Sterility by Design

Isolator System

Vikram Shukla

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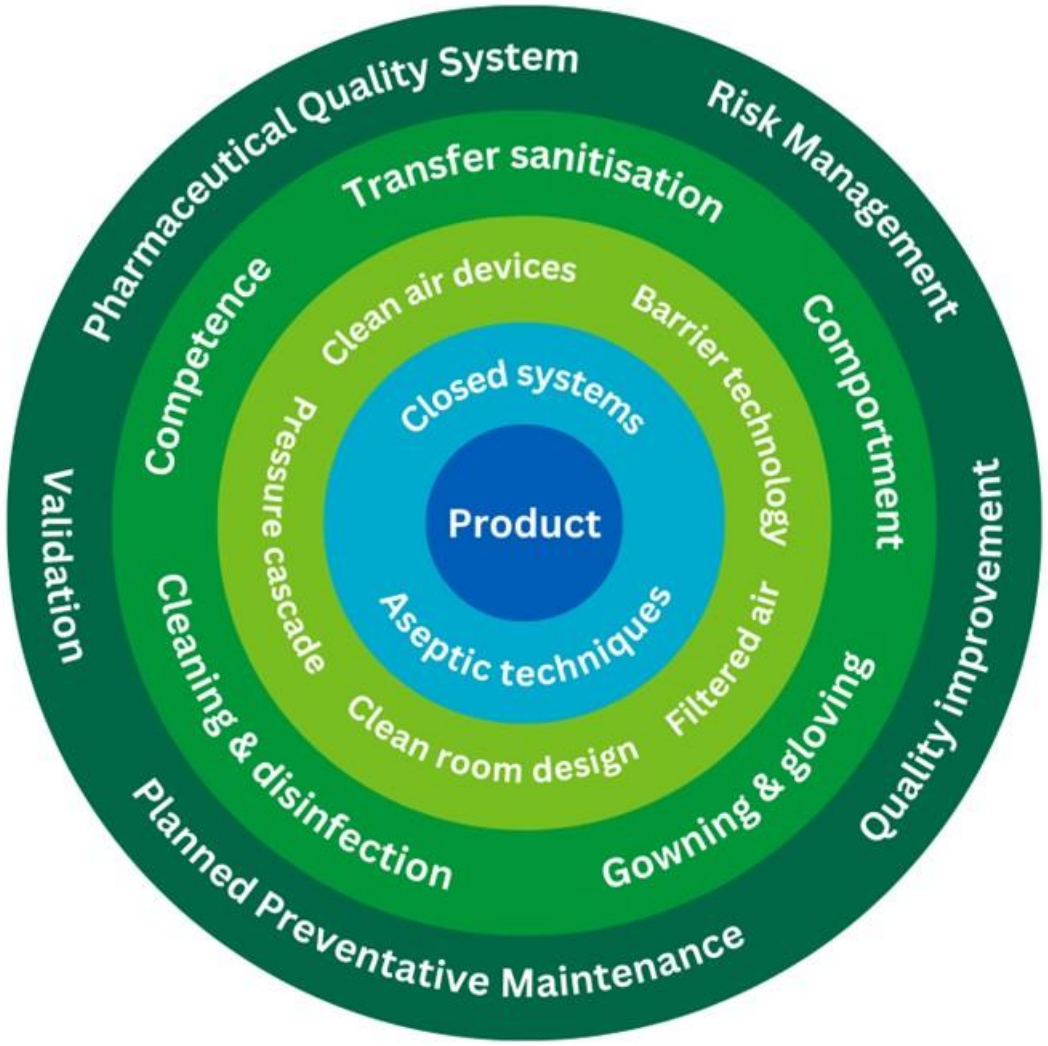
Isolator Key for Sterility of the Product



Key focus area to ensure product sterility by design

- Design of Isolator
- Operational Controls
- Cleaning and disinfection
- Decontamination
- Validation
  Operator competency
- Interventions
- Maintenance

Isolator if designed, Operated and maintained well will deliver sterile product

Isolator Design

Isolator System

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Types of Isolator

There are two major types of aseptic isolators:

1

Closed isolator

- Closed / sealed during the entire operations
- material transfer via aseptic connection to auxiliary equipment

2

Open isolator

- Allow for the continuous or semi-continuous ingress and/or egress of materials during operations through one or more openings.
- Openings are engineered (e.g. using continuous overpressure) to exclude the entry of external contaminant into the isolator.

Key Design Considerations

Ergonomics/ Diversity

Cleanability

Intervention

First Air

Glove port

Material Transfer mechanism
In closed conditions
Piping transitions (Liquid)

Differential Pressure
DP Control provision
Continuous monitoring and alarms

Dynamic VHP
Automatic during cycle

Temp and Humidity
Control provision
Continuous monitoring and alarms

CIP / WIP
Based in the need

MOC of all parts within
Hydrogen Peroxide resistant

Computational Fluid Dynamics

Isolator: Key Operational Considerations

Isolator System

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Disinfection and Decontamination

Is Cleaning and disinfection of Isolator required prior to VHP Decontamination**?**

Manual Cleaning
- Remove residues, spills, and visible contamination

Disinfection
- Apply approved disinfectant (e.g., sporicidal agent)

Drying / Visual Inspection
- Ensure no residual moisture before VHP exposure

HP Decontamination Cycle
- Automatic cycle for sporicidal decontamination

Disinfection and Decontamination

Key expectations

All surfaces that that are in **any way** or at **any time** exposed to the critical zone should be sterilized or subjected to a validated sporicidal process. This includes the **resident surfaces** of the isolator and **transient surfaces** of materials moving into and out of the isolator.

Cleaning prior to the Decontamination process.

Independent monitor of critical parameter or an assured and confirmed reliability of installed monitors.

Understanding of how gas generator works (Part of training)

Critical parameters related to its operation should be identified and recorded throughout the process.

The delivery of the correct gas at the validated concentration to the isolator and/or leaving the exhaust system should be confirmed

Sterilization or decontamination of **cooling zone**.

Disinfection and Decontamination

Cleaning before Decontamination: Key considerations

- Reason for cleaning (Cross contamination, particle etc)
- Cleaning agent
- Ease of cleaning
- Tools for cleaning
- Effect of cleaning material and its residue if any
- Hold time establishment

What are unexposed parts? Does RA identify it and take necessary actions

Disinfection before Decontamination: Key considerations

- **Unexposed** parts - To decontamination process
- Complexity, Variability and effectiveness of cleaning process
- Method of disinfection

Key Points

- Quality Risk assessment of Disinfection and Decontamination process
- Pre-VHP Decontamination Bioburden

Disinfection and Decontamination

Are the isolator cleaning techniques adequate?

Disinfection and Decontamination

Decontamination Key considerations

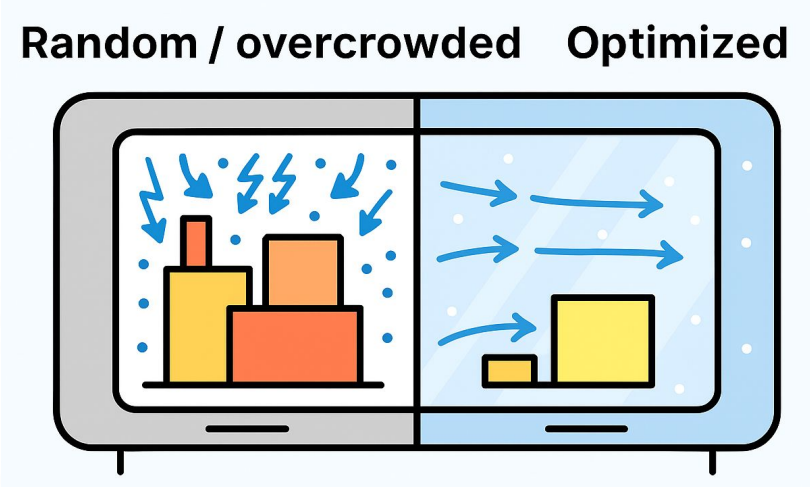
- 🤖 Automated
- 🧪 Recipe based
- 📊 Critical Parameters monitored
- 🧱 **Load pattern**
- ☁️ Decontamination
- 🌬️ Removal of decontamination agent / Aeration
- 🧠 Validated
- ✅ 6 Log reduction

📈 **Empty chamber** – Temp and Humidity studies

Cycle Development → Cycle Qualification

Should each load pattern of VHP be qualified?

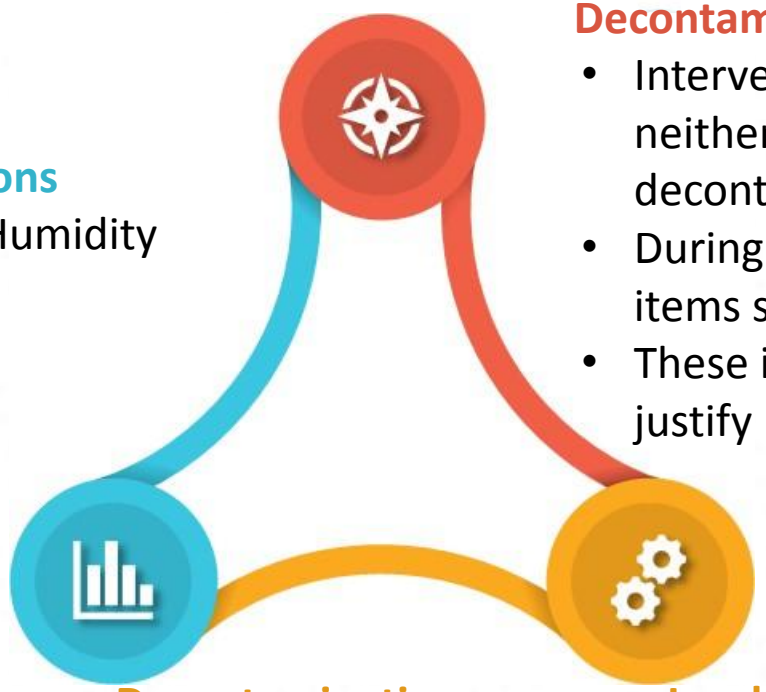
Is Load pattern important in VHP decontamination**?**



Disinfection and Decontamination

Decontamination process - Key considerations

- Environmental condition (Temperature, Humidity range, Variation)
- Fan speed / Blower speed
- Decontamination agent concentration
- Dose level
- Rate of application
- Hold time post VHP decontamination



Decontamination Key considerations

- Intervention that exposes the surface which is neither sterilized or may not have been decontaminated should be avoided.
- During batch breakdown handling which exposes items should be assessed.
- These interventions should not be simulated to justify

Decontamination process – Load configuration

- Maximum Load
- Minimum Load
- Extensions of gloves
- Position of doors and opening
- RTP ports opening
- Charecteristic of material

Disinfection and Decontamination

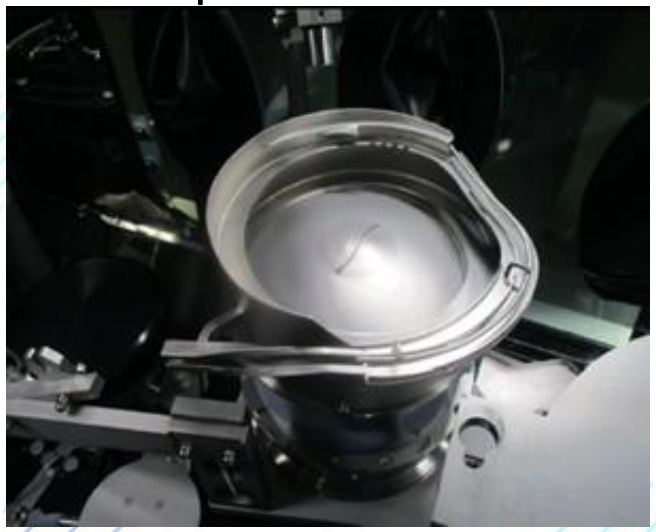
Should we know the Bio-load of Isolator and its items before Cleaning or Before Decontamination?

Disinfection and Decontamination

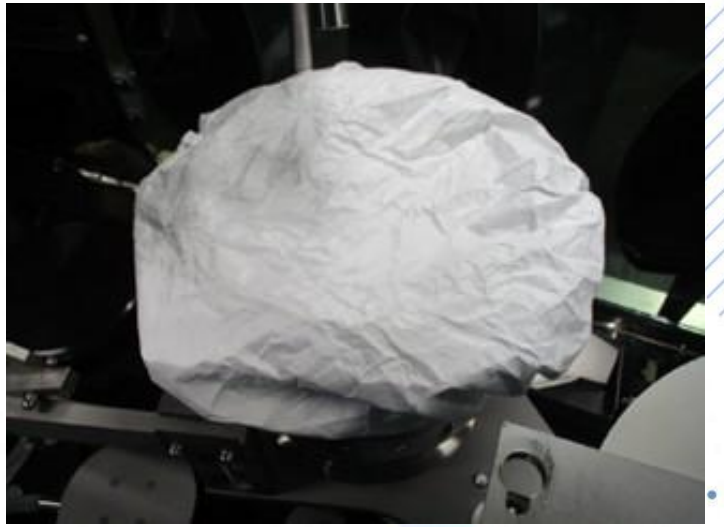
Which is better:

A sterilized In-direct product contact surfaces (E.g. Bowls, Scissors et) installed with the cover

Cover opened before VHP?



Cover opened after VHP?



Disinfection and Decontamination

Importance of Glove Extenders during VHP - Decontamination

Material Transfer-Loading

Direct Product Contact
E.g. Needles, Product Line, Filters

Options
- CIP / SIP :
- COP, Steam sterilized, Transfer and aseptic assembly post decontamination

Material transfer Criteria:
- Reduce Particulate contamination
- Reduce Microbial contamination risk

Direct Product Contact
Rubber stoppers, Tubs-Vials or PFS

Rubber Stoppers
- Steam sterilized – Transfer – Decontamination *(Integrity of wrapping)*
- RTU – Transfer via RTP
- RTS - Steam sterilized – Transfer via RTP
- Inhouse stopper processing
  - Wash – RTP bags - Steam sterilized – Transfer via RTP
  - Automatic processor – Transfer via RTP

Tubs
- E-Beam
- VHP Passbox

Key Points
- Risk assessment of transfer of material
- Packaging material (Integrity, VHP ingress, VHP compatibility,

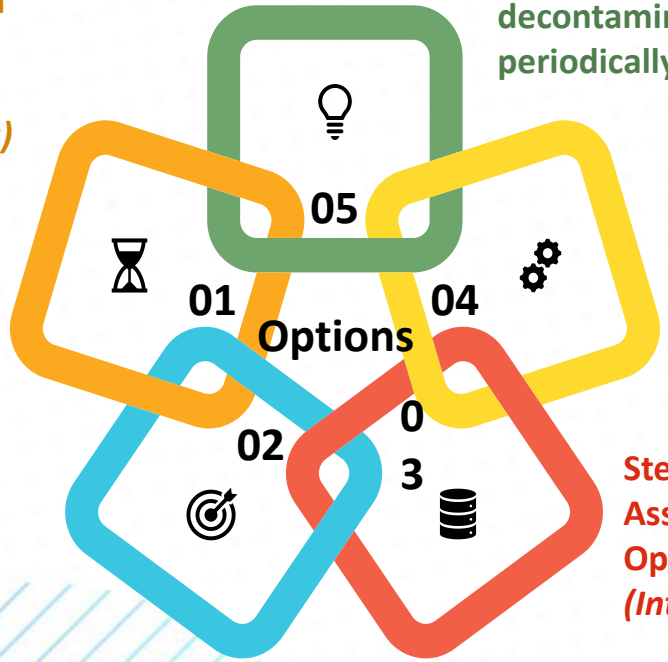
Control of Packaging material

Material Transfer-Loading: Operational Key Points

Indirect Product Contact
E.g. Stopper bowl, Tracks etc.

Steam sterilized and aseptically transferred via RTP port decontamination *(Integrity of wrapping)*

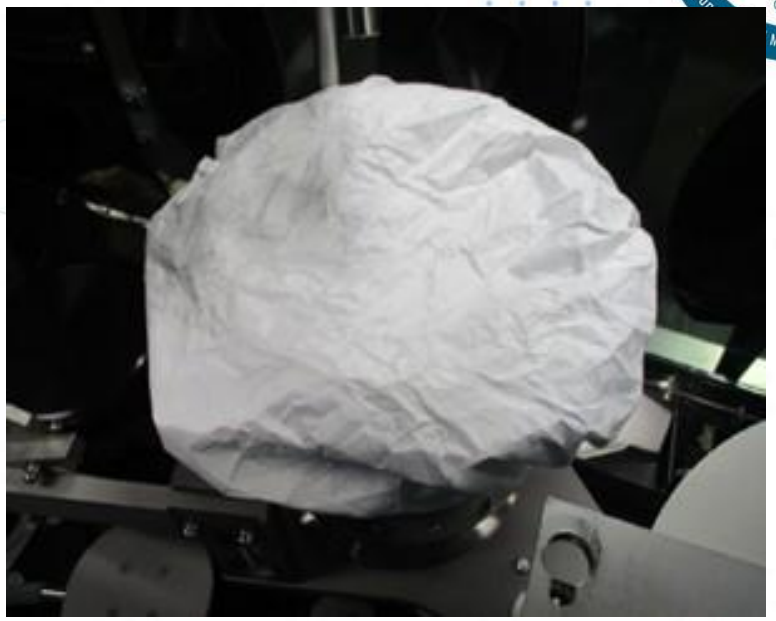
Sanitized - Installed-Open during decontamination but periodically *Sterilized*

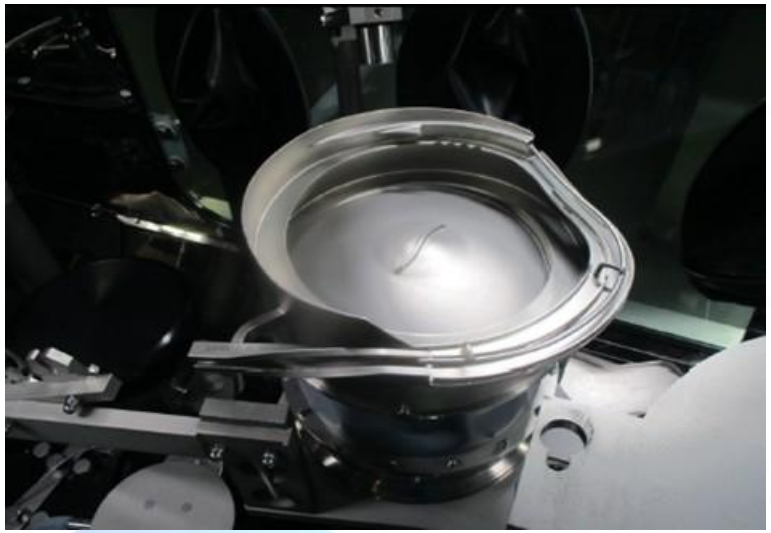


Sanitized - Installed-Open during decontamination.

Steam sterilized – Transfer - assembled - Opened during decontamination

Steam sterilized – Transfer – Assembled / Not assembled - Opened post decontamination *(Integrity of wrapping)*





Key Points

- Risk assessment of transfer of material
- Packaging material (Integrity, VHP ingress, VHP compatibility,

Material Transfer-Loading

🛡️ Sterility Assurance & Risk Management

- **Validated decontamination** of RTP surfaces and containers.
- **Double-bagging** and **protective sleeves** for sterile items.
- **Inclusion in APS** for realistic risk evaluation.

🧪 Material Transfer Risks

- **Bag integrity** must be inspected before transfer.
- **Sharp tools or fingernails** can pierce gloves or RTP seals.
- **Sunlight exposure** can degrade glove and seal materials over time.

🔧 Design & Ergonomic Risks

- Poor glove port positioning can lead to **awkward handling**, increasing contamination risk.
- **Crowding of materials** near RTP can block airflow and decontamination vapor distribution.
- **Excessive reaching/stretching** during RTP operations may compromise aseptic technique.

⚠️ Seal-Related Risks

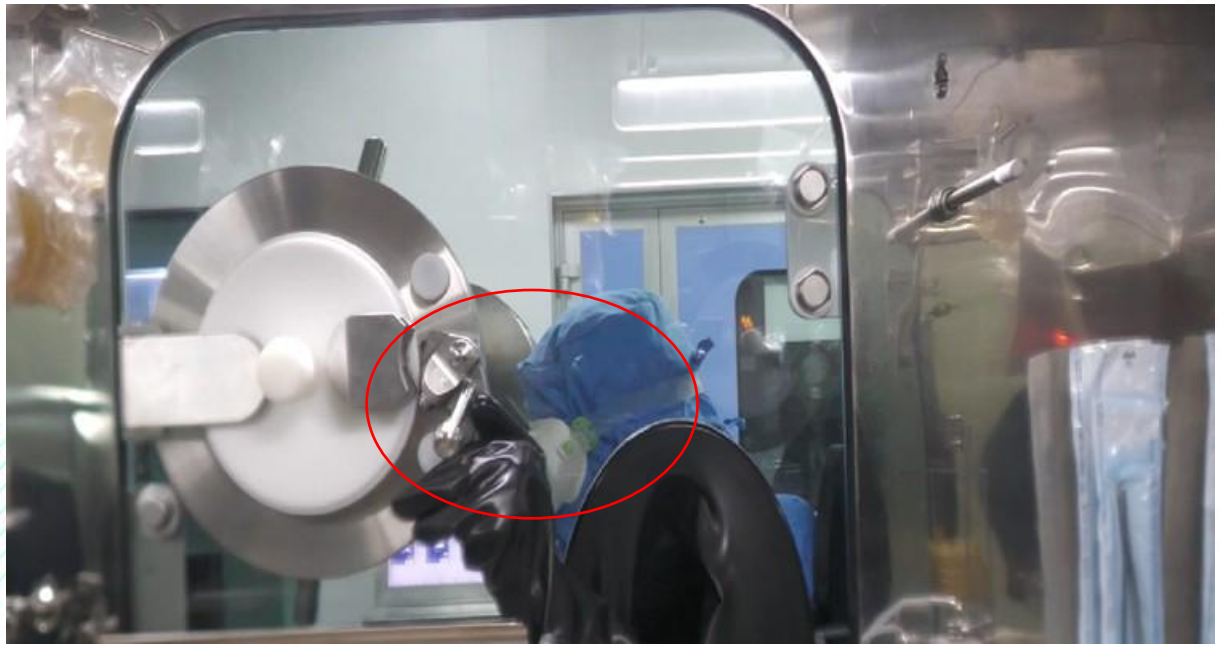
- **Inadequate sealing** between RTP α-port and β-container can compromise sterility.
- **Seal wear or damage** due to repeated use, poor alignment

🌬️ Airflow & Decontamination Risks

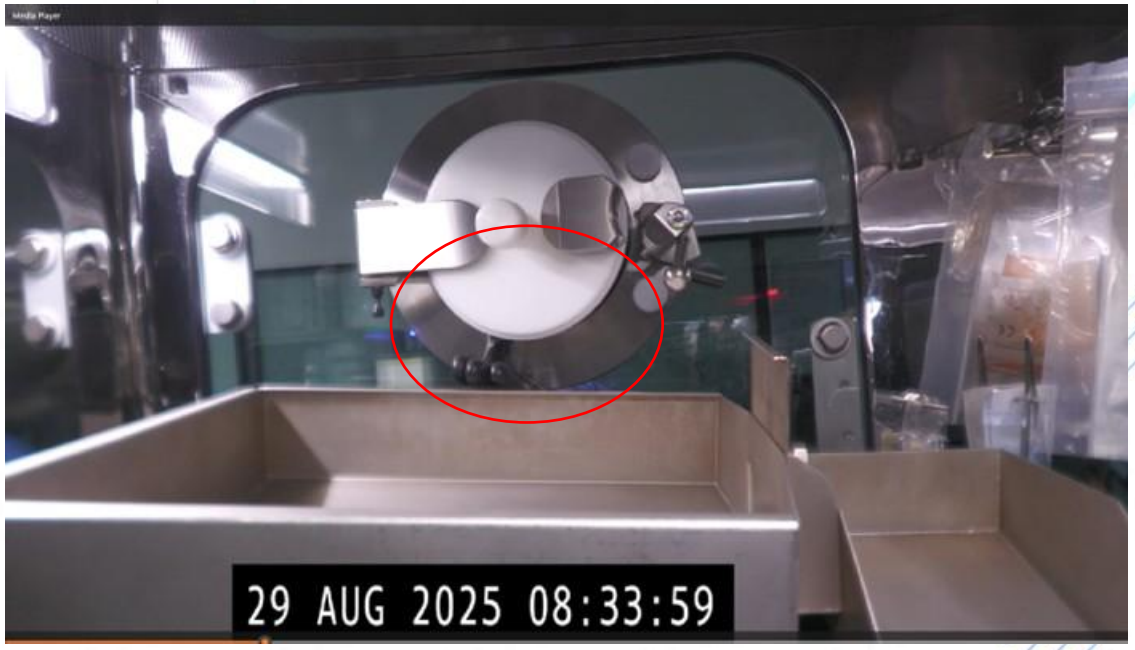
- RTP operations may **disrupt unidirectional airflow**, especially near exposed sterile product.
- **Turbulent airflow** caused by rapid movements or poor layout can spread contamination.
- **Unexposed surfaces** during VHP cycles (e.g., behind seals, under bags, or obstructed areas) may retain microbial load.

Material Transfer-Loading

Difficult to access the RTP port during material transfer



Poor design of glove port to access RTP port



Gowning

Any specific requirements for personnel working in Isolator for gowning (Grade C and when Isolator is open)?

Gowning

Should garments be “Sanitized” or “Steamed” or “Sterilized” when working in Isolator?

Gowning

Should garments be monitored for Bio load when working in Isolator?

Gowning

Are goggles required in Grade C when working in Isoaltor?

Gowning

Should gloves be sanitized before entering the gloved hand in Isolator glove?

Gloves

Are Sterile gloves (Non Isolator gloves) required when working inside isolator (When open before VHP)?

Gloves

Is Cleaning and sanitization of the Isolator glove required before VHP?

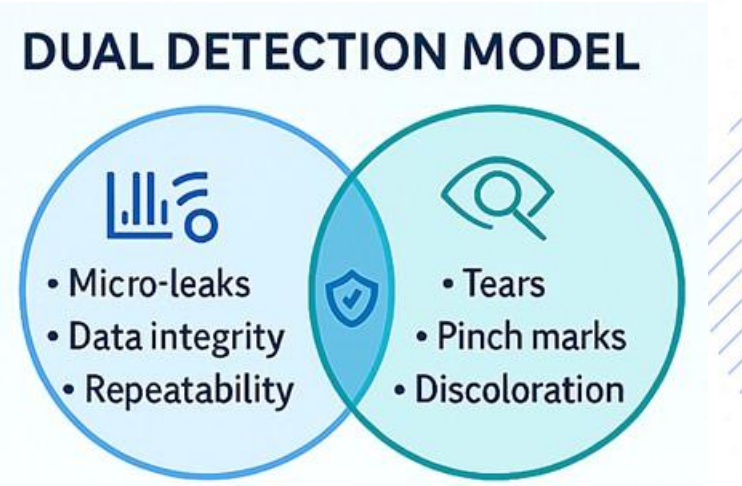
Gloves

What should be the frequency of Glove integrity testing?

Gloves

What is better:
Automated glove integrity testing?
Visual Inspection?
Both?

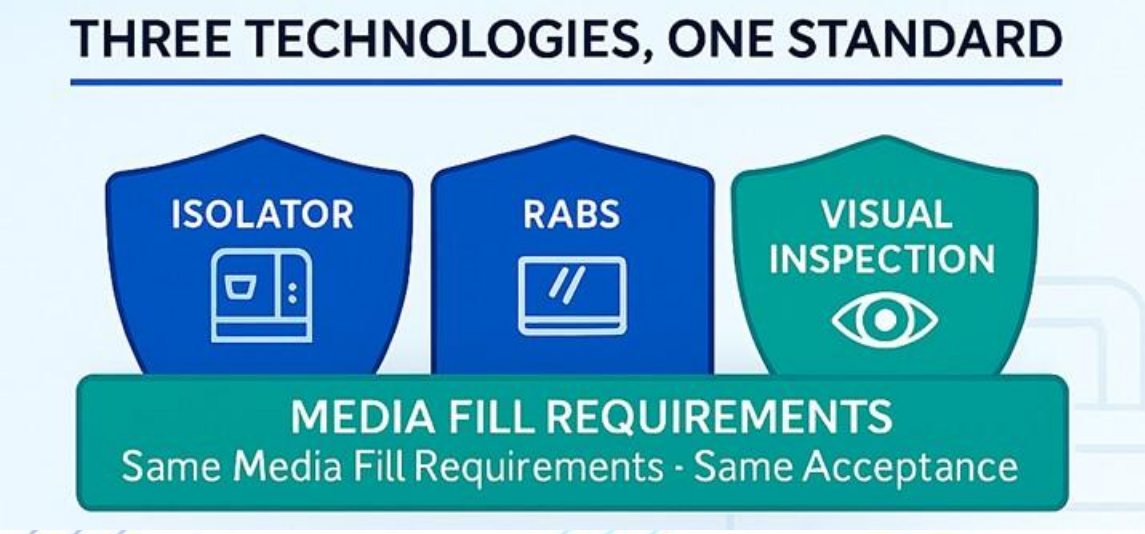
Qualification of Automated glove integrity testing and Glove Visual Inspector is important.

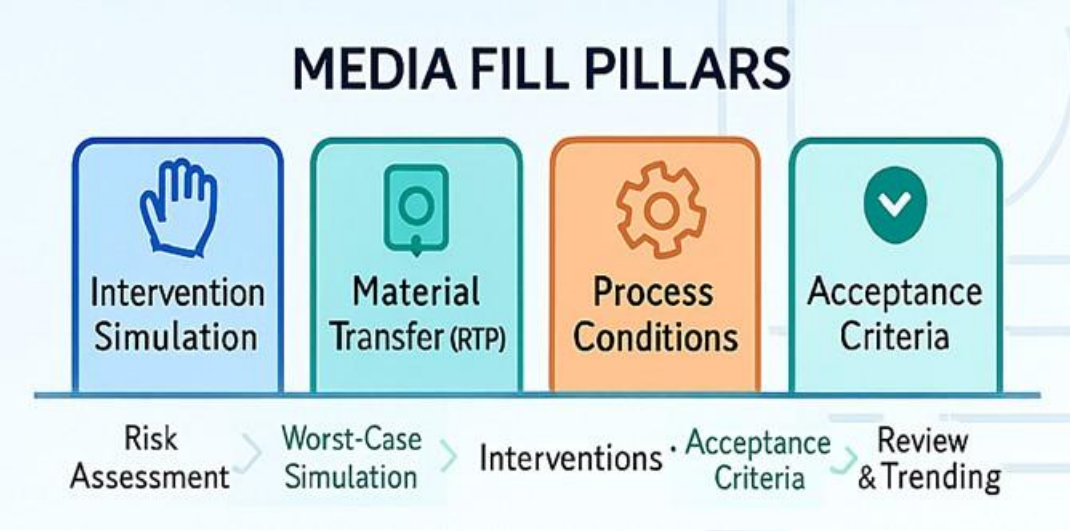


Media fill (Isolator)

Key considerations

- Same as for RABS
- All points required as per Regulatory guidance to be considered.
- No difference in RABS / Conventional media fill vs Isolator media fill. Risk based approach to be followed
- **Same acceptance criteria (Target is Zero)**

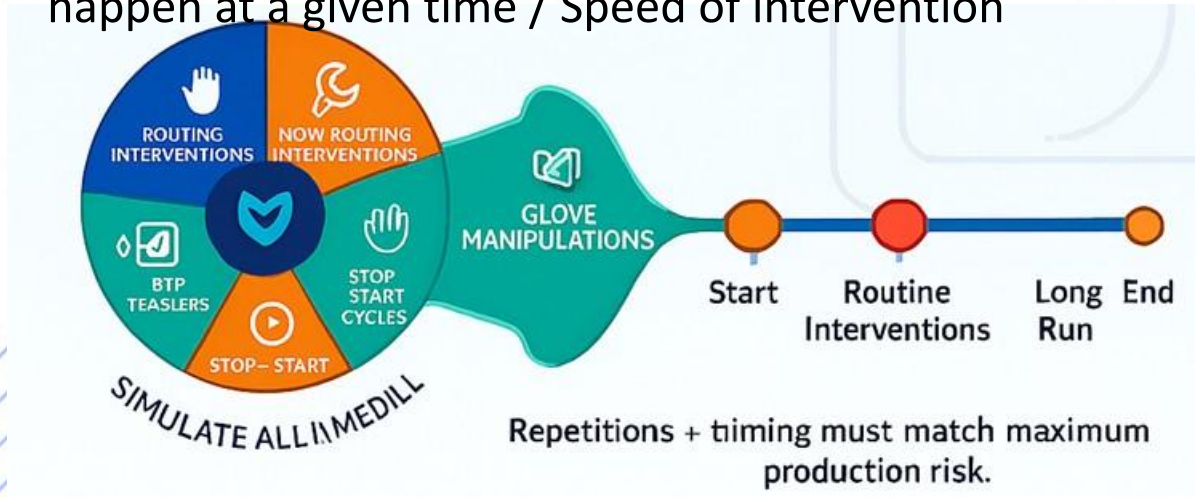




Media fill (Isolator)

Interventions for simulation

- Risk assessment / RPN
- Grouping of Interventions (By Risk or by zone or by type). Complexity of performing the interventions should be one criterial in RA
- Frequency of simulation of these interventions can done by groups or RPN number
- Some related to out side of the Isolator can be avoided (E.g. Number of persons present etc)
- No. of interventions or glove entry which can happen at a given time / Speed of intervention



Key Interventions in Isolators

- Important interventions (Not limited to)
  - Placement of hand
  - Transfer of material after VHP
  - EM
  - Staging of material post VHP
  - Assembly post VHP
  - Sampling and removal of vials
  - Component addition
  - Other corrective interventions

Bets Practice

Documented evidence that efforts were made to reduce interventions

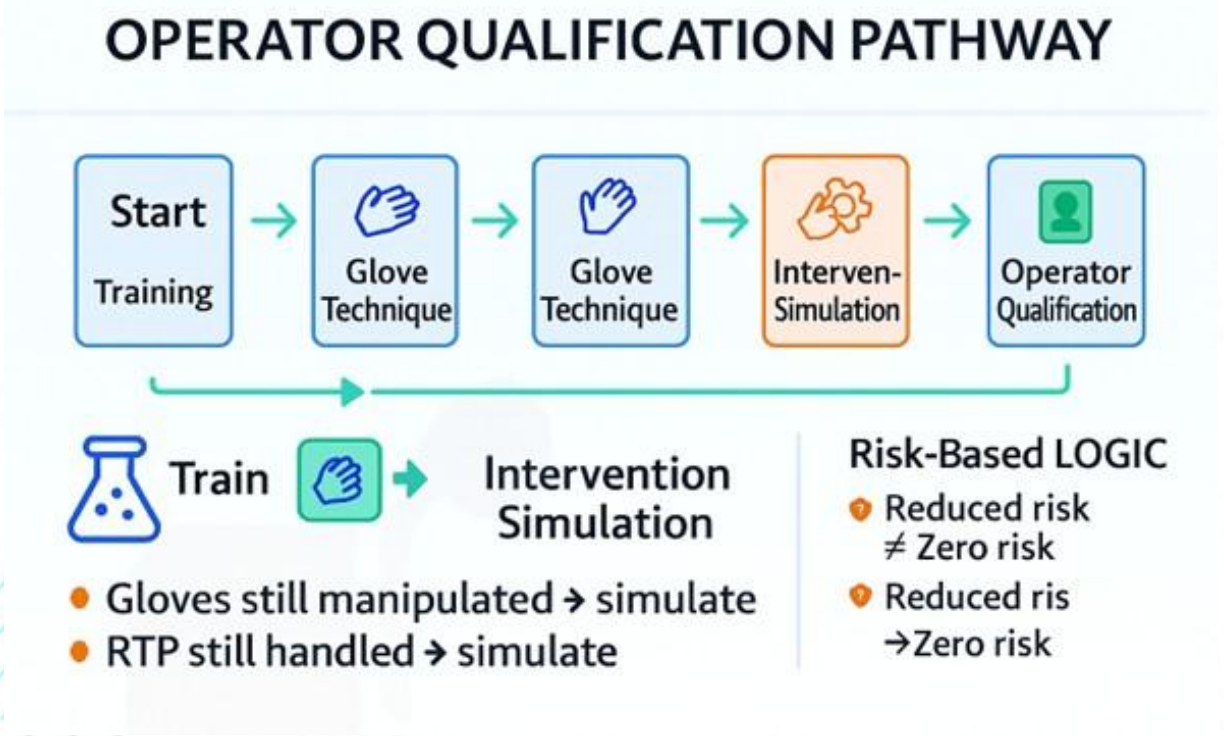
Media fill (Isolator)

Should all intervention be simulated in Isolator media fill vs Conventional / RABS media fill including repetitions and time of interventions?

1. **Operator qualification should be part of APS**

Media fill (Isolator)

Should Operator qualification should be part of APS for Isolators



Media fill (Isolator)

Should integrity be verified pre and post batch for the following

Isolator?

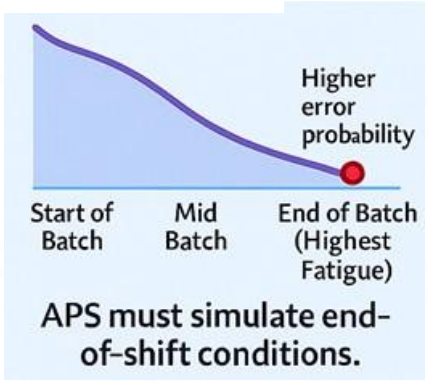
Gloves?

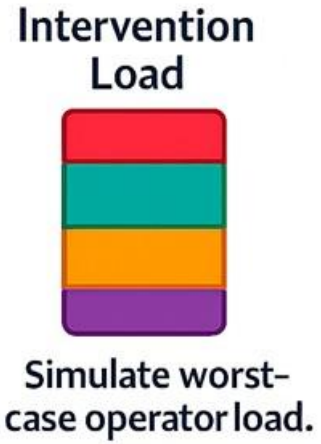
RTP containers?

All three — Isolator, Gloves, and RTP Containers — require integrity verification pre- and post-batch to maintain sterility assurance and comply with PDA, Annex 1, and industry expectations

Media fill (Isolator)

Does operator fatigue simulation important in Isolator?





“Operator Fatigue & Human Factors Still Matter in Isolators”

Media fill (Isolator)

Does Isolator provide more confidence in establishing longer fill time in multiple shifts and Multiple days?

Media fill (Isolator)

Power failure studies in be simulated Isolator?

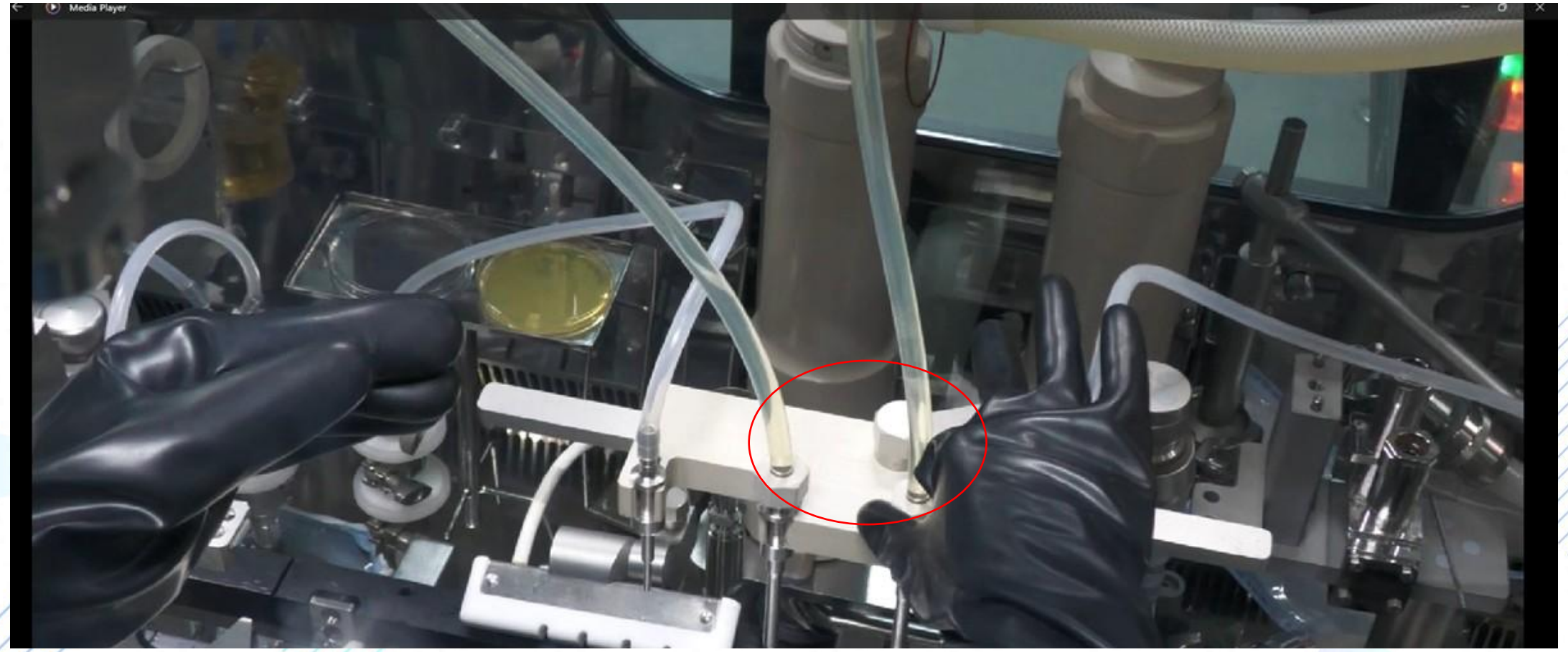
Media fill (Isolator)

Is same aseptic practices required in Isolator as is expected in conventional clean rooms?

Break in first air?

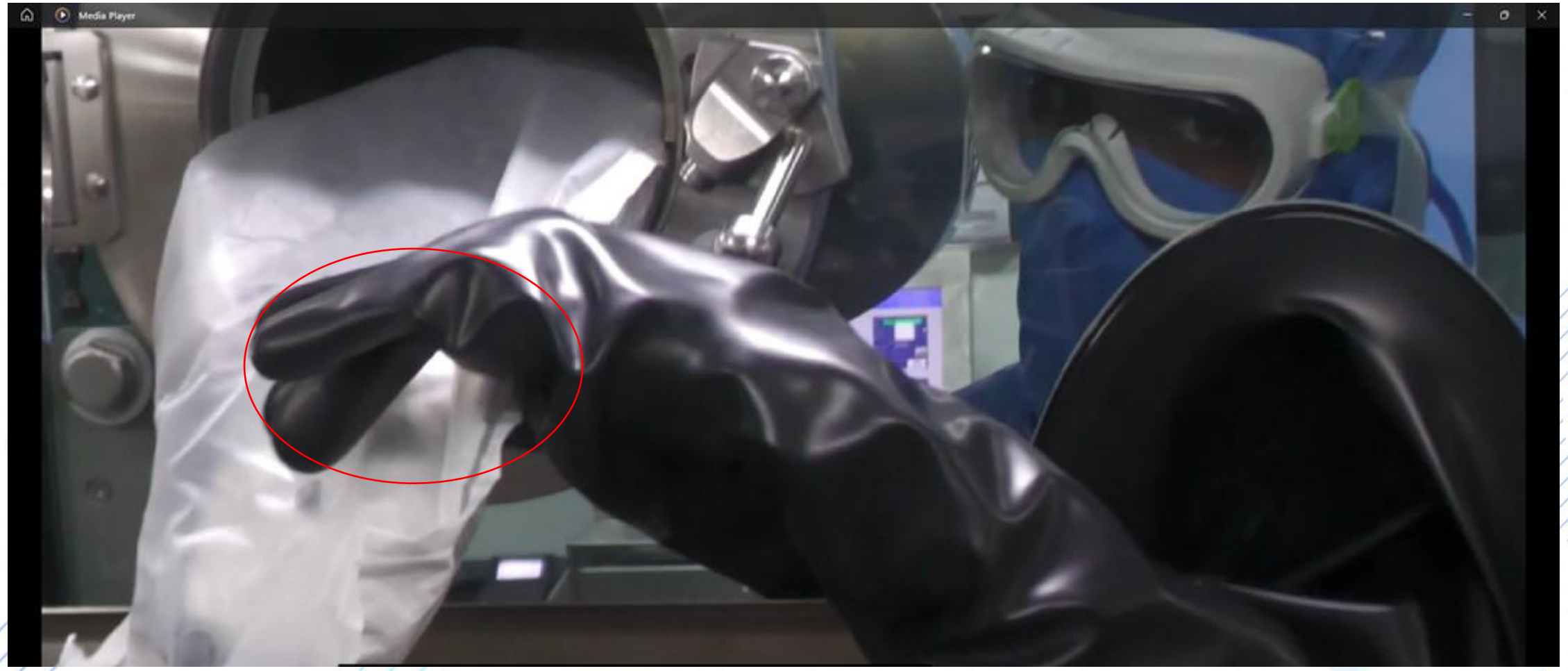
Media fill (Isolator)

Break in first air during filling assembly activity



Media fill (Isolator)

Break in first air during rubber stopper charging activity



Media fill (Isolator)

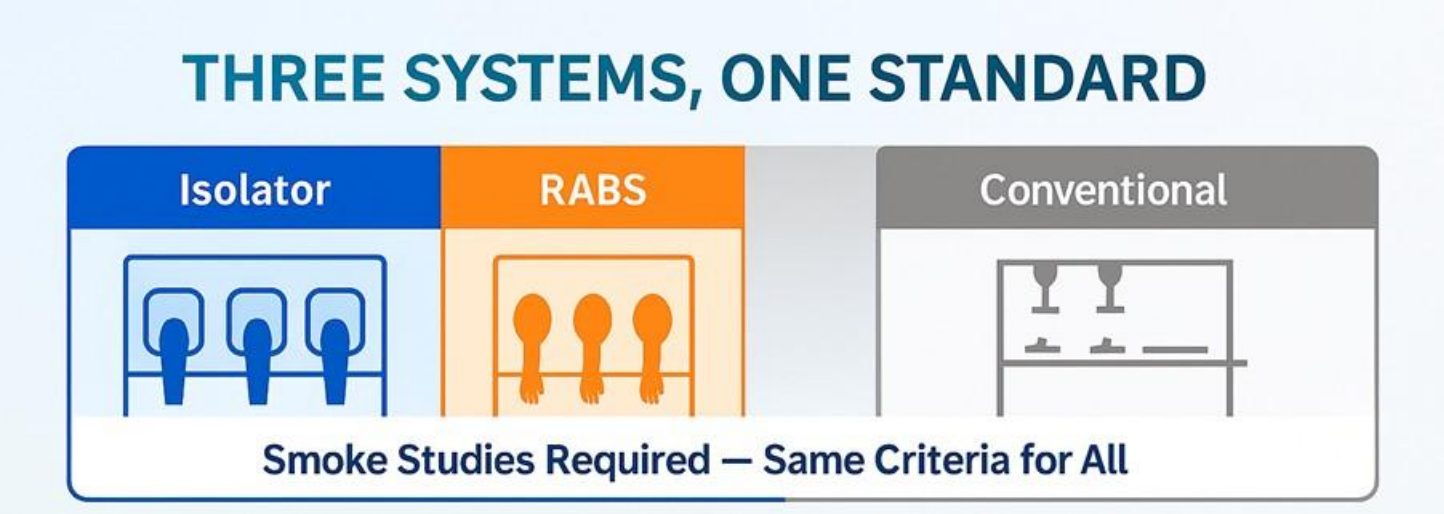
Is it acceptable that the VHP decontaminated gloves comes in contact with the Sterilized product contact parts**?**

Media fill (Isolator)

During filling assembly process Isolator glove directly touch to sterile product contact parts

Smoke studies

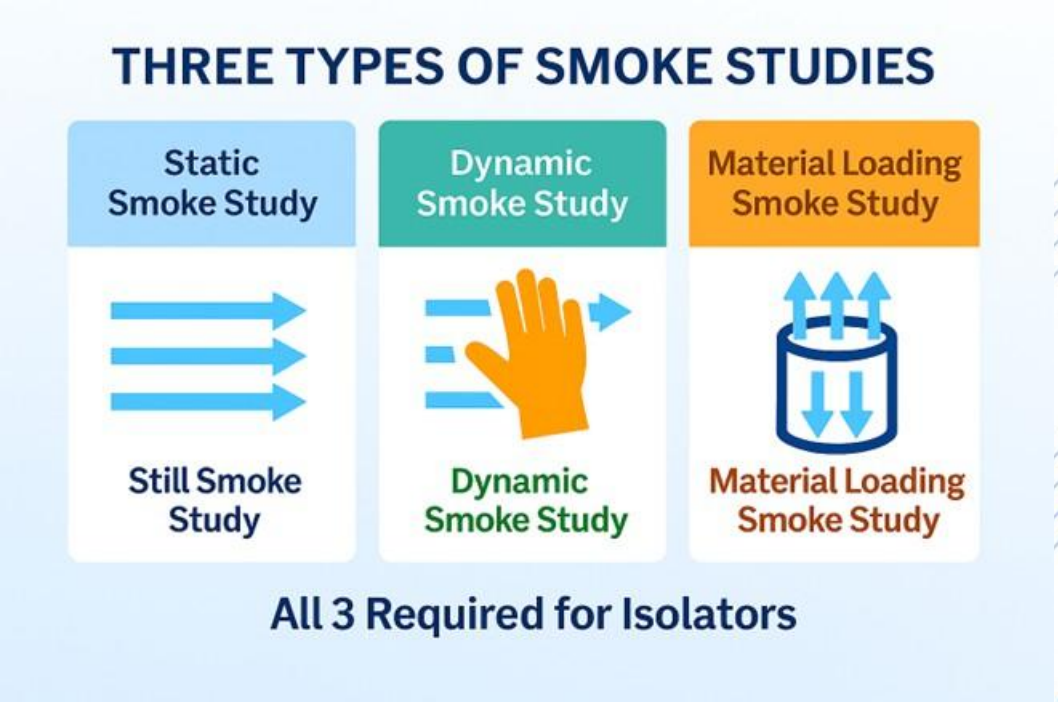
Do smoke studies be similar to Isolator and Conventional / RABS lines**?**



Smoke studies

Any additional smoke studies requirement we should perform for Isolator?

1. Static Smoke studies?
2. Dynamic smoke studies?
3. Material Loading
4. Material movement smoke?



Smoke studies

Static Smoke Studies (At-Rest)

✔ Mandatory

✔ Required for all aseptic systems (Conventional / RABS / Isolator)

Purpose:

- Verify **unidirectional airflow** integrity
- Confirm **uniform sweeping airflow** inside isolator
- Identify **dead spots**, vortices, recirculation zones around:
  - Corners
  - Behind equipment
- Confirm **proper airflow from HEPA to product contact surfaces**
  - Under filling needles
  - Stopper bowls, transfer trays

Dynamic Smoke Studies (In-Operation)

✔ Mandatory

✔ Must include **operator glove interventions**

- Visualize airflow during **worst-case glove movements**
- Evaluate impact on **first air protection**
- Ensure **no turbulence** or backflow when performing:
  - Aseptic adjustments, Sensor alignment, Jam clearance
  - Component handling

Confirm airflow remains Grade A **while line is running**

Material Loading Smoke Studies (Isolator-Specific)

✔ Strongly required

✔ More critical in isolators than in RABS/conventional systems

Purpose:

- Demonstrate that loading via **RTP, β-containers, transfer chambers, sliding doors** does not:
  - Disrupt first air, Introduce turbulence in the product path,
  - Create recirculation at load points
- Confirm airflow protection during:
  - Component bags introduction
  - Tub ready component loading
  - Tool introduction
  - Pre-fill staging
- Show how airflow behaves when **heavy or large components** are placed inside isolator

Environmental Monitoring: Operational Key Points

Types
Air and Surface

Frequency
Risk based. But similar to RABS

Number of samples
Risk-based, CCS-driven. Continuous viable & non-viable EM

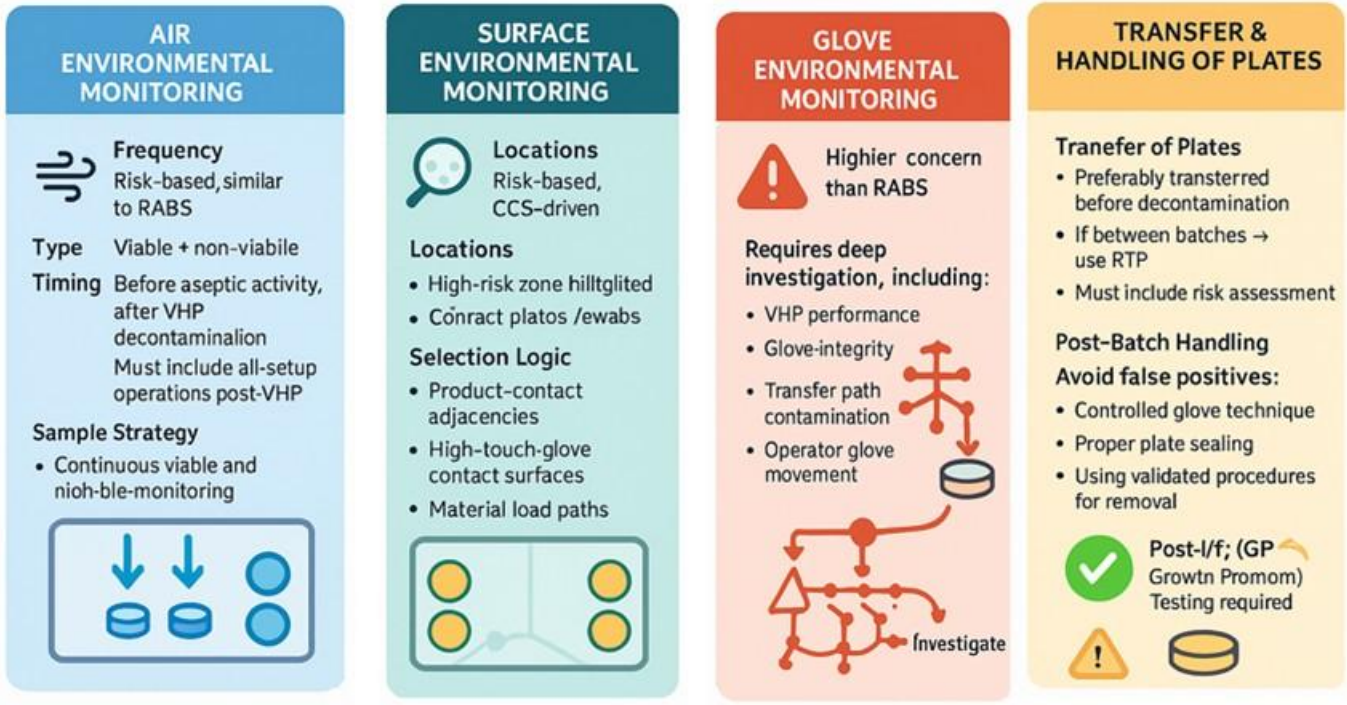
Time
Air: Prior to start of aseptic activity after decontamination.
Should include any set up activity after decontamination

Surface:
End of the batch.
How to select the locations?

Gloves:
End of the batch.
All Gloves?
Opening the Isolator?

Microbial Data Deviations in Isolator
Higher concern than RABS
Requires deeper investigation and assessment of cause

Transfer of plates
Preferably prior to decontamination
If between batches, RTP to be used including Risk assessment
Additional studies of GPT post decontamination
Handling of plates post batch to avoid false positive



Biological Indicators

1. How many locations for BI placement is right?
2. Is it OK to have 1 of the 3 BI failure at location?
3. How to identify the worst case location for BI placement?

Biological Indicators

1 BI TYPE & SPECIFICATION

Bacillus stearothermophilus

10^6 spores per carrier

I must have validated

D-value (e.g..1,5–2,0 min at 121°C)

Z-value

Rouge 81

CARRIER MATERIAL & COMPATIBILITY STUDIES

Carrier must be same as isolator material:

- Stainless steel coupons (SS316L)
- If polymer carriers used → equivalence studies required

VHP penetration studies for SS vs polymer

Carrier roughness impacts BI recovery

BI MONOLAYER / ROUGE BI

LOCATION SELECTION & WORST-CASE JUSTIFICATION

BI placement must be risk-based

Worst-case locations include:

- Shadowed areas
- Behind equipment
- Corners, under conveyors
- Largest load areas
- Airflow stagnation zones

Use airflow visualization to define worst cases

INCOMING RECOVERY & STORAGE REQUIREMENTS

Incoming BI recovery must be verified

BI must be stored under controlled temperature

Avoid heat, humidity, VHP exposure before use

Storage deviation shown with red exclamation icon

5 NUMBER OF BIs & TRIPLICATE STATEGY

Multiple BIs at each location recommended

- Triplcicates required
- Difficult-to-decontaminate areas
- Worst-case locations

3x BI

New Automation

Feature	Traditional Isolator	Robotic Gloveless Isolator
Human Interaction	Operators must insert their gloved hands into the isolator to manipulate materials.	Robotic arms or automated systems handle materials inside the isolator without human contact.
Sterility Control	Contamination risk is higher due to human interaction, despite gloves.	Enhanced sterility due to zero human contact, reducing contamination risks.
Efficiency	Less efficient, as human intervention is required for every task.	Highly efficient with automation and robotics handling tasks, reducing time and human involvement.
Precision	Dependent on human dexterity, which can lead to variability in operations.	High precision and repeatability due to robotic systems and automation.
Regulatory Compliance	Traditional systems may have limitations in documentation and automation of processes.	Designed for easier compliance with GMP and regulatory standards due to automated monitoring and traceability.
Scalability	Scaling can be labor-intensive and may require additional human operators.	Scalable with minimal human labor, as robotic systems can handle larger volumes.

Aseptic Filling Machines: Gloved vs. Gloveless | AST

- Gloveless Isolator

Isolator: Myths

Isolator System

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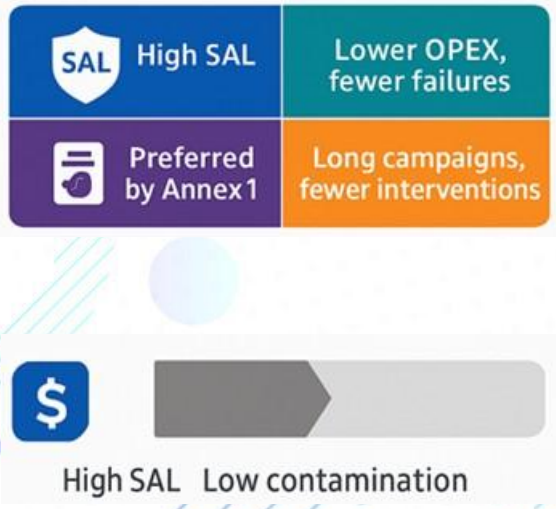
Cost vs Benefits

Myth 1: Isolators is very costly with not much advantages?



Feature	Isolator	RABS	Conventional Cleanroom
Initial Cost	High	Moderate	Low
Operational Cost	Moderate	Moderate	High
Human Intervention	Minimal	Moderate	High
Contamination Risk	Very Low	Low to Moderate	High
Regulatory Acceptance	Very High	High	Moderate
Cycle Time	Longer	Shorter	Shortest
Flexibility	Moderate	Moderate	High
ROI Timeline	Long-term	Mid-term	Short-term

Isolator - Myths

Myth: If we set up a facility with isolator, there will not be any regulatory risk.

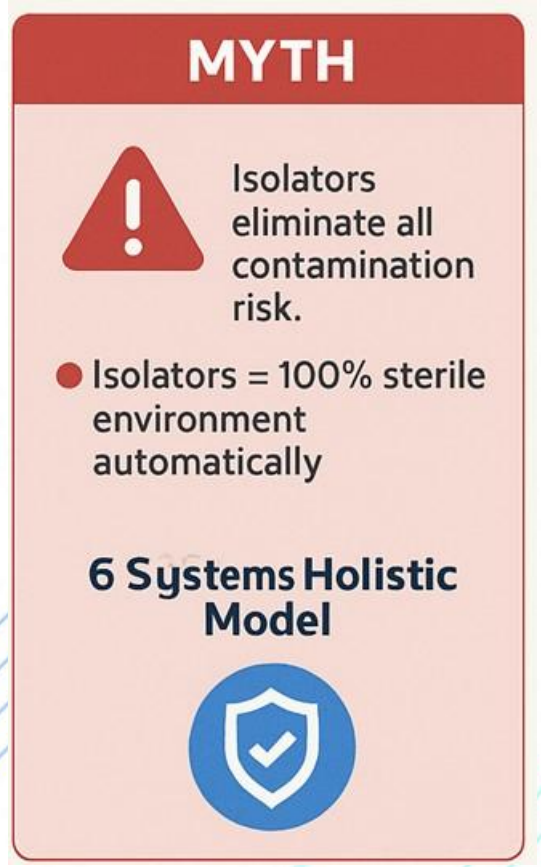
Reality:

- FDA Recognizes Isolators as a Superior Containment Technology
- But FDA Approval Is Not Automatic
- Isolators Must Be Properly Designed, Qualified, and Maintained
- FDA Has Rejected Sites with Isolators
- If isolators are poorly maintained, improperly validated, or used with inadequate aseptic practices, FDA can issue Form 483 observations or Warning Letters.
- Isolator use does not exempt a site from scrutiny.

Isolator - Myths

Myth: Isolators eliminate all contamination risks.

Reality: Isolator reduces some risk of contamination, but regulatory inspections are holistic in nature and will require overall 6 systems to be implemented correctly to reduce risk

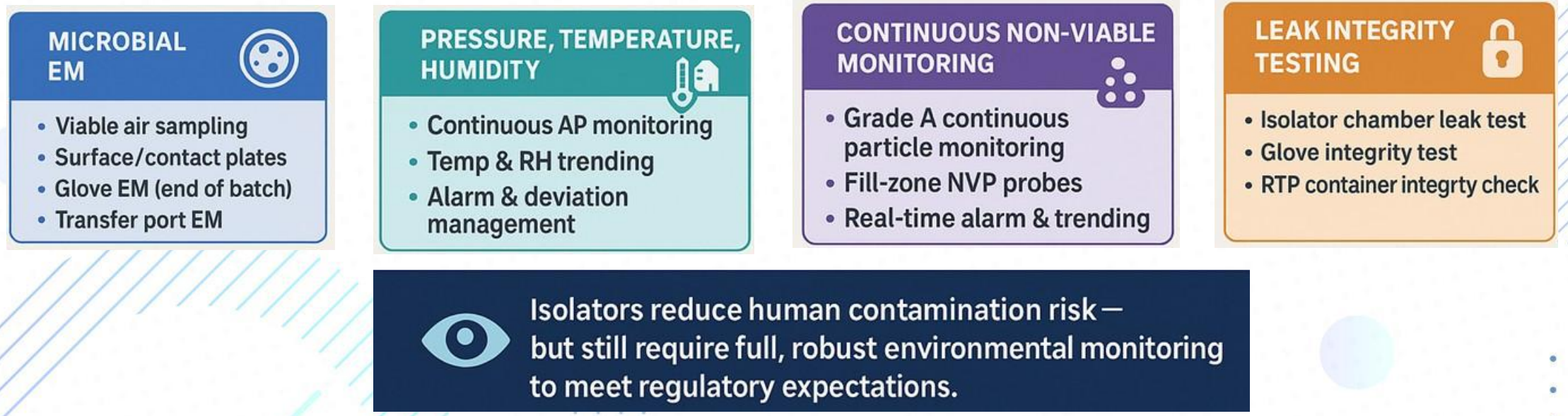


Isolator - Myths

Myth: Isolators don't require environmental monitoring as thorough as done for conventional and RABS systems

Reality: Isolators **do require monitoring more or less same** EM as done for conventional and RABS systems

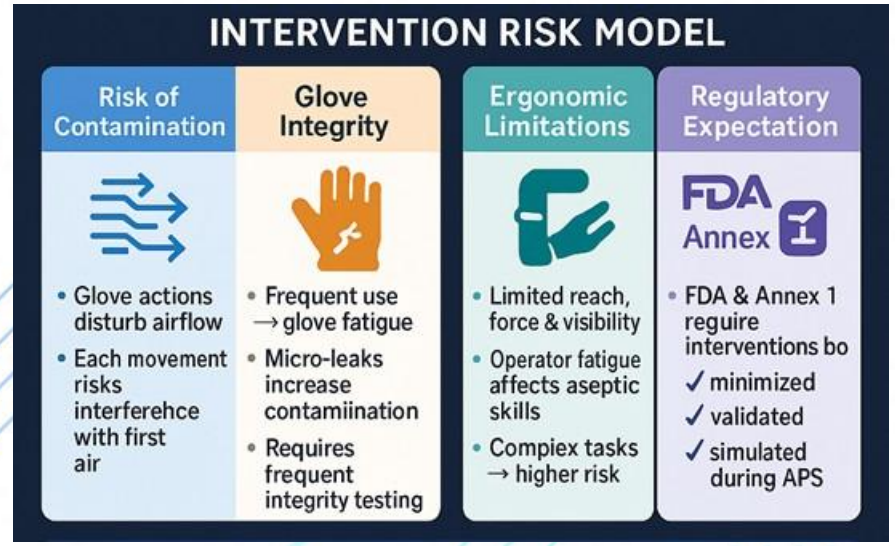
- Microbial EM
- Pressure differential monitoring, Temp, Humidity
- Leak testing
- Continuous non-viable particle monitoring



Isolator - Myths

Myth: We can do Unlimited Interventions in an Isolator as it is decontaminated by a validated method.

- Even though isolators allow remote manipulation via glove ports, interventions are not unlimited and must be minimized and controlled.
- Each intervention increases contamination risk, especially if gloves are damaged or improperly used.
- Glove fatigue and ergonomic limitations can affect operator performance and sterility.
- Design processes to be as automated and intervention-free as possible.
- Use Rapid Transfer Ports (RTPs) for material movement.
- Implement glove integrity testing and airflow visualization to ensure aseptic conditions are maintained

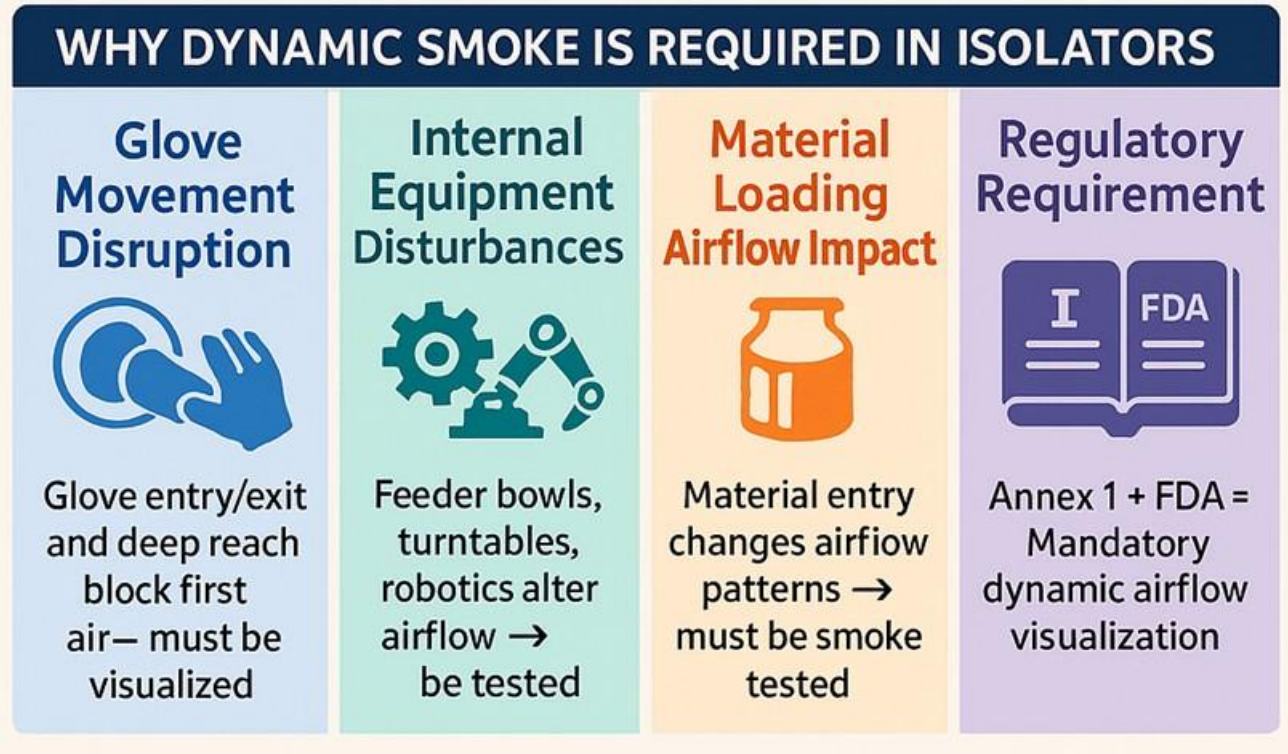


Isolators reduce contamination risk – but interventions must still be minimized and fully controlled.

Isolator - Myths

Myth: Dynamic Smoke Studies in Isolators are not required.

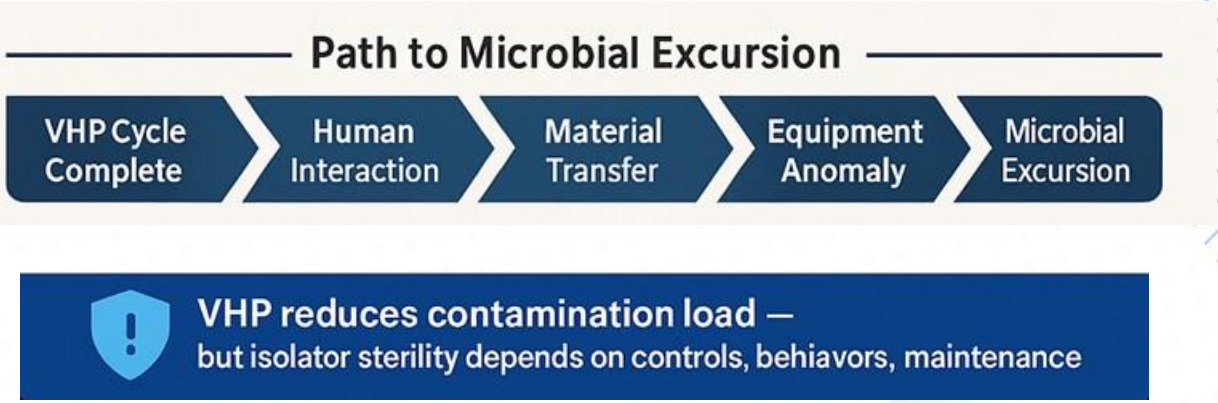
However, **regulatory guidelines and best practices** clearly emphasize the need for **airflow visualization studies (AVS)** — including **dynamic smoke studies** — even in isolators.



Isolator - Myths

Myth: Once VHP is validated, microbial excursion cannot happen inside an isolator.

- While Vaporized Hydrogen Peroxide (VHP) is a highly effective surface decontaminant, it does not guarantee a permanently sterile environment. Here's why:
  1. VHP is a Decontamination Process — Not a Permanent Sterilization
  2. Human Interventions via Glove Ports
  3. Material Transfer and RTPs
  4. .Cycle Drift or Equipment Failure



Isolator - Myths

Myth: If isolator is VHP decontaminated, first air compliance is less important

- Any intervention (e.g., troubleshooting, equipment adjustment) must follow **strict aseptic protocols**.
- Even minor deviations can lead to **microbial excursions**, especially if gloves or surfaces are compromised

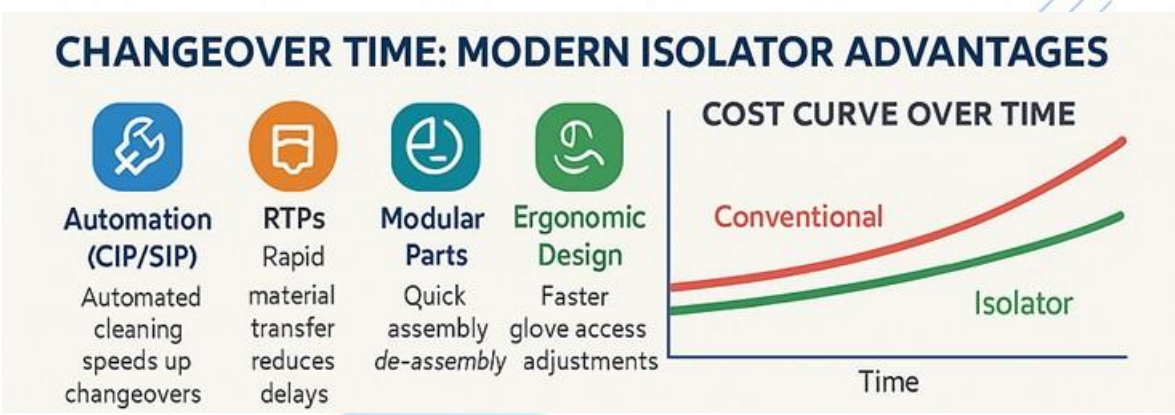
Isolators reduce contamination risk — but First Air remains your primary aseptic protection during operation.

Isolator - Myths

Myth: Isolator increases operational cost and changeover time is very high

1. While isolators may have higher initial costs, they often lead to lower operational costs and optimized changeover times when properly designed and managed
   - Initial Cost: Isolators typically cost 30–50% more than RABS due to their complex design and integrated decontamination systems.
   - Operational Savings:
     - Lower HVAC requirements:
     - Reduced gowning and cleaning:
     - Lower environmental monitoring burden:

2. Changeover Time: Not Necessarily Higher
   - Modern isolators are designed with ergonomic access, automated cleaning, and modular components to minimize changeover time.
   - Well-defined SOPs, mock-up studies, and risk-based cleaning validation can streamline changeovers.
   - Advanced isolator lines can support multi-product campaigns with efficient turnaround.



483 Observations Related to Isolators

Failure to perform required environmental monitoring inside sterility-testing isolator.

- Missing viable air and contact plates during dynamic operations.
- Deviations not recorded or justified.

Incomplete qualification of oncology manufacturing isolator system.

- Inadequate validation of isolator–lyophilizer integration.

Deficient glove integrity testing for cRABS/isolators.

- No rationale for needle size.
- No worst-case glove test position study.

Smoke study revealed operator behavior compromising isolator Grade A boundary.

- Transfer hose removed from isolator without maintaining Grade A protection.

Inadequate pressure and airflow control in isolators and micro-environments.

- Unverified HEPA and pressure control under dynamic conditions.

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Zydus LifeScience