SEPARATION PROCESSES TO PREVENT AND REMOVE MICROORGANISM IN BIOPHARMACEUTICAL PRODUCTION PROCESSES

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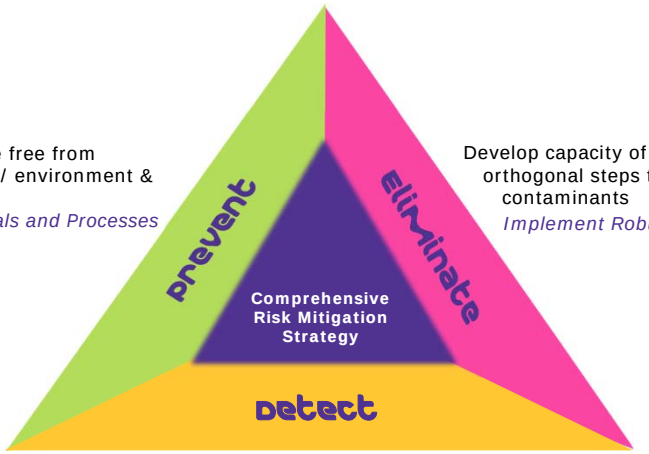
Developing a comprehensive microbiological biosafety clearance strategy

A multifaceted approach

Verify that raw materials are free from contaminants and processes / environment & people are protected
Ensure Safety of Raw Materials and Processes



Develop capacity of manufacturing process with orthogonal steps that remove or inactivate contaminants
Implement Robust Clearance Technologies

Verify absence of contaminants in process intermediates
Optimize Sampling and Test Methodologies

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Risk assessment and mitigation strategies

Appropriate Single-use System Applications

Routes of contamination in the process

Filter categorization

Moderately critical filters and risk approach

Critical filters and risk approach

Filter qualification

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RISK ASSESSMENT OVERVIEW

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3D System Risk Assessment Concept
Used to calculate practical severity

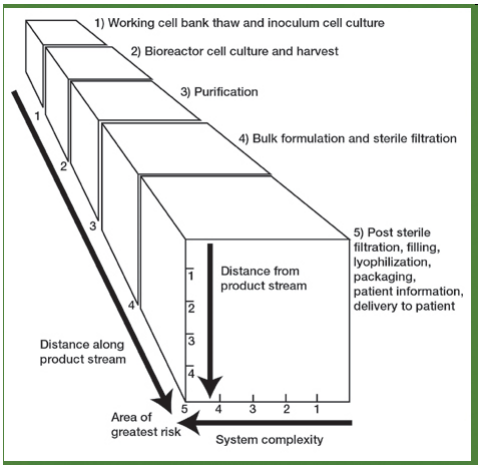
Considers

- System's distance from the process stream
- Location along the process stream
- System's complexity

Highest score is highest risk
(proximity x location x complexity)

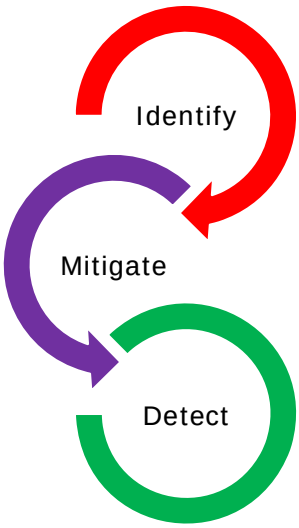
This tool is mainly used to assign a risk level to an overall system **before** assessing failure frequency and detectability (SOD / RPN)

Excellent for complex systems as part of "big picture" analysis to prioritize risk management

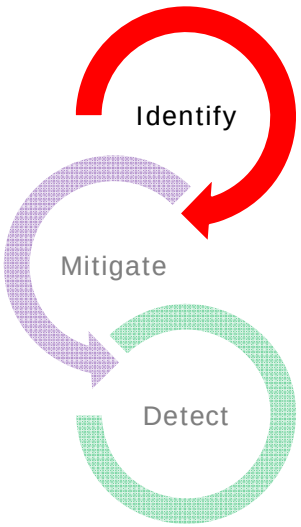


"A 3-D Risk Assessment Model", *Journal of Validation Technology* [Autumn 2008] pp70 - 76

Example of 3 Steps for Risk Assessment to Prevent Contamination



Risk Assessment: Identify



Assess extent of risk, ability to detect, and frequency of occurrence

Identify


Facility


Equipment

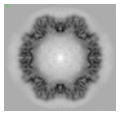

Process

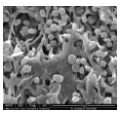

Materials

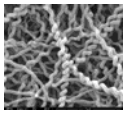

Utilities


Personnel

Each source is a potential entry point for microbial contamination such as;


Minute virus of mice (MVM)
~18-24 nm


Acholeplasma laidlawii
< 0.2 µm


Leptospira species
0.4 µm x >>5 µm


Bacillus species
1 µm x 4 µm

Case Studies of Microbial Contamination in Biologic Product Manufacturing
Suvarna, K., Lolas, A., Hughes, P., Friedman, R. Biotechnology Manufacturing Team, Division of Manufacturing and Product Quality, Office of Compliance, Center for Drug Evaluation and Research, Food and Drug Administration

Map Out the Process Flow of Raw Materials

Each step may introduce microbes into the process

- Handling
- Transport of materials in the facility
- Testing
- Sampling
- Transfer into different packaging
- Storage conditions
- Weighing
- Sieving
- Crushing
- Sifting
- Water transfer (cleaning, compounding)
- Compounding
- Mixing
- Hold times
- Dispensing
- Sampling
- Room Cleaning
- Equipment Cleaning
- Personnel Hygiene

How do I assess the risk of these parameters?

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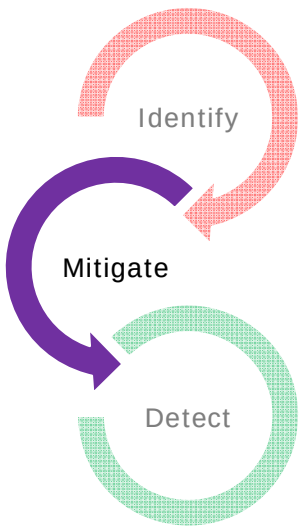
Risk Assessment: Mitigate

Eliminate source or reduce likelihood of occurrence



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Prevent Human Contamination

Strategies for prevention, mitigation and detection

Prevention

- Remove people from the environment

Mitigation

When people have to be in the environment

- Wear cleanroom attire
- Work in cleanrooms
- Properly trained personnel

Detection

- Viable air sampling
- Surface monitoring
- Personnel monitoring

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Prevent Raw Material Contamination

Raw Material Selection

Prevent

- Remove animal derived components
  - Caution! Serum-free does not mean mycoplasma free
  - Consider chemical free
- Select raw material quality grade
  - Pharmaceutical grade versus analytical grade
- Audit vendor

Mitigate

- Pre-treat components
  - Choose treatments effective for viral and bacterial reduct

Detect

- Screen raw material with rapid tests
  - Caution! Sample sizes versus kG to tons of material

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DCVMN Meeting – March 2017, Hanoi
Michael Payne, Merck Millipore

Considerations for bioreactor protection
Raw Material Pre-Treatment

Technology	Robust Clearance	Media Compatibility	Point of Use	Scalability	Cost Effective
HTST (~102°C ~10 sec)	Yes	Component dependent	Yes	Challenging for small to mid-scale	Yes at Large Scale
UV-C (254 nm)	Organism dependent	Component dependent	Yes	Challenging at large scale	Yes at Small Scale
γ Radiation	Organism dependent	Component dependent	No	Small batches	Yes
Downstream Virus Filters	If specifically claimed. Consistent LRV	Yes but designed for downstream fluids	Yes	Yes	Not for batch processes*
Upstream Virus Barrier Filters	Yes by size exclusion. Consistent LRV	Yes, specifically designed for upstream media	Yes	Yes	Yes

* *Downstream viral clearance filters, are designed for very clean feed streams and would not be cost effective on upstream bioreactor media and feeds.*

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Key Points
Mitigate

Prevention
- Best option wherever possible

Containment
- Personnel Control
- Single Use Technologies

Raw Material Selection
- Vendor qualification
- Pre-treatment

Downstream Processing
- Microbiological Clearance
- Filtration
- Sterilization, sanitization, cleaning and storage

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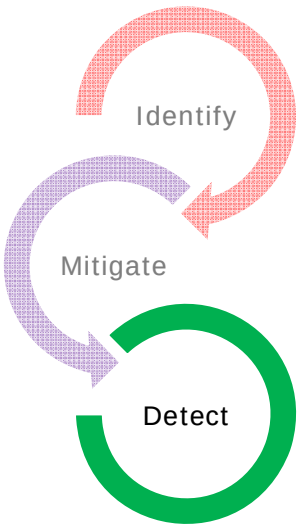
Risk Assessment: Detect



Determine location, frequency, and limits of detection

"Contaminant-free" is only as good as the detection method used

Microbiological Detection

Classical Methods

Most developed in the 19th century

- Microscopy
- Growth-based methods

Benefits

- Easy to implement
- Easy to qualify
- Larger sample volumes possible

Limitations

- No universal medium or growth conditions
- Only detect those microbes capable of replicating in the chosen test medium under the specified conditions
- Can take days to weeks for a result

Rapid Methods

Developed over the past 30 years but slow adoption rate

- qPCR
- TMA
- Microcolony growth detection

Benefits

- Rapid results
- Higher sensitivity for equal volume compared to classical methods

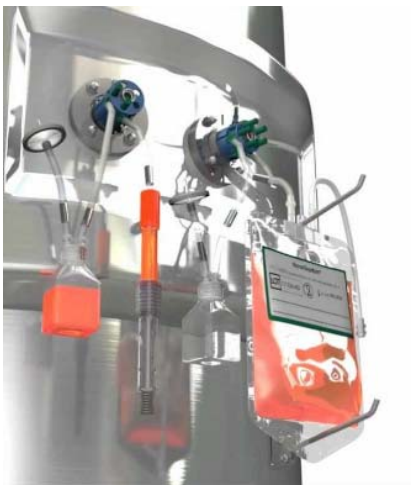
Limitations

- More extensive validation
- Higher expertise required
- False positives doesn't distinguish viable cells
- Small sample size
- Often destructive
  - Split samples needed for identification

Limits of Detection
Sampling Volumes

Sampling
- Vessel Liters to 10,000+ Liters
- Sample Volume
  - Less than 1 Liter

Assay
- Removed from sample volume
- Milliliter to microliter



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Limits of Detection

Sampling
Assume a 1 L sample from a 10,000 L Bioreactor
Assay requires a 1 mL sample for testing

CFU per Liter	10	1,000	10,000
CFU per mL	0.01	1	100
Probability an organism will **NOT** be detected in the sample	**0.99**	**0.9**	**0.37**

Assay Sensitivity
LOD PCR for *Leptospira:* 100 CFU (equivalent)
LOD PCR for Mycoplasma: 1-10 CFU (equivalent)
LOD by light microscopy @ 400 x: 10^5 to 10^6 cells

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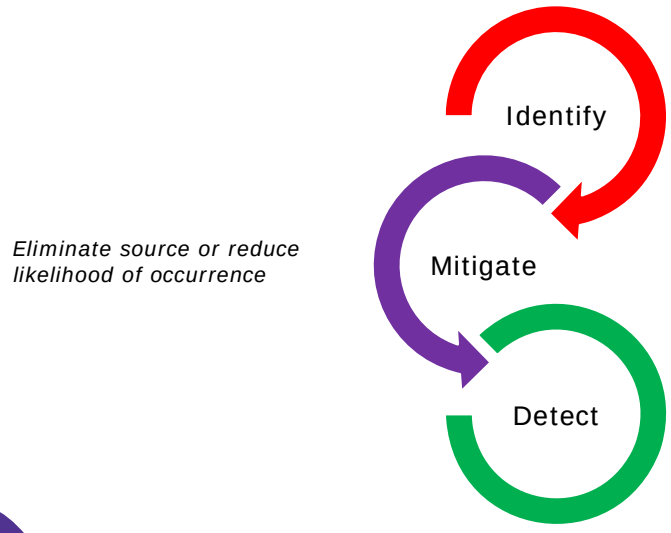
Risk Assessment to Prevent Contamination



Some Risk Assessment Considerations for Filters

Fluid classification
Fluids labeled “sterile” have the highest risk

Dosage form
Injectables without preservative have highest risk

Room classification
Lower grade brings greater risk if there is a breech

Location of filter in the process
The closer to the final product the greater risk

Detectability of poor filtration performance
No in-line testing has the highest risk

Contact time
The longer the contact time the greater the risk

Process conditions
The more aggressive the conditions, the greater the risk

Fluid pretreatment
Less pretreatment has greater risk

Fluid posttreatment
No downstream removal of low MW material has greater risk

Filter pretreatment
The more aggressive the pretreatment (e.g. SIP), the greater the risk

Prior history
If there have been previous filter related issues, the risk is greater

PDA Industry Aseptic Processing Survey 2001

Personnel

- Microbe Shedding # 1
- Human Error # 2
- Non-routine Activity # 3
- Aseptic Assembly # 4
- Mechanical Failure # 5
- Routine AP Activity # 7 (tie)
- Material Transfers # 8 (tie)

Environmental

- Airborne Contaminants # 6
- Surface Contaminants # 7 (tie)
- Failure of HEPA Filter # 8 (tie)

Sterilisation

- Improper Sanitization # 7 (tie)
- Failure of 0.2 Filter # 8 (tie)
- Improper Sterilization # 9

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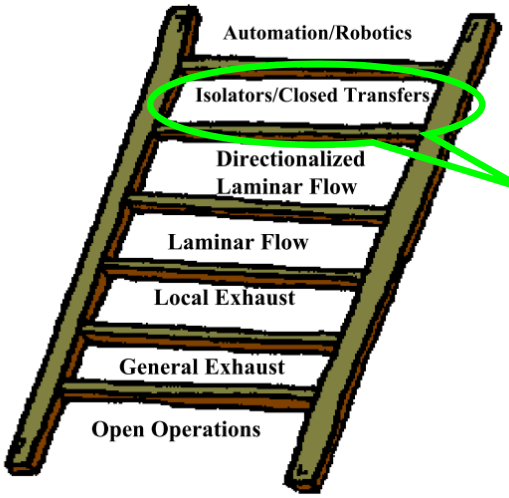
Hierarchy of containment technology



- ✓ Totally enclosed systems
- ✓ Secure connection technology
- ✓ RABS / Isolator technology

Single-use Products Offer Some Containment Opportunities

These products require components that need to be assembled

EXAMPLE OF APPROPRIATE USE OF SUS TECHNOLOGIES - SAMPLING

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Example of Appropriate SUS Technology - Aseptic Sampling System

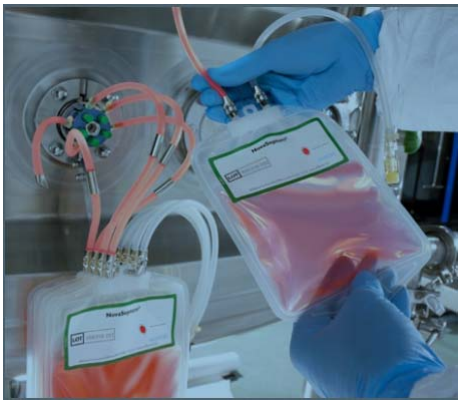
Increase sampling productivity, while reducing set-up, cleaning and flushing time whilst increasing biosafety

Traditional Sampling Method
- Lengthy CIP/SIP with each sample
- Additional steps over and over again

Connect → CIP/SIP

Sampling with Millipore
- Only one SIP
- Push to sample
- Separate & crimp
- One to 45 samples at once
- Unlimited possibilities

Push to Sample → 1 minute → Separate & Crimp



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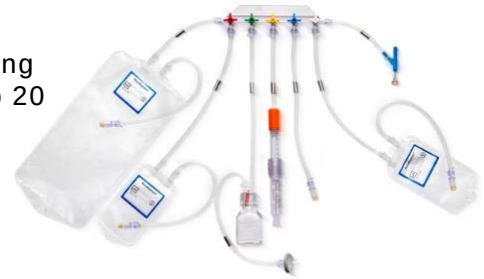
Safe and easy aseptic sampling and disconnection directly from the container

Variety of sterile sampling devices and volumes including small volumes from 1 mL to 20 mL



Safe and easy sterile disconnection

Increases yield
Reduces sampling deviations
Reduces risk of non-sterility and reduced biosafety

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EXAMPLE OF APPROPRIATE USE OF SUS TECHNOLOGIES - MULTIPLE STERILE CONNECTIONS

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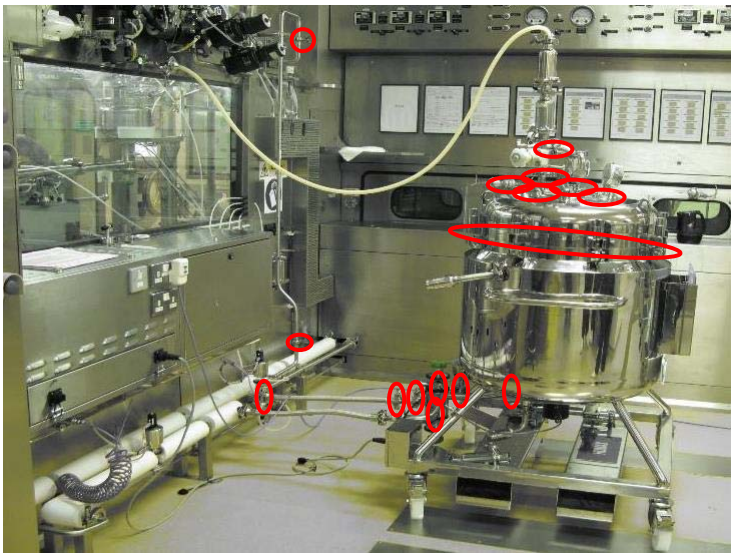
Previous SS System - Areas of Risk
Aseptic Build & SIP to Point-of-Filling



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Connection under Laminar Air Flow

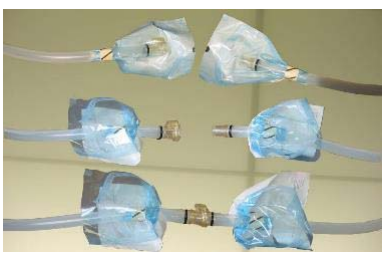
Opened connections

Advantages

- Ease of use
- Low cost

Challenges

- Connection needs to be performed in classified zone
- Risk of process design error
- Reduced risk of contamination



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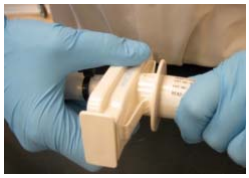
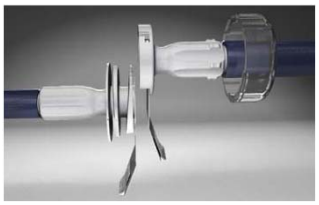
Sterile to sterile connectors

These connectors are designed to connect together sterile entities (container, tubing) in a non “classify A/B” environment.





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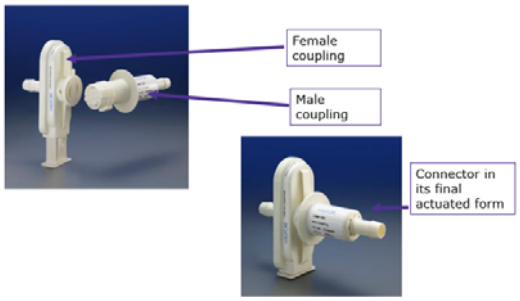
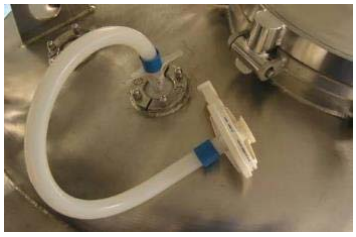
Sterile to sterile connectors

What they do:

An **operator independent and environment independent** sterile connection between
Gamma sterilized assembly *and* Gamma sterilized assembly
Gamma sterilized assembly *and* Autoclaved assembly
Autoclaved assembly *and* Autoclaved assembly





Validated to maintain sterile flow path in any environment

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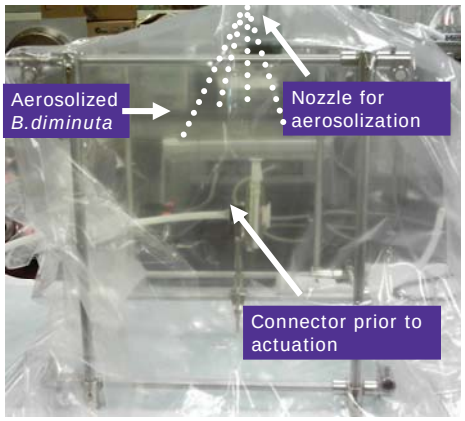
Retention of Sterile-to-Sterile Connector Aerosolized Testing of Connector in a Glove bag

(NB User environment would be grade C)



- Glove bag acts as isolator
- Suspension of *B. diminuta* is aerosolized into glove bag until total bacterial count is $>10^6$ cfu
- Connector is assembled and actuated under aerosolization
- Media transferred through connector under constant aerosolization of bacteria
- Media incubated and observed for turbidity over 7 days
- No turbidity or growth observed

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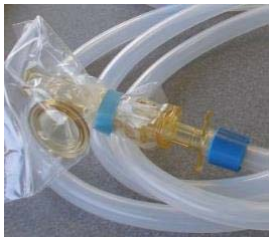
SIP valve to Sterile Single Use lines

What they do:

Enables integration of Steamable hard piped process equipment with single-use sterile fluid paths

- Multi-steam, allowing for steam, disconnection, and re-steam
- Autoclaving
- Gamma compatible
- Validated to maintain flow path sterility



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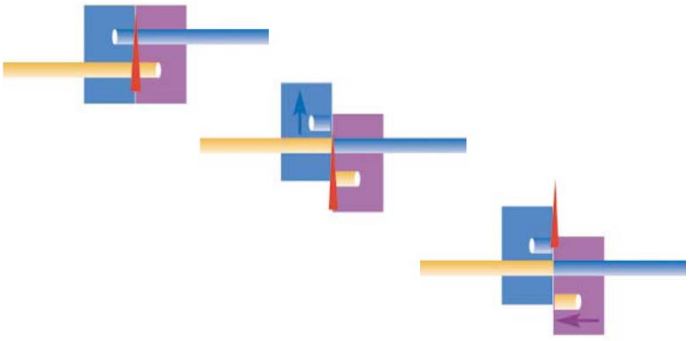
Challenge: Joining Two Sterile Lines - Tube Welder

A device where two sterile tubing lines are together heat welded

Tubing lines are inserted into holders. Then a heated blade cut the tubes allowing the lines to fuse together leaving a sterile fluid pathway.



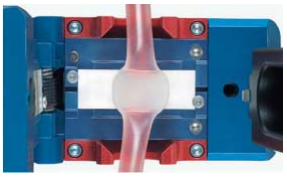
Challenge: Disconnecting Sterile Lines

Advantage

- Ease to use

Challenges

- Single use
- Maintaining sterility after disconnections
- Speed of seal
- Dry or fairly dry
- Matching tubing or sleeve to sealer
- Moving equipment to tubing



SUS Example: Sterile Connect AND Reconnect

Multiple, sterile connections, disconnections and reconnections of single-use assemblies

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IDENTIFICATION OF CONTAMINANT POINTS OF CONCERN

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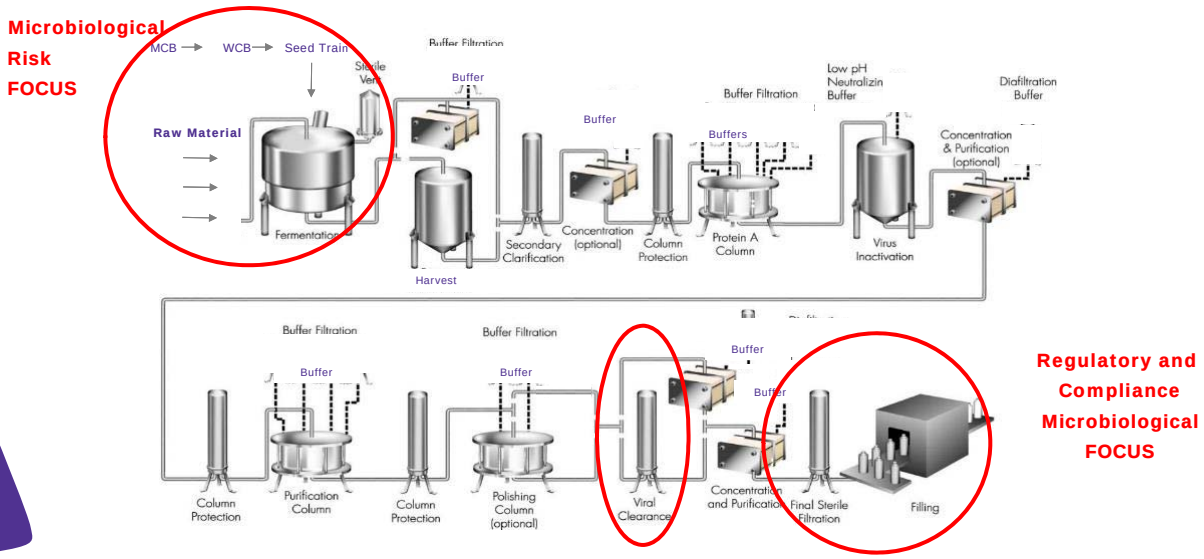
Overview of Generic Biological Manufacturing Process



MICROBIOLOGICAL RISK HAMMOCK

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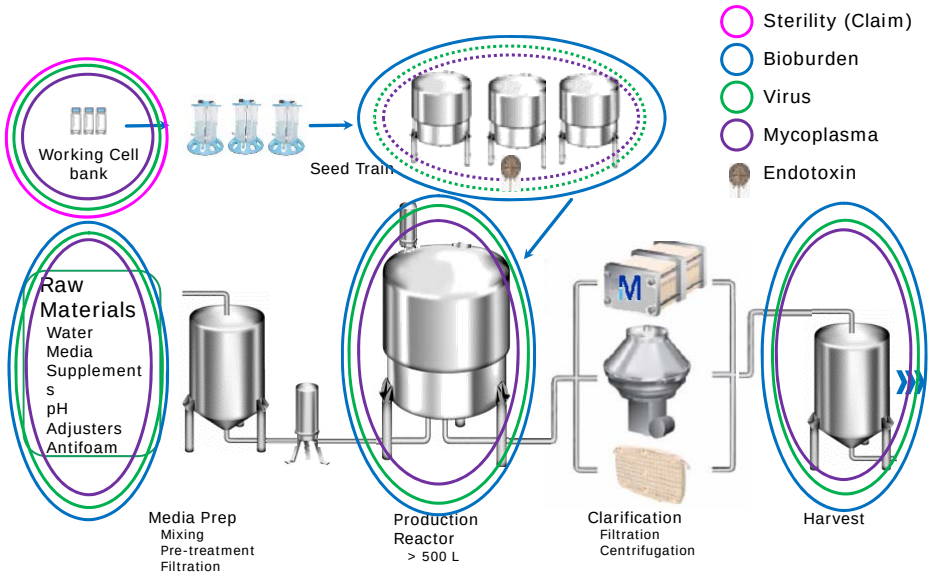
High microbiological risk
Little specific microbiological regulatory guidance

Biopharmaceutical Process

Upstream



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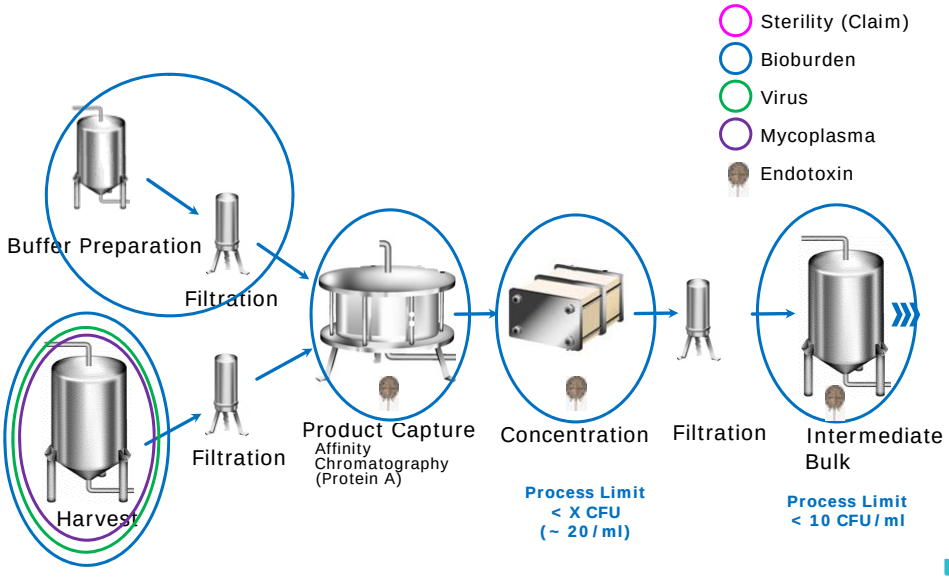
Biopharmaceutical Process

Downstream Purification (1)

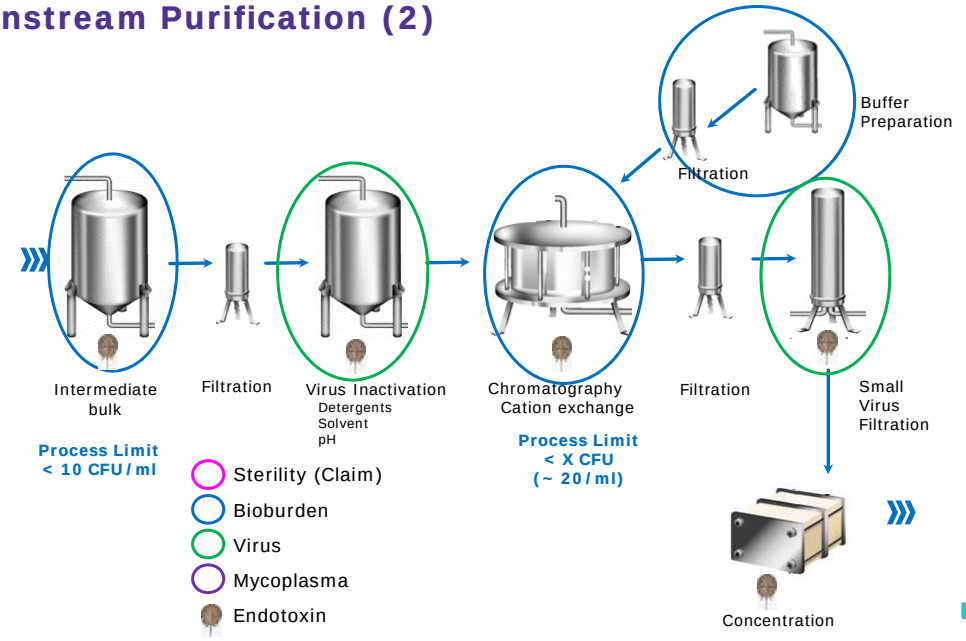
Moderate microbiological risk
No specific microbiological regulatory guidance



Biopharmaceutical Process

Downstream Purification (2)

Moderate microbiological risk
Little specific microbiological regulatory guidance

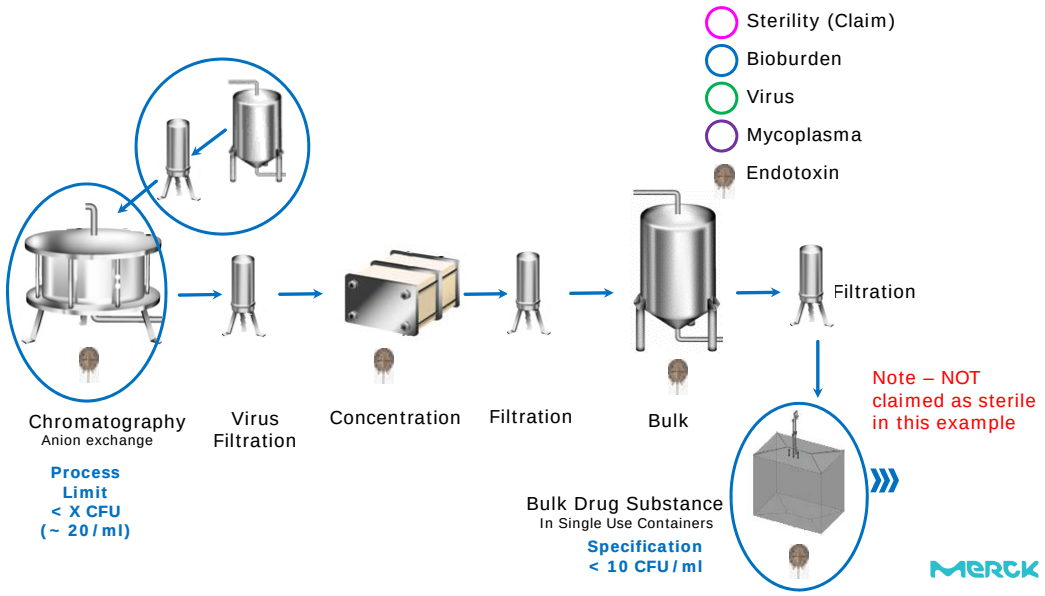


Biopharmaceutical Process

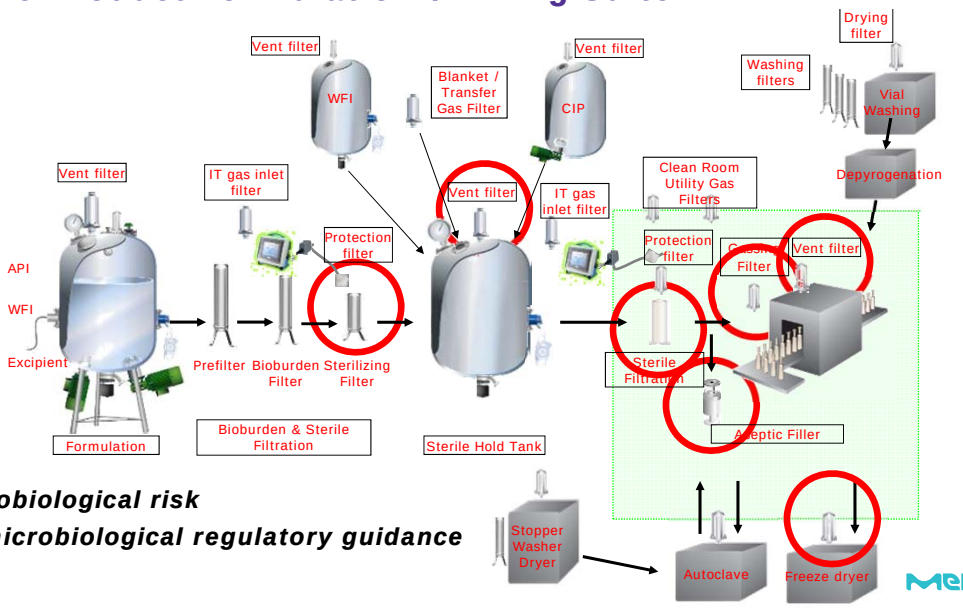
Downstream Purification (3)

Moderate microbiological risk

No specific microbiological regulatory guidance



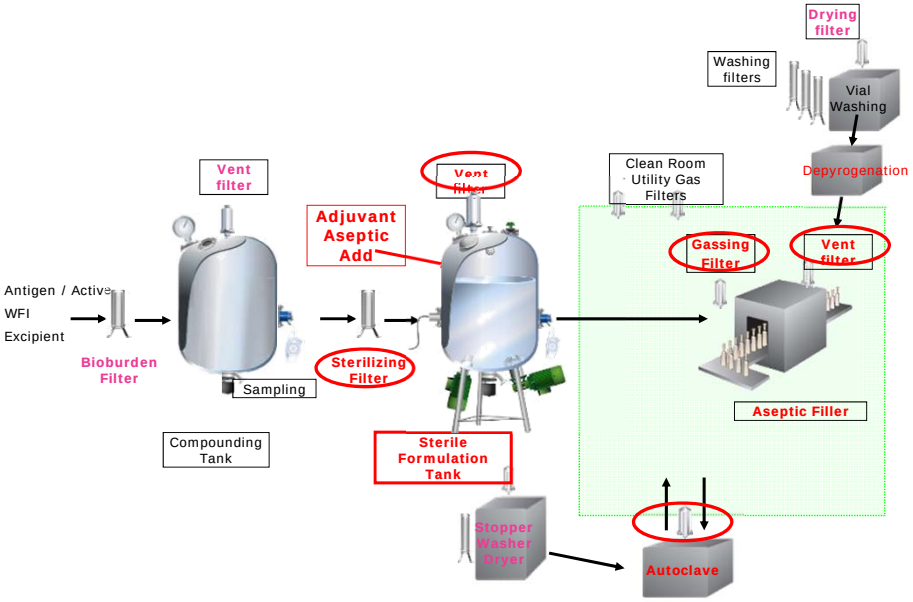
Filterable Product Formulation / Filling Suite



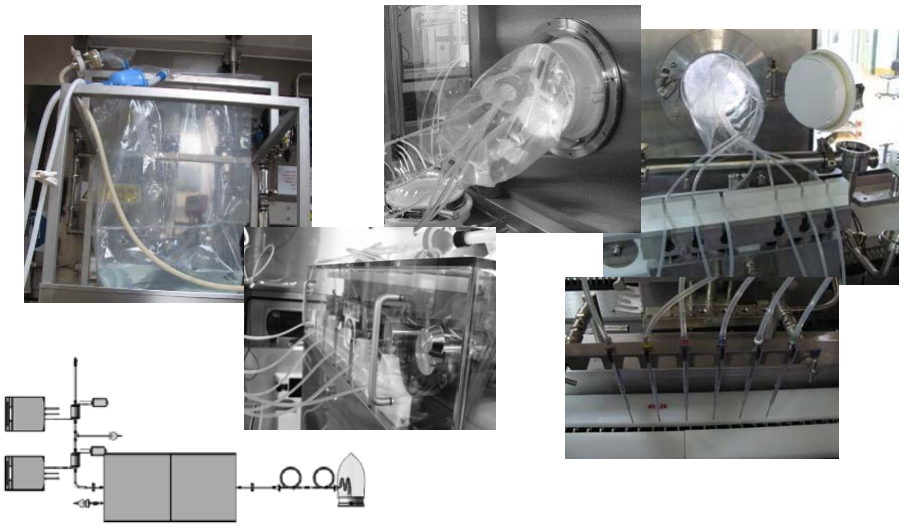
High microbiological risk

Specific microbiological regulatory guidance

Non- Filterable Product Formulation / Filling Suite



SUS Filling Assembly Example - SVP



Filters Can Be Divided into 3 Groups - Definitions

Service

The filter does not affect product quality

- Where process fluids come from facility-wide systems, are not tailored to a specific process and do not have contact with the drug substance or potential drug substance.
- Part of a No-Impact System - Where the equipment of system has no impact, direct or indirect, on product quality (ISPE Commissioning & Qualification Baseline Guide (2001))
  - **Examples: distribution gas filter, water prefilter**

Moderately critical

The filter indirectly affects product quality

- Where process fluids "will not be in direct contact with exposed sterile product or surfaces." (PDA TR40)
- Part of an Indirect Impact System - equipment or system expected to have incidental or secondary impact on product quality (ISPE Commissioning & Qualification Baseline Guide (2001))
  - **Examples: vent filter in a grade D/C area, bioburden reduction filter**

Critical Applications

The filter directly affects product quality

- Where process fluids "are in direct contact with sterile final product or critical surfaces of the associated equipment." (PDA TR40)
- Part of Direct Impact System - equipment or system that will have focused and immediate impact on product quality (ISPE Commissioning & Qualification Baseline Guide (2001))
  - **Examples: vent filter on a sterile hold vessel, sterile liquid filter**

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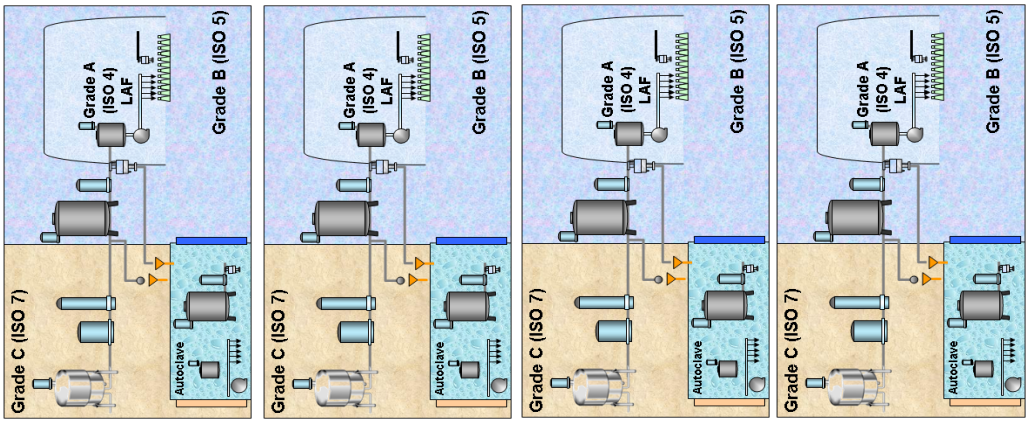
Advantages of Classification
- Multiple Process Lines or Bioreactor Trains



Categorize each filter in a line based on risk, then duplicate across the whole production area

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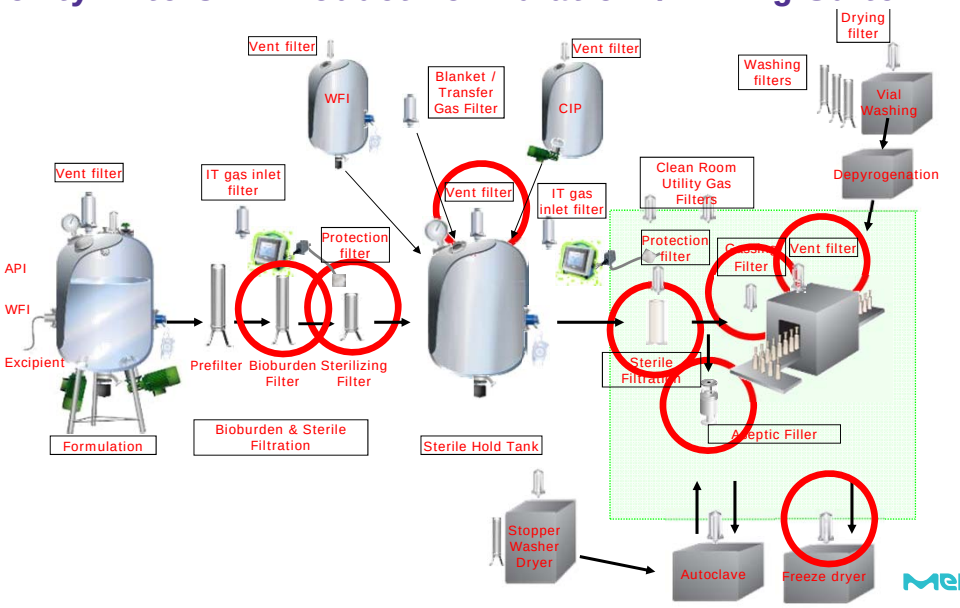
High Priority Filters in Product Formulation / Filling Suite



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Purpose of Moderately Critical Filtration

Removal of undesirable microorganisms from process fluids

- Prevent contamination of the fermentation
- Cell culture media and air
- Formulation and process tanks
- Chromatography systems
- Buffers, washing fluids
- Process intermediates

Reduction of bioburden in purification process steps

- Low bioburden means low endotoxin
- Low / controlled / specified bioburden may be a compliance requirement

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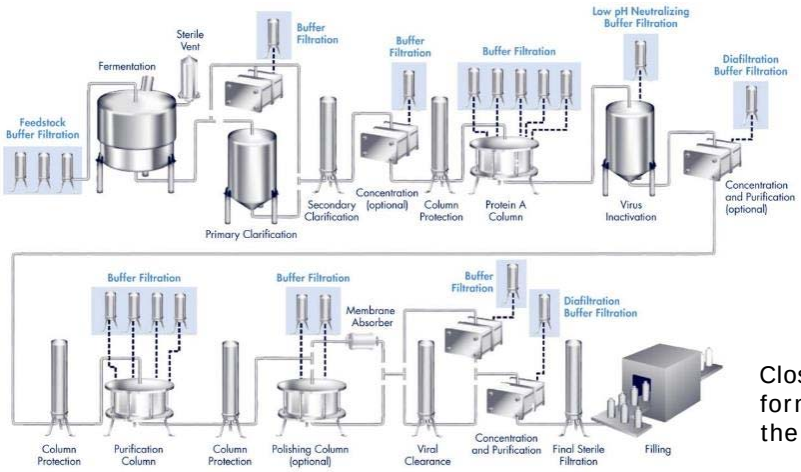
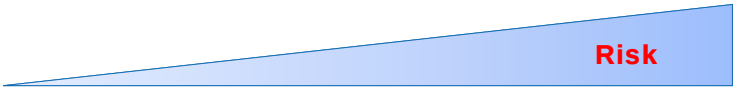
Buffers in a Generic Biologicals Process - moderately critical filtration



Closer to the formulation point, the higher the risk



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CRITICAL FILTERS AND RISK APPROACH

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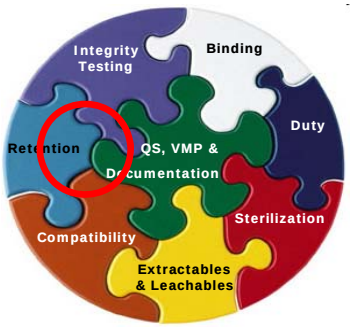
Retention: What are the requirements for sterile medicinal products

"All Sterilization Processes Should be Validated."
WHO Annex 6: Good Manufacturing Practices for Sterile Pharmaceutical Products section 5.4 page 273



"Whatever type of filter or combination of filters is used, validation should include microbiological challenges to simulate "worst case" production conditions. The selections of the microorganisms to perform the challenge
test (e.g. P. diminuta) has to be justified. The nature of the product may affect the filter and so the validation should be performed in the presence of the product....."
PIC/S Guide for Inspectorates: Recommendation on the Validation of Aseptic Processes

A summary should be provided containing information and data concerning the validation of the retention of microbes and compatibility of the filter used for the specific product.
US FDA Guidance on Sterilization Validation

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What Critical Filters need to be Qualified for a Sterile Medicinal Product

Sterilizing liquid filter

Bioburden reduction filter

Sterilizing gas filtration

Note: 0.22 or 0.2 or 0.1um rated filters may not be sterilizing grade.

Sterilizing grade filters are specifically labelled, qualified and documented. This is what to look for when choosing a critical filter

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FILTER QUALIFICATION

8 Elements of Sterile (Critical) Filter Qualification

Represent "worst case" process conditions, process fluid, filter performance and microbiological challenge

Prove the filter's bacterial retention capabilities with a non-destructive test.

Prove the filter does not unacceptably remove stream components.

Prove the filter removes bacteria from the stream compliant with ASTM 838-05 and regulations

Prove the filter meets all performance & duty requirements within product & process conditions.

Prove the sterilization method is effective and does not compromise the filter.

Prove the stream does not adversely impact the filter duty or process stream

Identify, quantify, and assess impact of compounds that migrate from filter to process stream.

Requirements for Filter Documentation

- ✓Suitability for duty
- ✓Process definitions
- ✓Bacterial / particulate retention
- ✓Integrity testing
- ✓Sterilisation process validation
- ✓Adsorption
- ✓Leachables / Extractables
- ✓Risk analysis approach to processing and product impact
- ✓Quality by design

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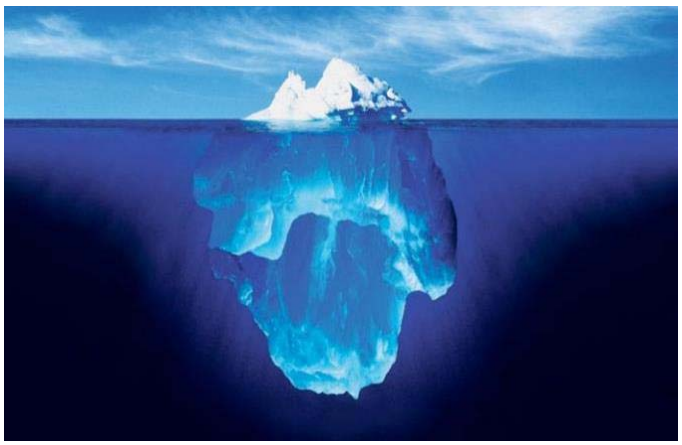
Thank You for your Attention!
May we be of Further Assistance?



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Annex 2 Manufacture of Biological active substances and Medicinal Products for Human Use

For biological materials that cannot be sterilized (e.g. by filtration), processing must be conducted aseptically to minimise the introduction of contaminants.

5. As part of the control strategy, the degree of environmental control of particulate and microbial contamination of the production premises should be adapted to the active substance, intermediate or finished product and the production step, bearing in mind the potential level of contamination of the starting materials and the risks to the product.

6. Manufacturing and storage facilities, processes and environmental classifications should be designed to prevent the extraneous contamination of products. Prevention of contamination is more appropriate than detection and removal

8 c) Live organisms and spores are prevented from entering non-related areas or equipment by addressing all potential routes of cross-contamination and utilizing single use components and engineering measures such as closed systems.

8 e) Environmental monitoring specific for the micro-organism being manufactured, where the micro-organisms are capable of persistence in the manufacturing environment and where methods are available, is conducted in adjacent areas during manufacture and after completion of cleaning and decontamination.

8 f) Products, equipment, ancillary equipment (e.g. for calibration and validation) and disposable items are only moved within and removed from such areas in a manner that prevents contamination of other areas, other products and different product

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Annex 2 Manufacture of Biological active substances and Medicinal Products for Human Use

13. Equipment used during handling of live organisms and cells, including those for sampling, should be designed to prevent any contamination during processing.

16. Air vent filters should be hydrophobic and validated for their scheduled life span with integrity testing at appropriate intervals based on appropriate QRM principles.

33. Given that the risks from the introduction of contamination and the consequences to the finished product is the same irrespective of the stage of manufacture, establishment of a control strategy to protect the product and the preparation of solutions, buffers and other additions should be based on the principles and guidance contained in the appropriate sections of Annex 1.

34. Where sterilization of starting and raw materials is required, it should be carried out where possible by heat. Where necessary, other appropriate methods may also be used for inactivation of biological materials (e.g. irradiation and filtration).

51. The growth promoting properties of culture media should be demonstrated to be suitable for its intended use. If possible, media should be sterilized in situ. In-line sterilizing filters for routine addition of gases, media, acids or alkalis, anti-foaming agents etc. to fermenters should be used where possible.

52. Addition of materials or cultures to fermenters and other vessels and sampling should be carried out under carefully controlled conditions to prevent contamination. Care should be taken to ensure that vessels are correctly connected when addition or sampling takes place.

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