A Quality by Design (QbD) Approach to Microbial Retention Validation

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Introduction

Regulatory guidance and manufacturing requirements exist to perform product-specific microbial retention testing on sterilizing filters. The implementation of a Quality by Design approach to microbial retention testing supports a paradigm that would obviate the need for product-specific testing for early-stage products, when the quantity of material for testing is limited. A review of sterilizing filter validation data was performed to identify process operating ranges that did not impact filter retention. In this work, process and product parameters were varied to determine their effect on microbial retention.

Regulatory Considerations

cGMP Manufacturing Regulations

Current Good Manufacturing Practices for Finished Pharmaceutical

21 CFR 211.113(b)

- Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of all aseptic and sterilization processes.

Regulatory Requirements for Ensuring Sterile Product

US: FDA Guidance for Industry CGMP for Phase 1 Investigational Drugs

- Product sterility is a critical element of human subject safety
- Implement appropriate controls for aseptic processing to ensure a sterile Phase 1 investigational drug

EU: EC Good Manufacturing Practice Medicinal Products for Human and Veterinary Use Annex 13 Investigational Medicinal Products

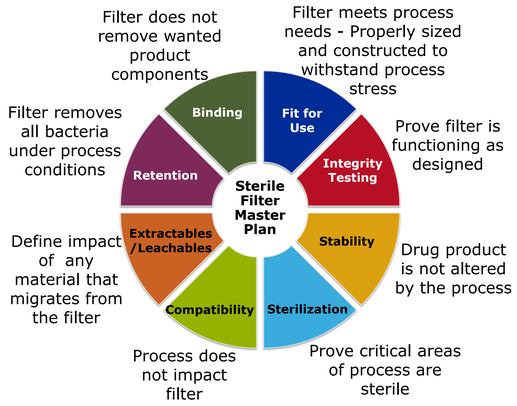
- Section 17. For sterile products, the validation of sterilising processes should be of the same standard as for products authorised for marketing.

Quality by Design (QbD)

An initiative from worldwide regulators, framed in ICH Q8, 2009

- Systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control based on sound science and quality risk management.
- A key element of this approach is a risk analysis of the manufacturing process for steps that might impact specific Critical Quality Attributes (CQAs), such as product sterility.

Elements of a Sterilizing Filter Validation



Sterilizing Filtration

PDA® Technical Report No. 26

Sterilizing filtration is the process of removing microorganisms from a fluid stream without adversely affecting product quality.

Sterilizing Filtration Validation:
Bacterial retention testing is conducted with process feed under process conditions at a challenge level of ≥ 1 x 10^7 CFU/cm² of effective filtration area. Quantitative retention of the entire challenge

ASTM F838:
"Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration"

Challenges in Performing Microbial Retention for Phase 1 Products

- Probability of clinical success → approval is 12%[1]
- During early stages of a project there are constraints on the volume of material available to perform microbial retention testing

A multivariable approach (following QbD principles[2]) was evaluated to determine the impact of process parameters on microbial retention performance of sterilizing grade membranes

References
[1]Dimasi, JA, et al., 2016. "Innovation in the Pharmaceutical Industry: New Estimates of R&D Costs." Journal of Health Economics, vol. 47:20-33.

[2]Finkler, C.; Krummen, L. Introduction to the Application of QbD Principles for the Development of Monoclonal Antibodies. Biologicals 2016, 44 (5), 282–290.

Quality by Design Matrix

Critical quality attribute: product sterility
Focus: recombinant proteins, primarily mAbs

Sterilizing-Grade Filters
- 0.2 µm Millipore Express® SHC hydrophilic polyethersulfone (PES) membrane
- 0.22 µm Durapore® hydrophilic polyvinylidene fluoride (PVDF) membrane

Data Sources
- MilliporeSigma published work
- Validation Services microbial retention studies
- Pfizer/MilliporeSigma specific testing
- Mechanistic studies demonstrating size exclusion

Retention Validation Data Review: Range of Process and Product Parameters

Durapore® 0.22 µm Membrane – ALL FULLY RETENTIVE

Parameter	# of Studies	Minimum	Maximum
Concentration (mg/mL)	31	1	150
Pressure (psi)	51	0.5	50
Temperature (°C)	31	2	30
Volume (L/m²)	20	493	79,710
Time (hr)	34	7	168
pH	13	4.0	8.0
Surfactant (%)	8	0.05	20

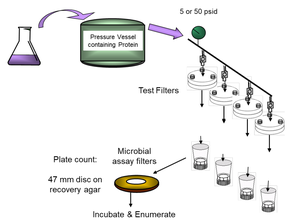
Millipore Express® SHC 0.5/0.2 µm Membrane – ALL FULLY

Parameter	# of Studies	Minimum	Maximum
Concentration (mg/mL)	19	1	150
Pressure (psi)	19	5	50
Temperature (°C)	19	2	30
Volume (L/m²)	19	133	10,870
Time (hr)	19	15	168
pH	19	4.9	7.6
Surfactant (mg/mL)	19	0.1	0.4

Experimental Overview

With customer feed under specific conditions

Challenge organism
- *Brevundimonas diminuta* (ATCC® 19146™ strain)
- Small (0.3-0.4 x 0.6-1.0 µm), water-borne, motile, "worst-case" organism



Sterilizing filter
- Millipore Express® SHC filters
- Multiple membrane lots

Process parameters bracketed to evaluate impact on retention
- Pressure
- Temperature
- Time
- Volume (L/m²)
- Product Concentration

[mAb1] g/L	Pressure (psi)	Temp (°C)	Time (hr)
150	50	4-8 (48 hr) + 18-23(48 hr)	96
1	50	4-8 (96 hr)	96
150	5	4-8 (48 hr) + 18-23 (48 hr)	96

Results

[mAb1] g/L	Pressure (psi)	Temp (°C)	Membrane	Volume (L/m²)	Bacterial Challenge (CFU/cm²)	LRV*
150	50	4-8 (48 hr) + 18-23 (48 hr)	0.2 µm, lot 1	259	8.5×10^7	≥9.1
			0.2 µm, lot 2	327	1.1×10^8	≥9.2
			0.2 µm, lot 3	333	1.1×10^8	≥9.2
			0.45 µm, control	324	1.1×10^8	1.3
1	50	4-8 (96 hr)	0.2 µm, lot 1	319	7.1×10^7	≥9.0
			0.2 µm, lot 2	310	6.9×10^7	≥9.0
			0.2 µm, lot 3	312	6.9×10^7	≥9.0
			0.45 µm, control	298	6.6×10^7	1.4
150	5	4-8 (48 hr) + 18-23 (48 hr)	0.2 µm, lot 1	330	8.0×10^7	≥9.0
			0.2 µm, lot 2	328	7.9×10^7	≥9.0
			0.2 µm, lot 3	316	7.6×10^7	≥9.0
			0.45 µm, control	341	8.2×10^7	1.6

*LRV (log reduction value)
"≥" indicates complete retention

All studies demonstrated complete retention of *B. diminuta* at a challenge level of ≥ 1 x 10^7 CFU/cm² of filtration area
Worst case processing conditions did not impact microbial retention
- **High pressure**
- **High temperature**
- **Extended duration**

Summary

- The use of QbD principles[2] can be used in microbial retention testing, supporting a paradigm minimizing the need for product-specific testing for early-stage products.
- A review of retention validation data defined a robust process design space over a range of product parameters that met the critical quality attribute of product sterility.
- These experimental results confirm process ranges for sterilizing filter retention performance for Phase 1 manufacturing.
- Partnering with your filter supplier can streamline testing plans and help develop a comprehensive strategy for validation and implementation.

Additional Information

A Quality by Design Approach to Microbial Retention Validation, Annie Leahy, Jennifer Juneau, Kathleen Souza, Corinne Miller, Nhung Nguyen, Herb Lutz and Parag Kolhe, PDA Journal of Pharmaceutical Science and Technology July 2022, pdajpst.2022.012739; DOI: https://doi.org/10.5731/pdajpst.2022.012739