 to 31st July 2032
- Covers use in Bioquell Hydrogen Peroxide Vapour systems
- PT2, PT3 (restricted – cages & cage racks) & PT4
- Large (>4m3 to unlimited) and small (0.25m3 to 4m3) enclosures
- Efficacy – all microorganism groups (bacteria, mycobacteria, spores, yeasts, fungi, viruses and bacteriophage)
- Cycle – in standardised NFT 72-281 or EN 17272 test = 35 min dwell time

Application of HPV in closed systems (isolators)

....*should be* ***automated***, ***validated*** *and controlled within* ***defined cycle parameters***

- Hydrogen peroxide vapour systems are automated
- How do you produce a defined validated cycle?





Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

How do you validate an isolator cycle?

- Hydrogen peroxide generator qualification
  - Documented Installation and Operational qualification (IQ / OQ)
- Determine the isolator set-up
  - Pre or post production cycle vs in-process cycles
  - Identification and justification of the worst case load
  - Determine and organise the elements that will impact the isolator decontamination cycle (airflows, temperatures, etc)
  - Determine challenging locations from a kill perspective
- Cycle development
  - Determining cycle parameters required to achieve desired kill
- Performance qualification (PQ)
  - 3 replicate cycles achieving 100% BI inactivation for the given load

Load organisation

Load organisation and positioning is critical to ensure a successful decontamination

Must ensure:

- repeatable positioning
- minimal occlusion
- good vapour distribution
- sufficient space to work

Cycle development

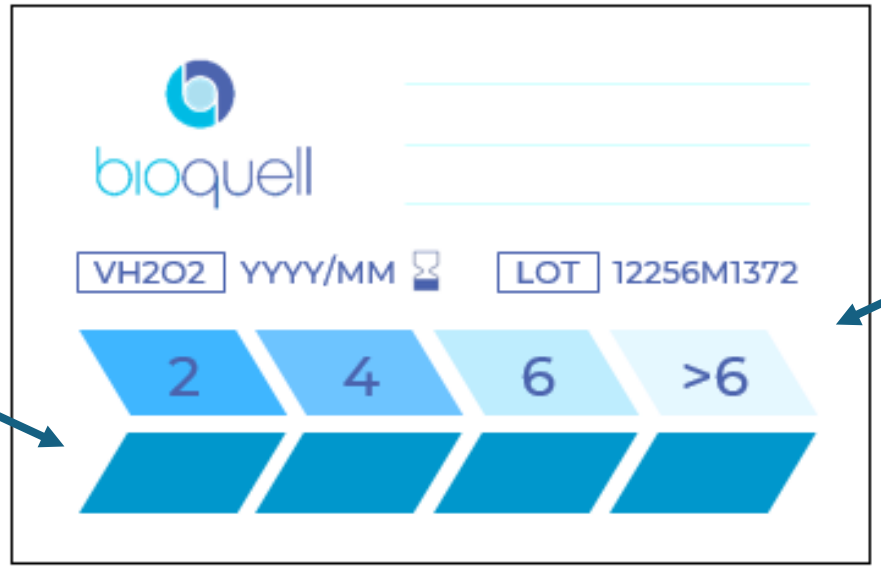
Historically gassing cycle development (GCD) approached as cycle fractional removal or lethal / sub lethal cycles

GCD could take weeks due to cycle repeats and waiting for BI grow out times (even if using 24 hour readings)

➡ Bioquell has been using a different approach for many years and we are seeing this approach gaining traction in the industry / with our customers

Cycle development - a faster approach (range finder cycles)

Reactive ink panels – convert from blue to white on progressive exposure to hydrogen peroxide



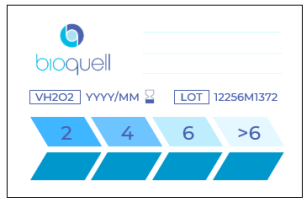
Comparison panels show the equivalent BI log reduction achieved

Provides visual real-time read of cycle progress & vapour distribution – instantaneous feedback, no need to wait 1 – 7 days!

Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

Performance Qualification



Development cycle

PQ cycle (1,2 or 3) single BIs

+'ve BI ?

NO

Continue PQ

3 clear cycles

YES

+'ve BI >2 ?

NO

Conduct evaluation cycle

YES

Investigate cause

Establish new development cycle

Investigation concludes inappropriate cycle

Understanding Hydrogen Peroxide Vapour (HPV) and its application

John Chewins Ecolab (Bioquell)

Single vs triplicate BIs?

Standard Cycle

Investigation / Evaluation Cycle

- Rogue BIs can occur in a population – a manufacturer can't test every BI!
- Bioquell use single BIs in a cycle – if 1 location shows growth, conduct a repeat with triplicate BIs at the location. If all 3 BIs show zero growth – supports Rogue, run replacement cycle. If not, redevelop cycle

Thank you for your attention!

Slovenian Clearoom Society
Dimičeva street 13 I 1504 Ljubljana
T: +386 1 5898 312 I F: +386 1 5898 317 I E: ptz@gzs.si
https://scs.gzs.si

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Panel Discussion & Q&A to wrap up the day's proceedings

Moderator: Conor Murray, Principal, 3dimension Cleanrooms, Irish SME and Head of Delegation for NSAI, Irish national standards body, Ireland

Govorci

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

Concluding remarks & Day 1 summary

Nataša Štirn

Expert for Cleanroom Technology & Quality Systems, Klimer Štirn & Co d.n.o., Slovenia, President SCS

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA

Slovenian Cleanroom Society

PTZ Opportunity Tradition Knowledge
CHAMBER OF SMALL BUSINESS AND TRADE

Gospodarska zbornica Slovenije
Chamber of Commerce and Industry of Slovenia

International Conference

CLEANROOMS TODAY AND TOMORROW:

INNOVATION, SUSTAINABILITY, EXCELLENCE AND REGULATORY COMPLIANCE

30 – 31 MARCH 2026

BRDO PRI KRANJU, SLOVENIA