Sterile Filter Validation:

Evolving Expectations and Dual Filter Validation Strategies

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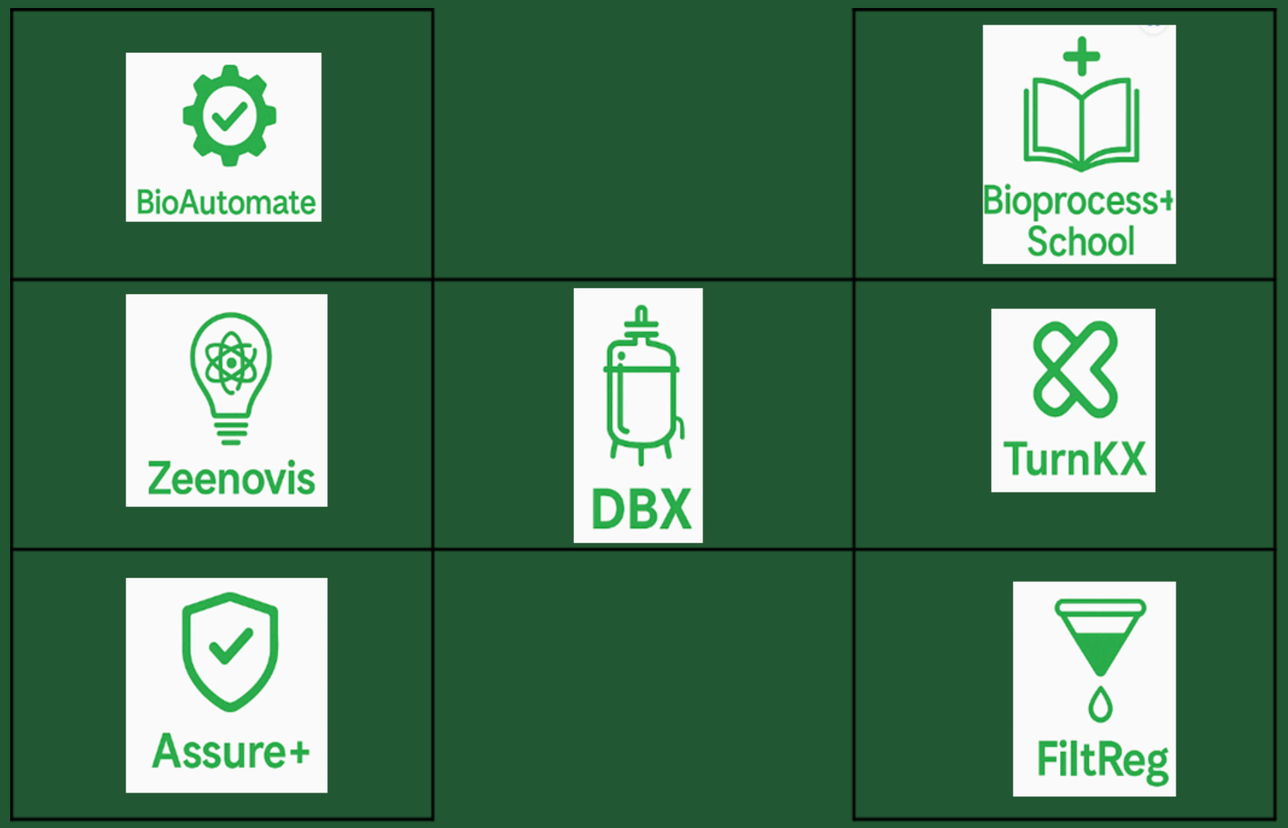
BUSINESS VERTICALS



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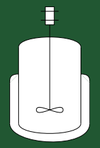


Bioprocess Technology	BIOPROCESS ENGINEERING	REGULATORY AFFAIRS	AUTOMATION	TECHNOLOGY TRANSFER

HIGHLIGHTS

- GMP modelled Pilot-Scale Facility
- Expert Trainers
- Customizable Modules
- Real Time Case Studies

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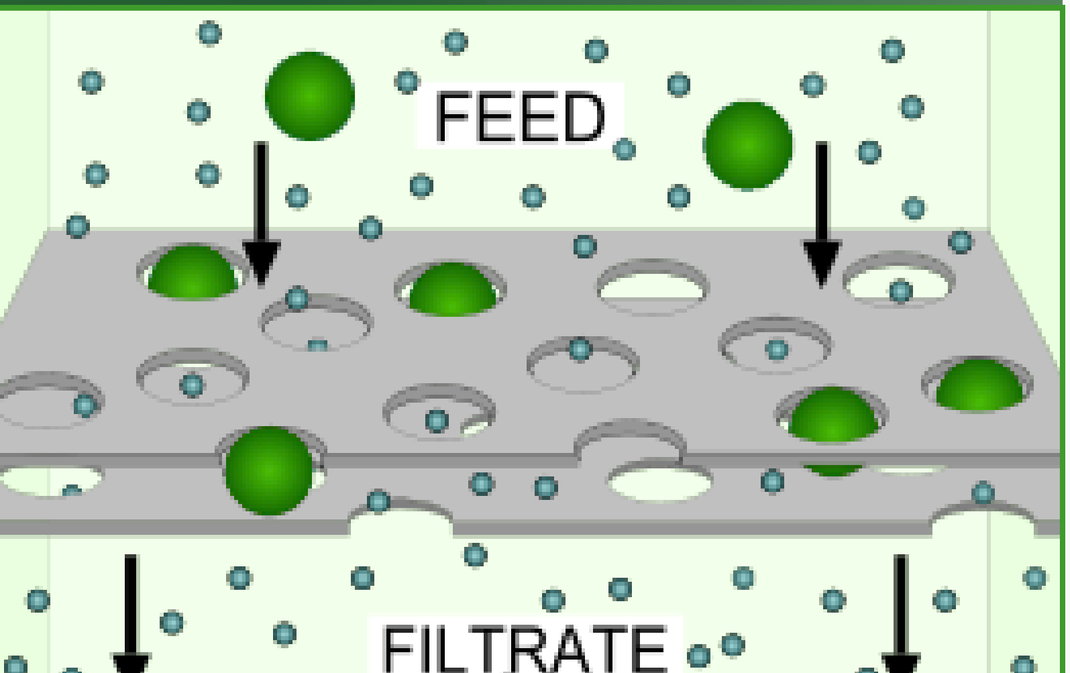
Sterile Filter



Sterile Filtration Plays a crucial role in Biopharmaceutical Manufacturing

- Sterile filtration is the critical step to ensure sterility of biopharmaceutical products
- Used widely in biologics, vaccines, cell therapies, parenterals, and other sterile injectable
- Especially to sterilize heat–labile substances

Why sterile filter validation?

- Sterile filtration ensures sterility in the product
- Failure in sterile filtration = batch rejection, product recall, or patient risk.
- Global regulators demand scientifically justified and validated filter performance

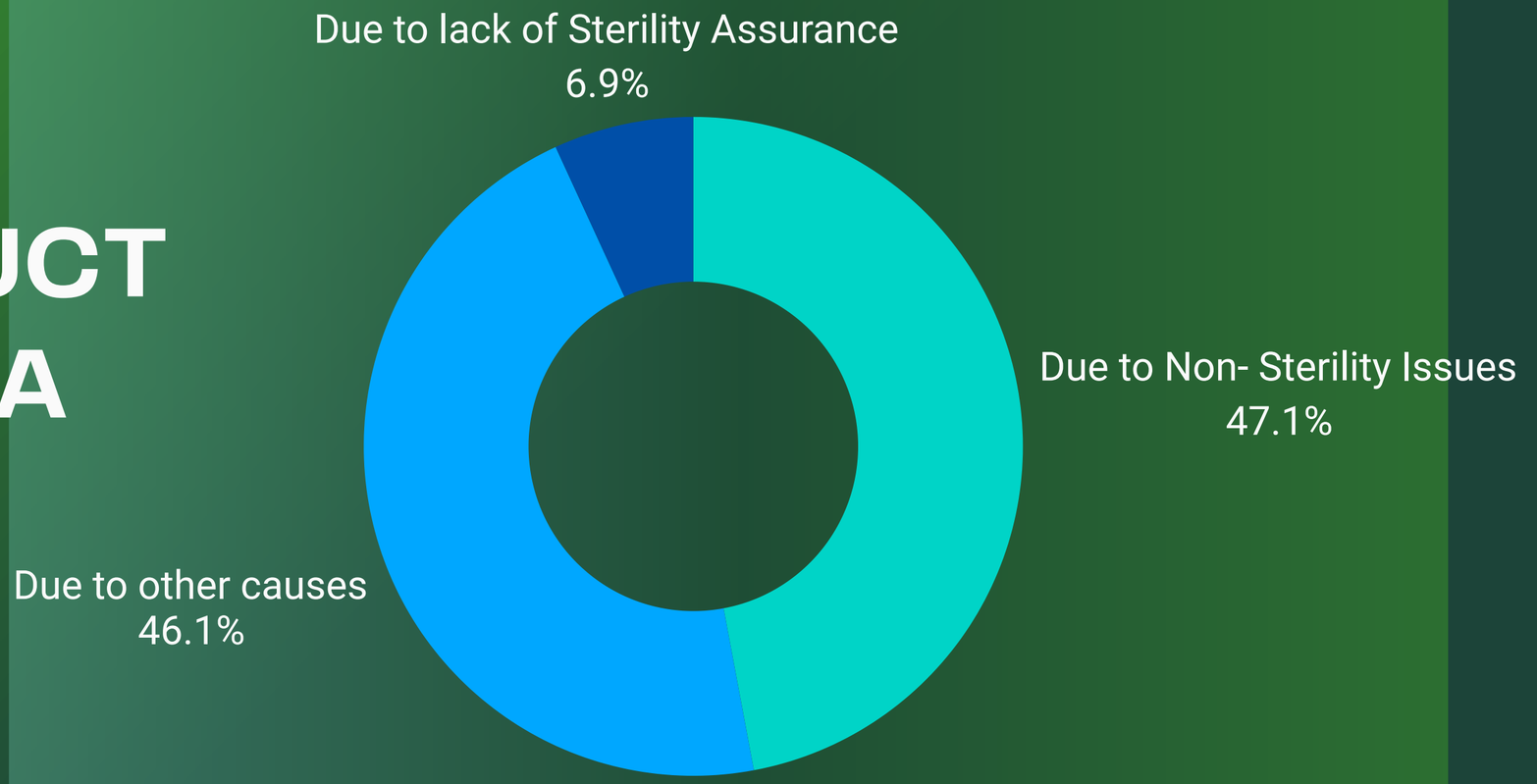
Importance of sterile filter validation

- ~70% of sterile drug products in the global market undergo sterile filtration as the primary method of sterilization (since many biologics are heat/ radiation sensitive).

- A single product recall due to filter integrity issues can cost companies millions of dollars, aside from reputational damage.

STERILE PRODUCT RECALLS BY FDA

(2012- 2023)



Sterilizing grade filters

The FDA has defined a sterilizing filter as one that retains the classic challenge of at least $1X10^7$CFU of *Brevundimonas diminuta* ATCC-19146 per cm^2 effective filtration area (EFA) at pressures up to 30 psi (2 bar).

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Hydrophilic Filters

MoCs: Cellulose Acetate (CA), Nylon, Polyethersulfone (PES), PVDF (Hydrophilic Treated Version)

Applications:
Sterile Filtration of Liquids:
Media, buffers, WFI (Water for Injection), culture broths, proteins, final drug formulations.

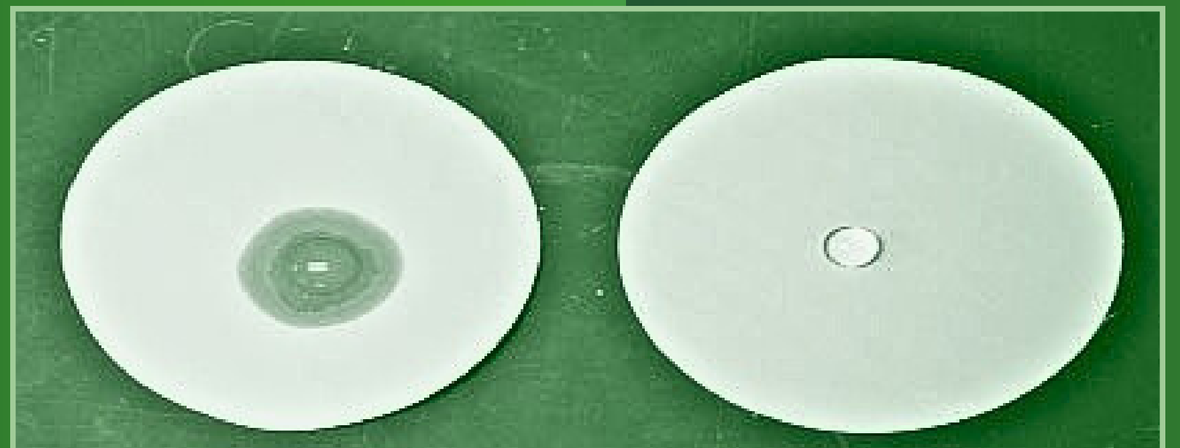
Clarification / Prefiltration:
Removal of particulates before sterilizing-grade filter.
Virus Filtration: Some specialized hydrophilic membranes used.

Hydrophobic Filters

MoCs: PTFE (Polytetrafluoroethylene) → most common.
PVDF (hydrophobic-treated).

Applications
Gas and Vent Filtration:
Sterilizing compressed air, nitrogen, or CO_2 used in bioreactors and fermenters.

Vent filters on tanks, fermenters, bioreactors, and sterile holding vessels.



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What is filter validation?

Documented evidence that a sterilizing-grade filter or process filter consistently removes or retains microorganisms/particles under actual process conditions.

It ensures that the filter chosen is suitable, effective, and reliable for its intended use.

Validation Strategies

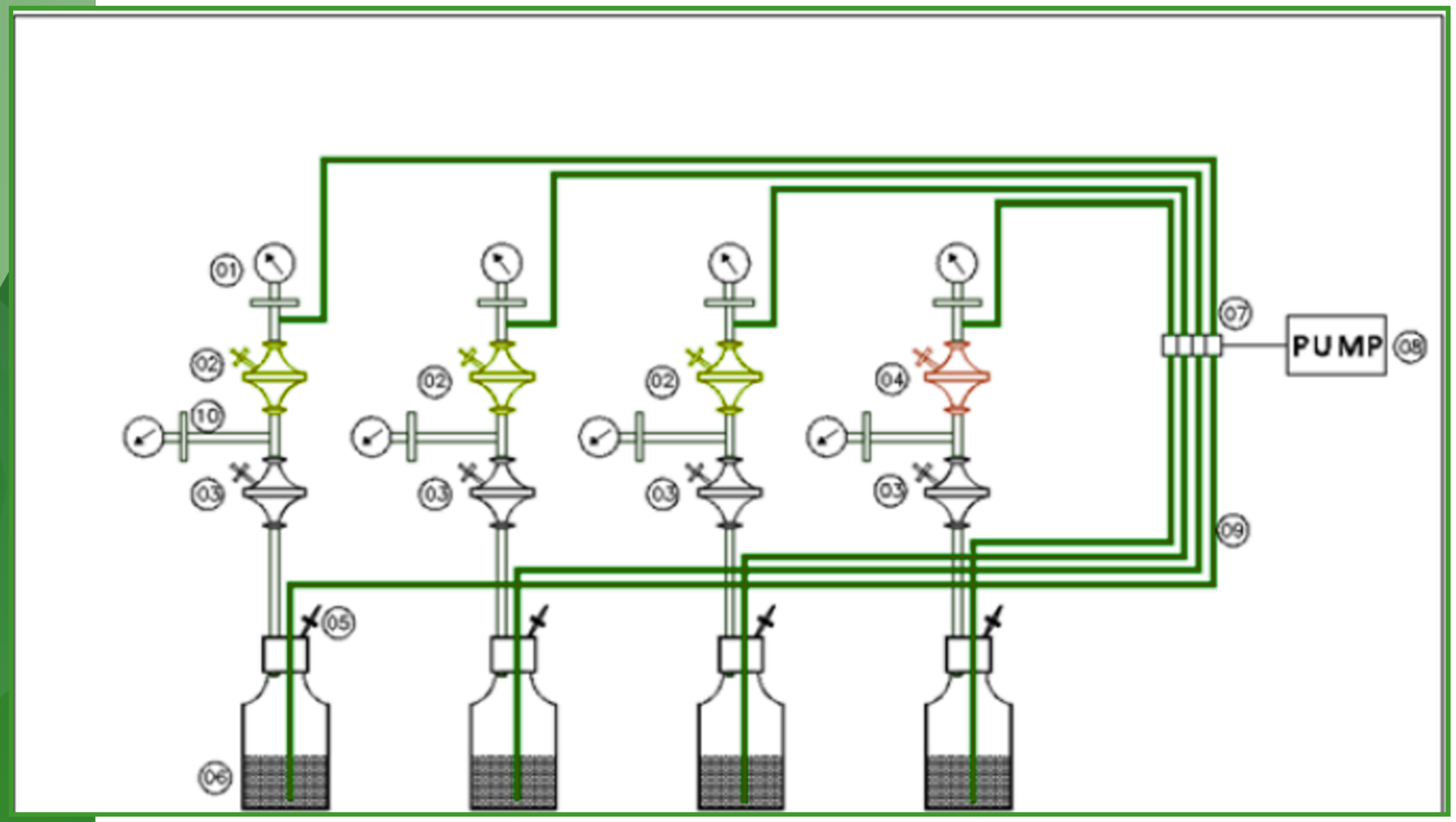
- Bacterial Retention Test
- Integrity Testing
  - Bubble Point Test
  - Air Diffusion Test
  - Water Intrusion Test

Filter Integrity Testing

Pre-use Integrity Testing – ensures filter integrity before filtration

Post-use Integrity Testing – confirms no filter failure during processing.

Bacterial Retention Test



Log Reduction Value (LRV)

$$\mathrm{LRV} = \log_{10}\left(\frac{N_0}{N}\right)$$

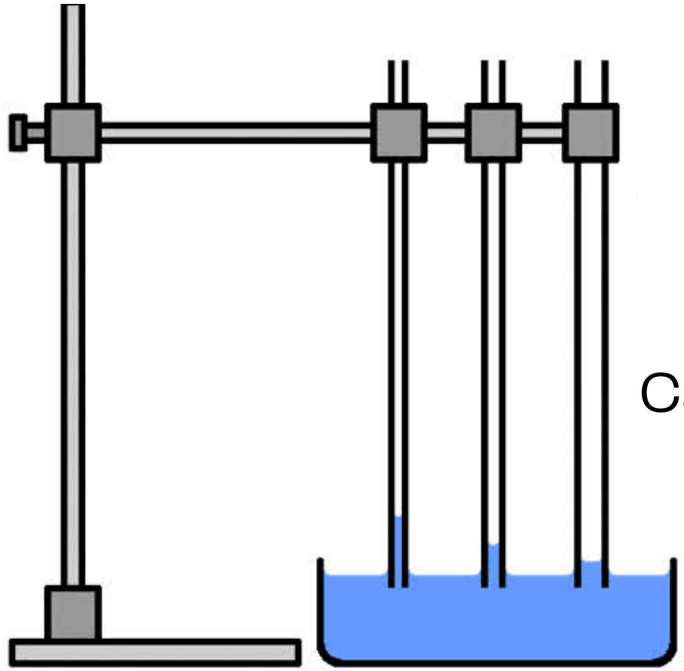
Where:

N_0= number of microorganisms (challenge organisms) before filtration

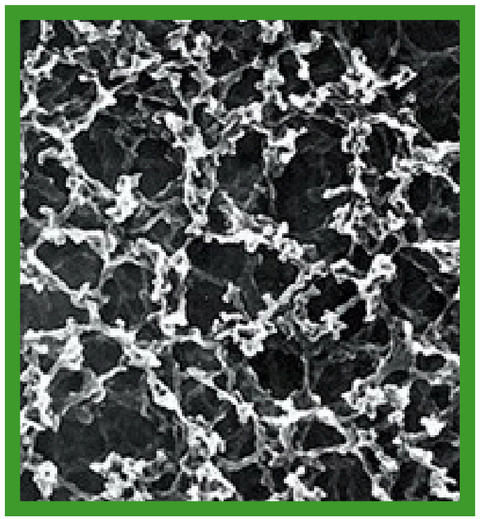
N = number of microorganisms after filtration (that passed through the filter)

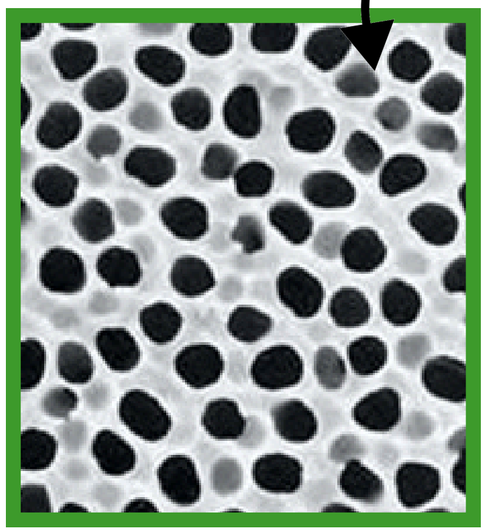
Sterilizing-grade filters must demonstrate ≥ 7 LRV against *B. diminuta* under validated conditions (As per FDA, PDA TR26, and EU GMP guidance).

Bubble Point Test- Principle



Capillary Action

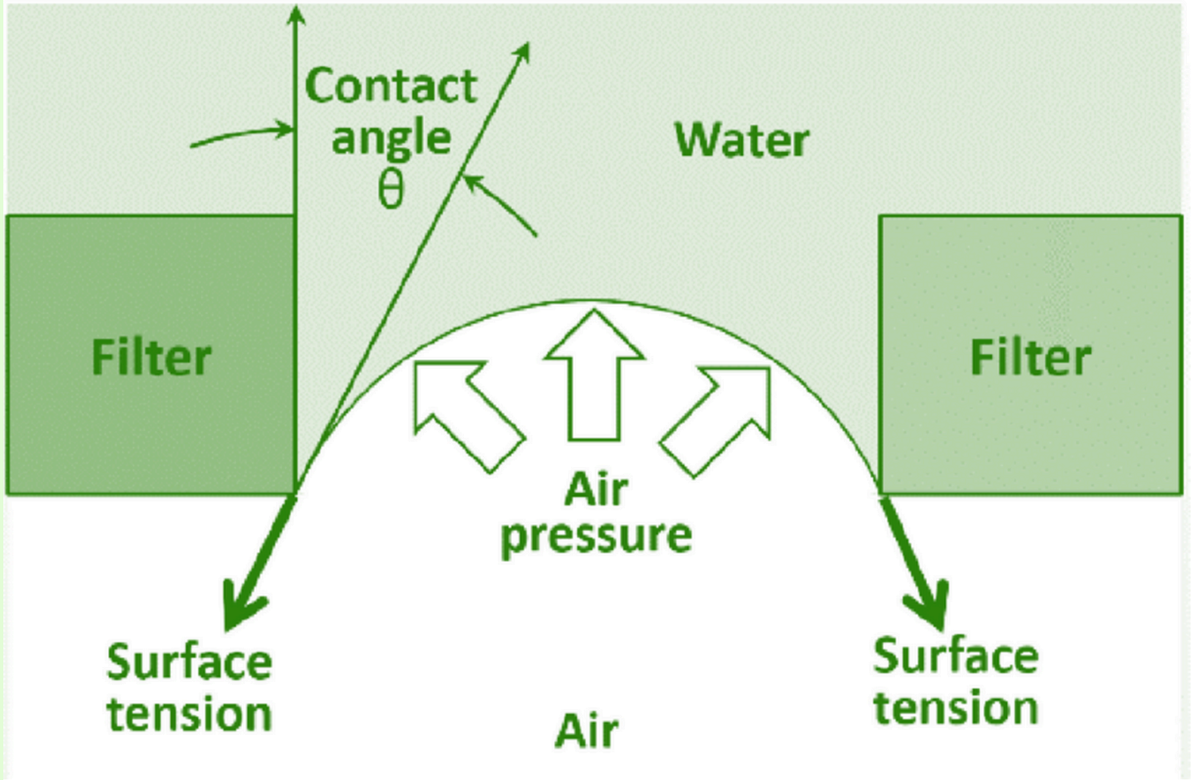




Source: Cytiva, 2021

Conducting The Test

Bubble Point Test



Source: Lindsley et al., 2016

$$BP = \frac{4 \cdot k \cdot \gamma \cdot \cos\theta}{d}$$

Where

k = shape correction factor

Y = surface tension

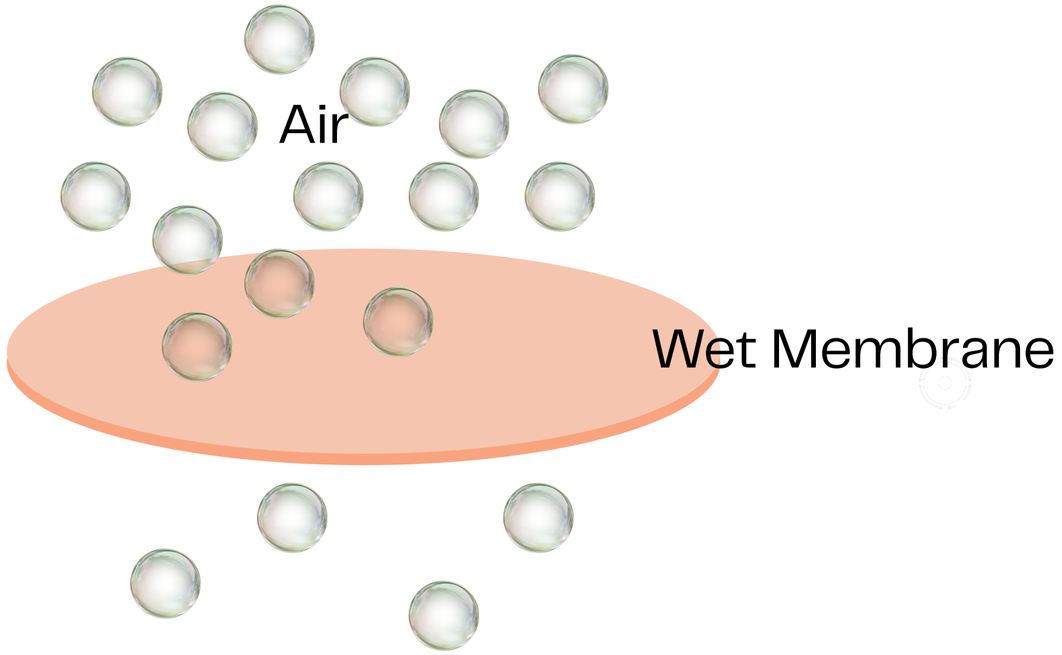
ɵ = contact angle

d = pore diameter

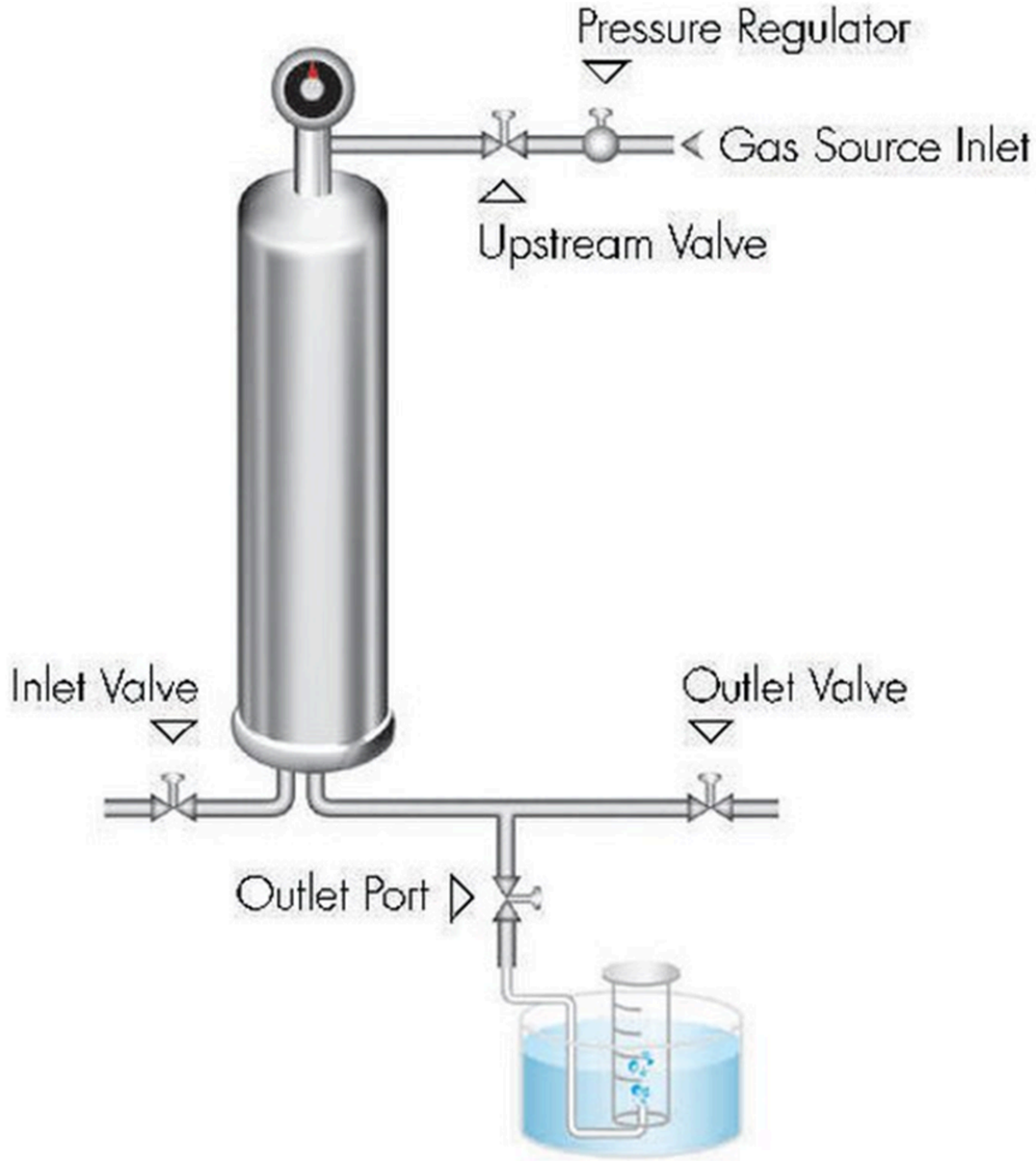
Air Diffusion Test

Gas molecules migrate through the water-filled pores of a wetted membrane- Fick's Law of Diffusion

At a pressure approximately 80% of the minimum bubble point, the gas which diffuses through the membrane is measured to determine a filter's integrity



Air Diffusion Test



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Integrity Testing of Hydrophobic Filters

Water Intrusion Test

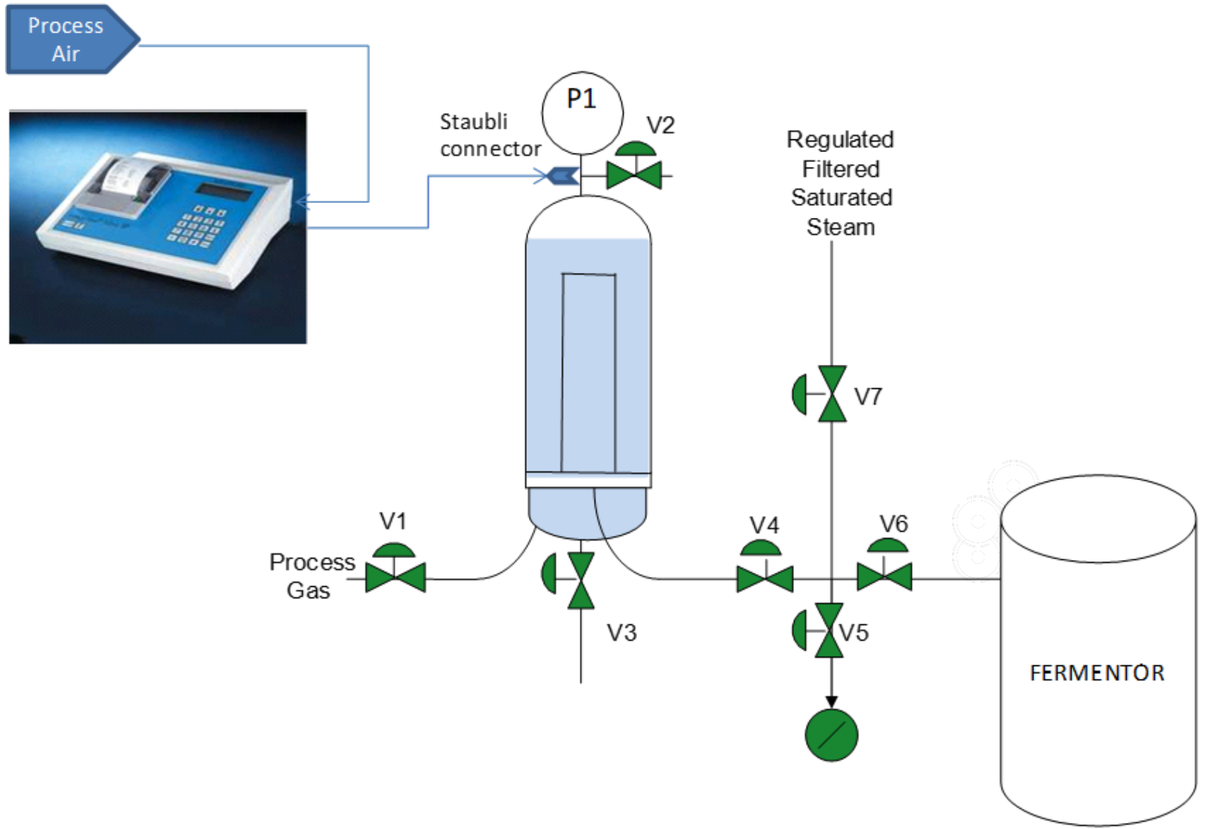
Wetting Liquids for Hydrophobic Filters

- Less hydrophilic filters– liquids of lower surface tensions
- Aqueous alcoholic solutions – Suitable for Hydrophobic
- ~60–70% v/v, methanol, ethanol, isopropanol, or (rarely) 25% v/v tertiary butanol
- Challenges:
  1. Subsequent disposition of the wetting liquid
  2. Complex plumbing arrangements, or by risking asepsis downstream of the filter

Water Intrusion Test

Measures the decay rate of a pressure level imposed upon a hydrophobic filter enveloped by water.

Automated integrity tester– a particular decay level is identified as the point at which water enters the largest pores of the filter.



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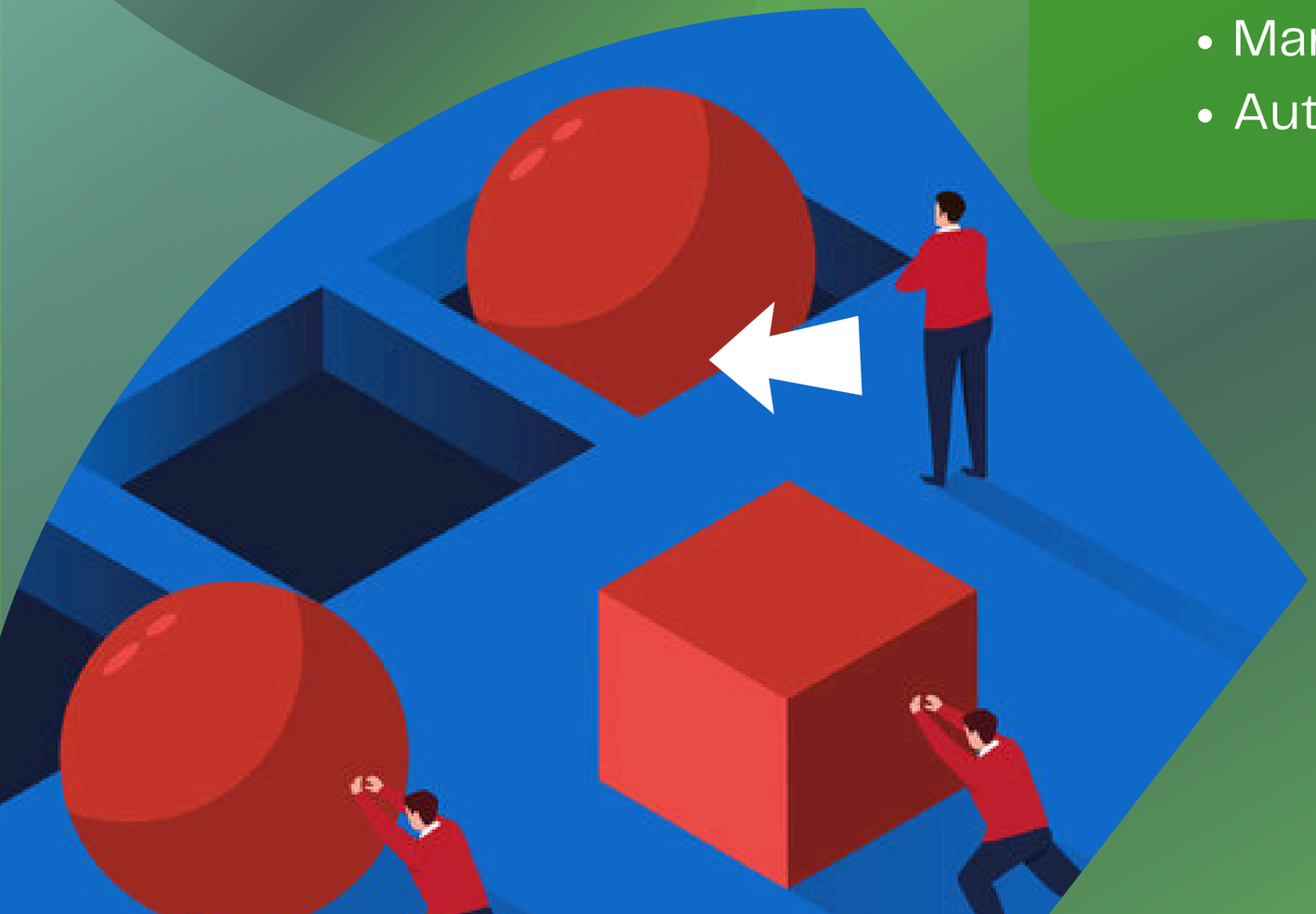
Common pitfalls

- Improper Wetting
- Wrong Wetting Liquid
- Manual testing can introduce variability
- Automation reduces error



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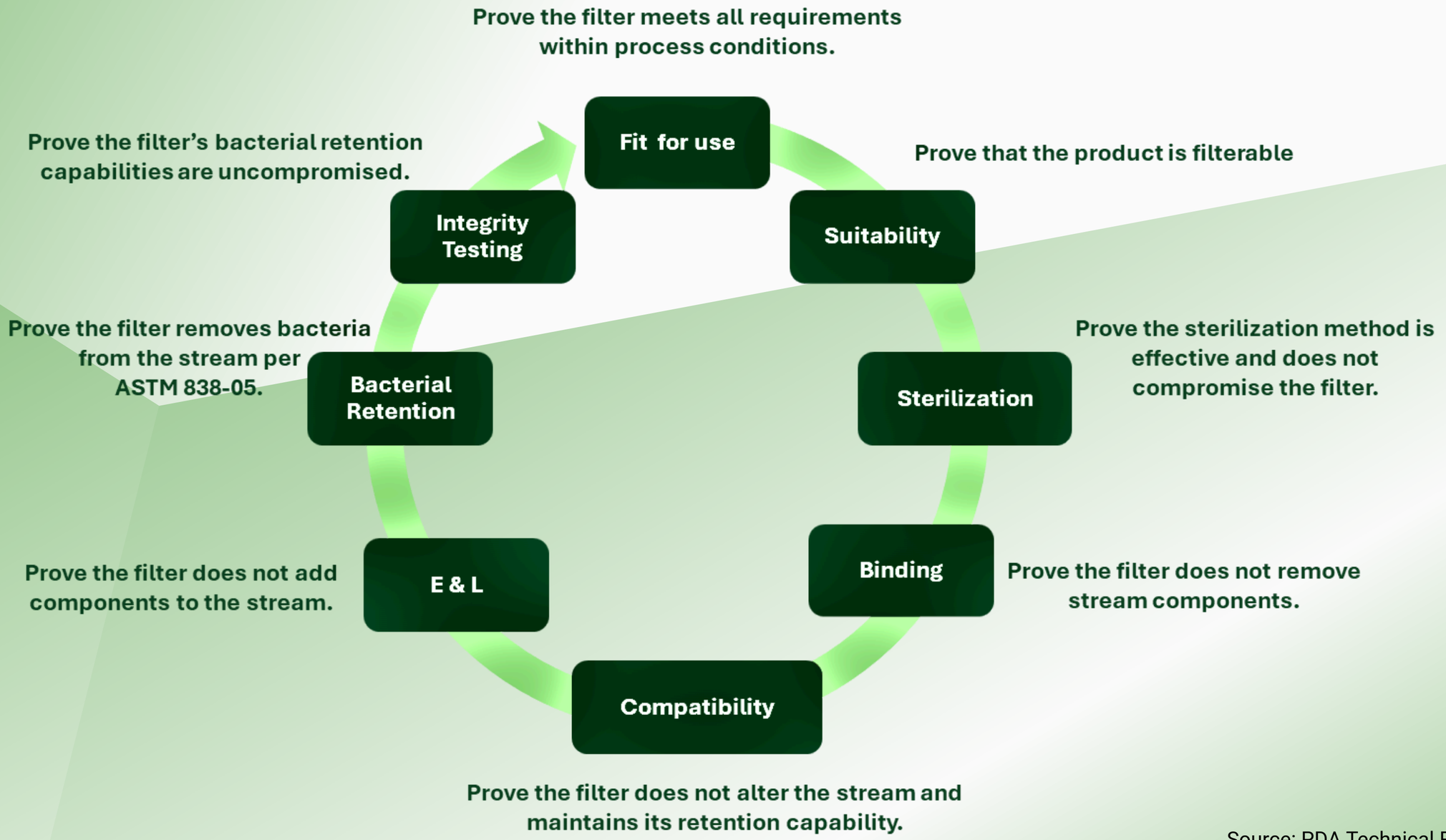
POLL QUESTION 1

Sterile Filter Validation Approach



Source: PDA Technical Report No. 26

Evolving Expectation in Filter Validation

Dual Filter Validation	21 CFR Part 11 Compliance	Bracket Approach Δ P	Flowrate & Pressure-simultaneously	PUPSIT (Pre-use/Post-sterilization Integrity Testing) & Avoid Masking effect

POLL QUESTION 2

PUPSIT

PUPSIT(Pre-use/Post-sterilization Integrity Testing)	
Parameter	**Description**
Definition	**A filter integrity test performed before use and post sterilization, but prior to filtration**
Purpose	**To verify that the filter remains intact and functional after sterilization.**
Importance	**Ensures filter integrity has not been compromised by sterilization or handling, and prevents false positive results.**
Risk Control	**Helps avoid false positives (e.g., masking of filter damage) and ensures sterile filtration reliability.**

PDA Tech Report 26 – BRT Requirement

6.2 Bacterial Retention Validation Studies

The goal of bacterial retention validation studies is to have documented evidence demonstrating that the filtration process will consistently remove a high level of a standard bacterium or relevant bioburden isolate, suspended within the product or surrogate fluid, under simulated process conditions.

The decision to test membrane discs or a full-size process filter is dependent upon the study goal. If the study is to validate the bacterial retention efficiency of a particular membrane material, the use of a small test membrane disc is generally regarded as sufficient. The test methodology used for determining the physical integrity of process filters should yield results that are meaningful in terms of bacterial retention testing. If different test methods are used, a relationship between the two should be demonstrated.

Process time and pressure drop can affect bacterial retention test results. Bacterial challenge over the full process time assesses the potential for time-dependent factors, such as filter compatibility, maintenance of integrity and the occurrence of time-dependent penetration.

The pressure differential across the test filter during validation of the bacterial challenge test should meet or exceed the maximum pressure differential permitted during processing (within the filter manufacturer's design specifications). The actual process flow rates should be incorporated when designing the model challenge conditions. It may not be possible to mimic pressure differential and flow rate simultaneously during validation. The filter user should determine which parameter is more relevant to the specific process and develop a rationale to support the decision.

Advancement in Filter Validation – Automated Bacterial Retention Skid

21 CFR part 11 Compliance:

Bacterial Retention Test		
Parameter	Automated	Manual
21 CFR part 11 complaint	✓	✗
Data integrity	✓	✗
Manual intervention	✗	✓
Online flow rate monitoring	✓	✗
Pressure and its flow rate simultaneous monitoring	✓	✗
System qualification as per Industrial standards	✓	✗
Data monitoring minute by minute	✓	✗
Industrial process mimicking: CIP-SIP-filtration-CIP	✓	✗
Online delta P monitoring	✓	✗
Test data capture upon reaching/exceeding the set value	✓	✗

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Automated Retention Skid – Report

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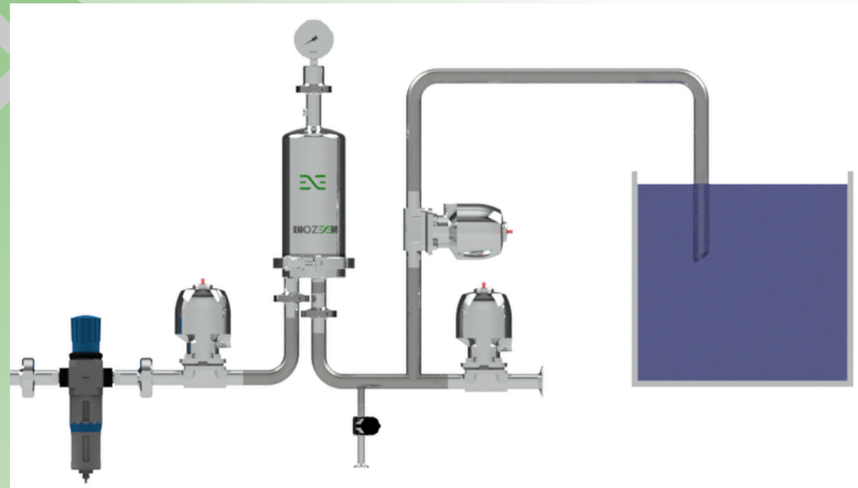
Enabling Easy approval of your dossiers

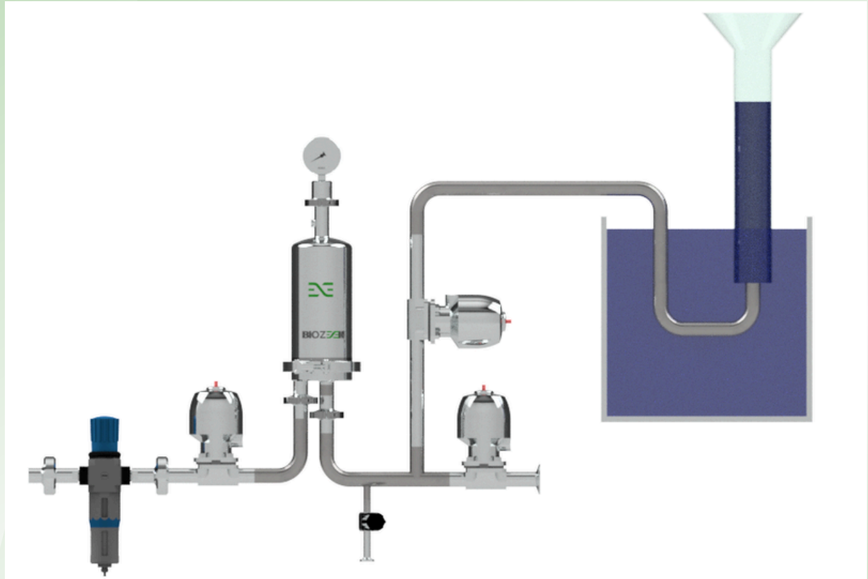
Online Data snapshot

Date Time	Step	TT01_PV	PT1_PSI	PT2_PSI	PT3_PSI	PT4_PSI	FT01_PV	FT02_PV	FT03_PV	FT04_PV	Delta_P01	Delta_P 02	Delta_P 03	Delta_P 04
09-06-2014	01 : Operator Message	23.1	0	0	0	0	0.32	0.32	0	0	0	0	-0.02	0
09-06-2014	02 : Stabilizing	23.7	0	0	0	0	0	172.96	0	0	0	0	-0.02	0
09-06-2014	03 : Recirculation	24.3	29.24	20.98	25.75	7.08	244.74	215.65	223.56	265.93	2.02	1.31	1.56	0.29
09-06-2014	03 : Recirculation	24.2	30.25	21.92	25.99	6.75	257.71	219.13	229.57	288.06	2.09	1.37	1.58	0.25
09-06-2014	03 : Recirculation	24.3	31.49	22.63	26.39	6.98	259.92	228.62	236.52	300.4	2.17	1.42	1.6	0.26
09-06-2014	03 : Recirculation	24.4	31.29	22.36	27.16	7.76	259.92	227.98	239.37	309.57	2.16	1.39	1.65	0.3
09-06-2014	03 : Recirculation	24.4	33.67	23.54	26.32	7.79	263.4	229.57	244.43	312.73	2.32	1.47	1.59	0.3
09-06-2014	03 : Recirculation	25	32.13	22.8	27.66	8.12	266.25	231.46	244.11	316.84	2.22	1.42	1.69	0.31
09-06-2014	03 : Recirculation	26	32.4	23.27	27.43	7.86	266.56	230.51	246.01	320.63	2.23	1.45	1.67	0.29
09-06-2014	03 : Recirculation	26.4	33.91	23.27	27.13	8.39	271.62	228.93	244.74	321.26	2.34	1.45	1.65	0.33
09-06-2014	03 : Recirculation	23.8	33.77	23.84	27.3	8.12	275.42	234.94	242.85	342.13	2.33	1.49	1.66	0.3
09-06-2014	03 : Recirculation	23.9	33.1	23.23	28.1	8.33	273.2	229.88	242.85	331.38	2.28	1.45	1.71	0.32
09-06-2014	03 : Recirculation	24.1	0	0	0	0	2.21	8.85	1.9	0	0	0	-0.02	0

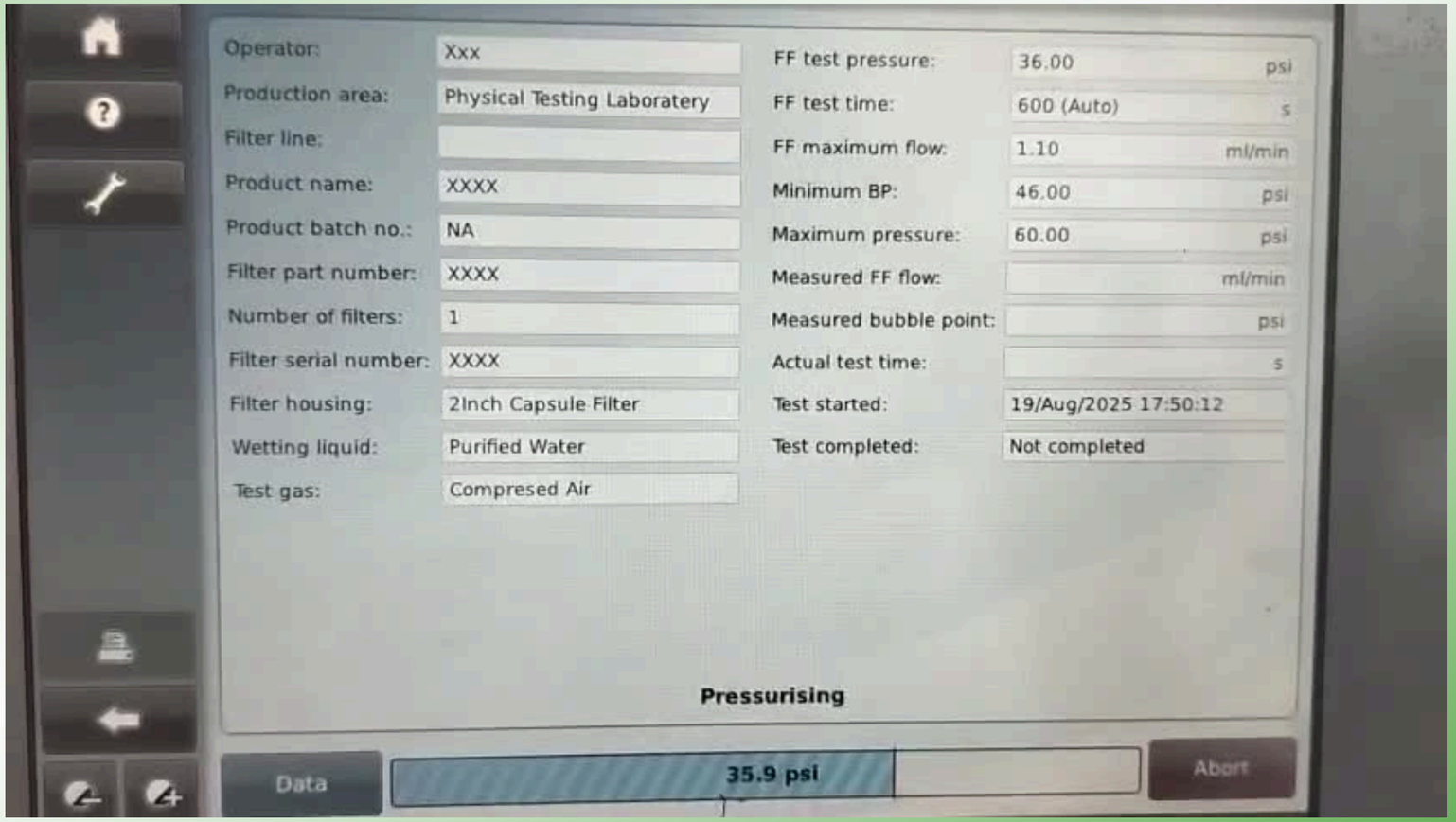
Types of Filter Integrity Tests



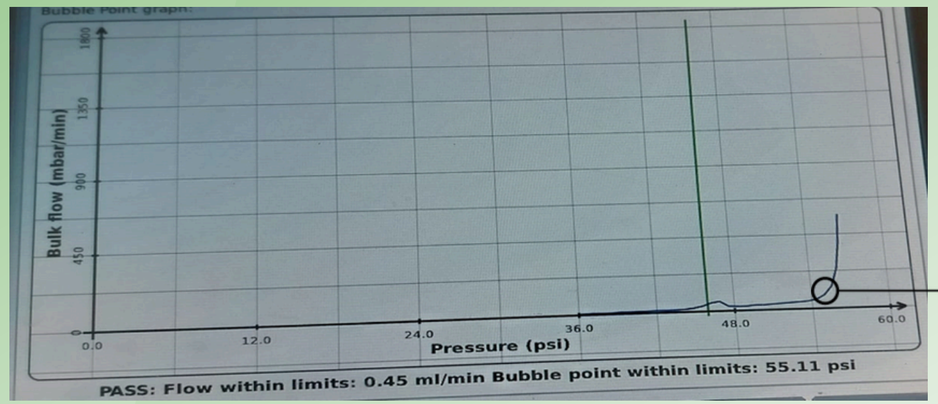
Manual Bubble Point Test



Manual Diffusion/ Forward Flow Test

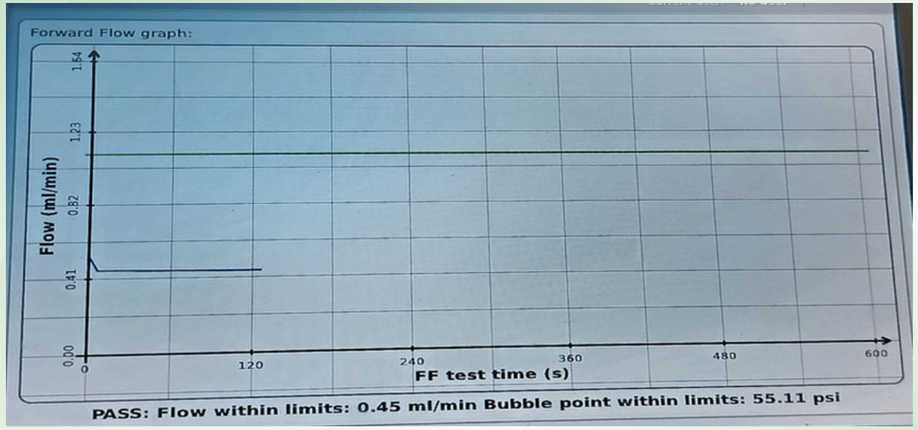


Plots of Bubble point and diffusion

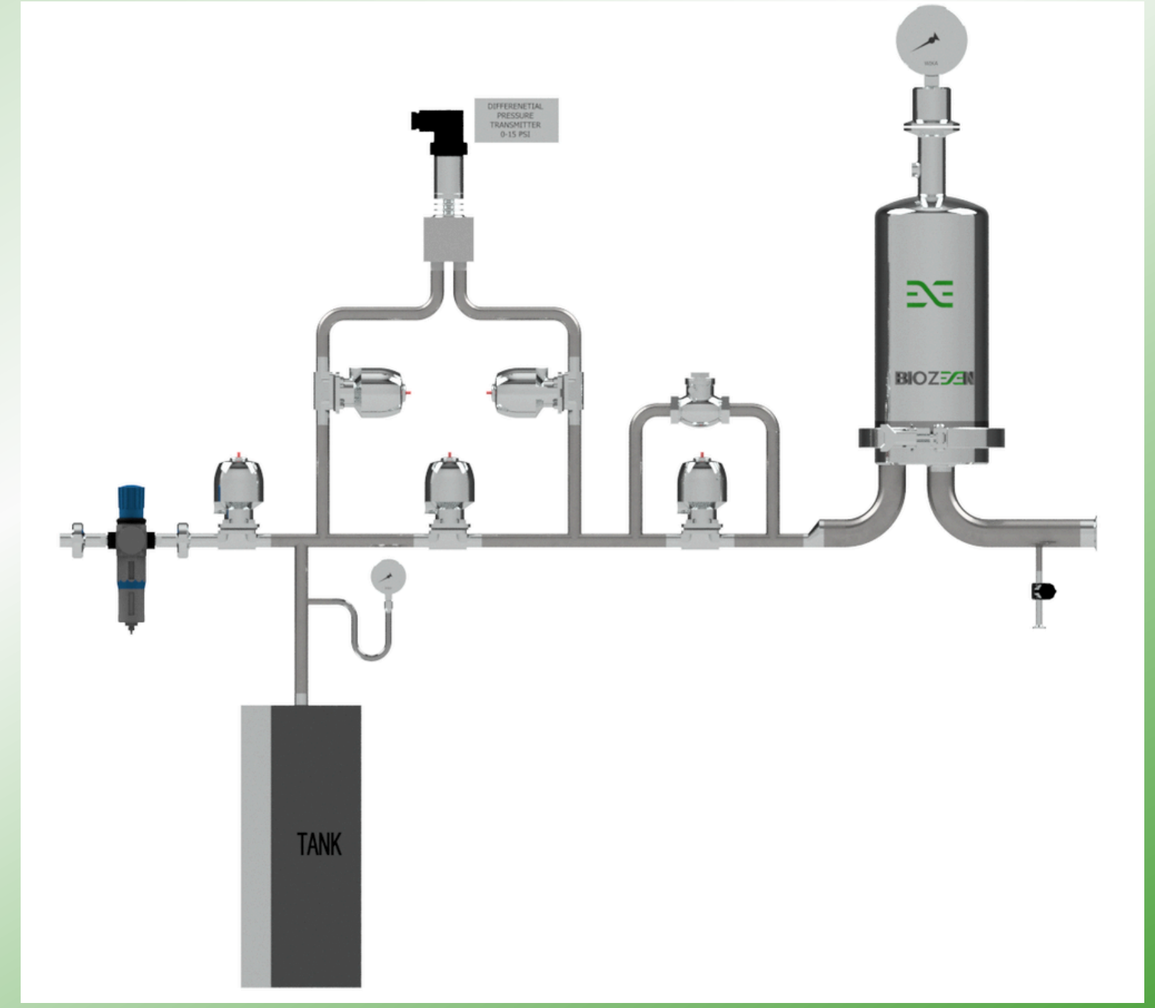


Bubble Point

Plot showing the result of Bubble point test



Plot showing the result of Diffusion test



POLL QUESTION 3

Common Faults During Testing

- Wetting Issue
- Wrong program selection
- Leakage Issues
- Temperature Influence
- Choice of gas
- Thermal Stress on the filter
- Bubble not detectable/ obtainable
- Instrumentation Should be calibration valid
- Correct Wetting Fluid
- During Reestablishment Water Bubble Point Failure
- Proper assembly and functioning of the test set up
- Use of correct filter housing if cartridge filter

FTOS

Filter train optimization is the process of selecting the correct combination of filters for the maximum throughput.

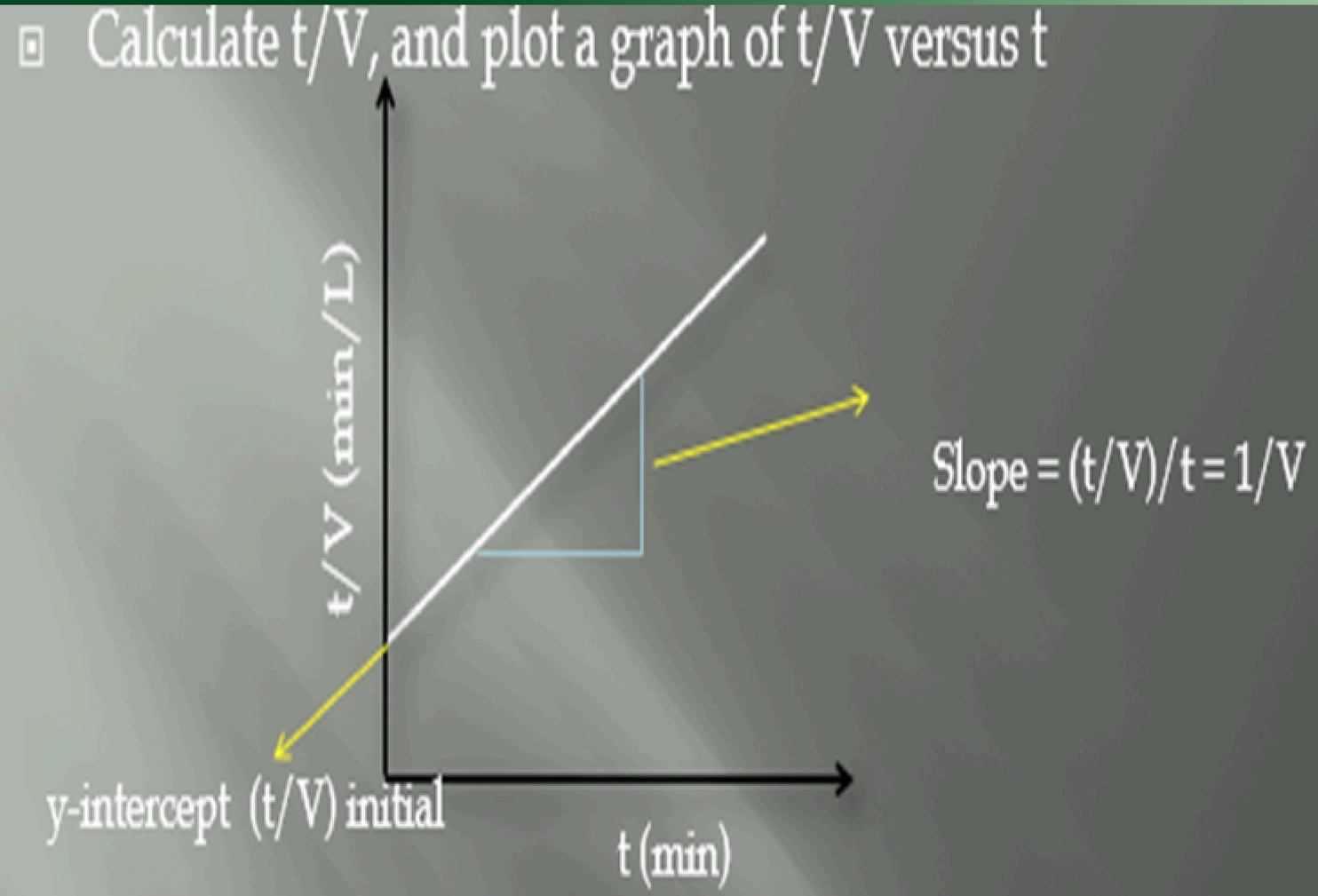
In this method the pressure is kept constant and, volume end point is found.

In this method is applicable only for gradual pore plugging model.

During the test, the cumulative weight is recorded as a function of time and the maximum volume that will be filtered is calculated using density of product
Generally, filter area selected is larger than Amin to have additional safety factor.

FTOS

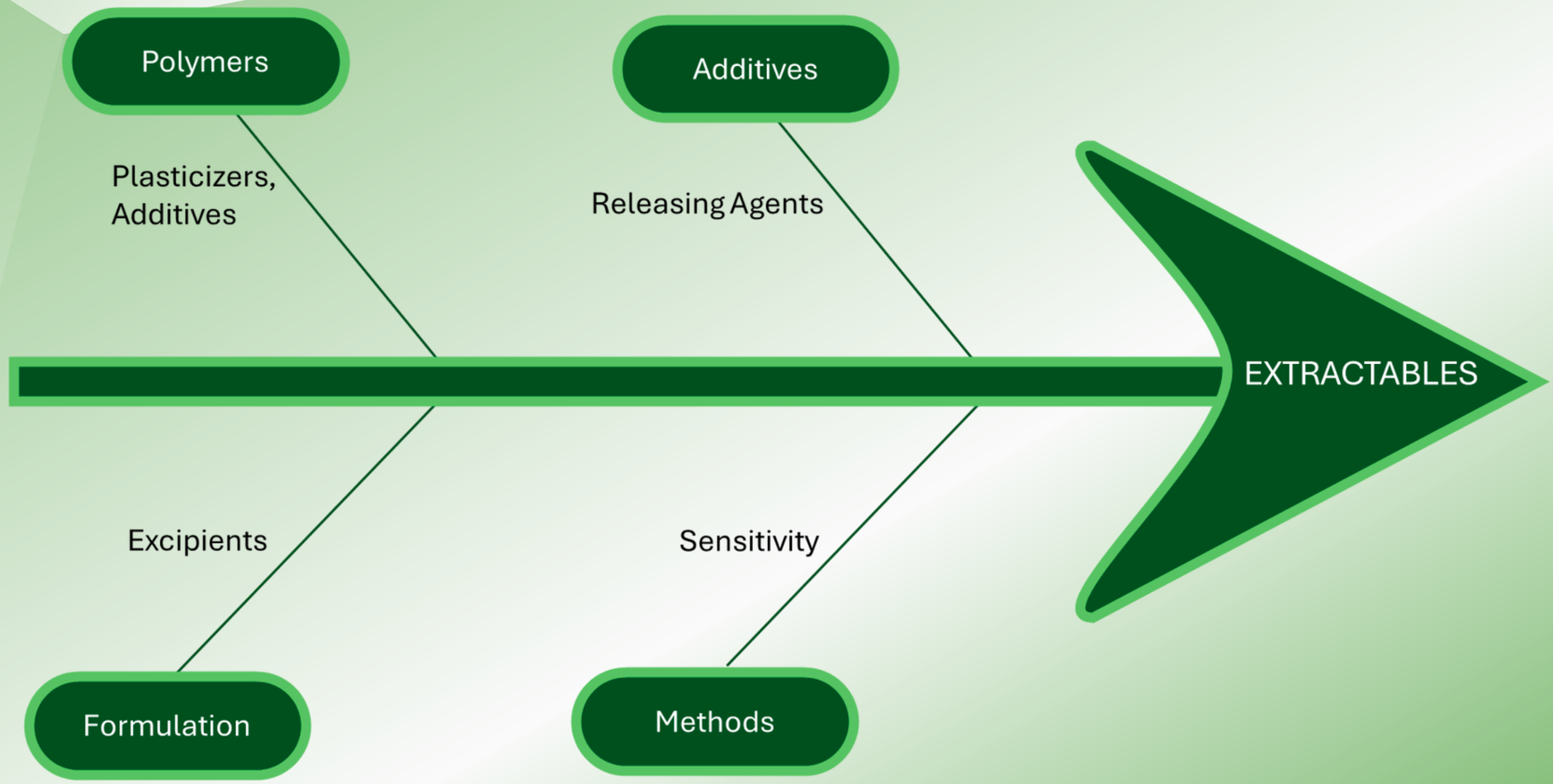
- Calculate t/V, and plot a graph of t/V versus t



Data Table		
TIME	VOLUME	T/V
(min)	(mL)	(min/mL)
0.08	2	0.0408
0.17	4	0.0434
0.25	5	0.0510
0.33	6	0.0561
0.42	8	0.0536
0.50	9	0.0567
0.58	11	0.0538
0.67	12	0.0570
0.75	14	0.0546
0.83	15	0.0564
0.92	16	0.0587
1.00	18	0.0567
1.08	19	0.0580
1.17	20	0.0597
1.25	21	0.0607
1.33	22	0.0617
1.42	23	0.0630
1.50	24	0.0638
1.58	26	0.0620
1.67	27	0.0631
1.75	28	0.0638
1.83	29	0.0644
1.92	30	0.0653
2.00	31	0.0658
2.08	32	0.0663
2.17	33	0.0671

Source: Principles of Fermentation Technology, Peter F Stanbury, Chapter 5, p324

Risk Analysis Perspective – Extractables



Note: Model Solvent Selection is based on the product pH and Functional Groups

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Changes to Consider	Bacterial Retention	Product Bubble point	Product Diffusion	Compatability	Extractables
Change in filter membrane (type/materials of construction)	✓	✓	✓	✓	✓
Same membrane - Change in device type (from a capsule to a cartridge for instance)				✓ If original test is a full-device test	✓
Same membrane - Change in pore size	✓	✓	✓	✓ May need a letter with data to update the original report	✓
Same membrane - Change in filtration surface area	✓ If filtered volume / surface area is increased				✓ May need a letter with data to update the original report

Changes to Consider	Bacterial Retention	Product Bubble point	Product Diffusion	Compatability	Extractables
Increase in flux ie. increased flow rate per surface area					
Increase in total filter/product contact time				 If it exceeds contact time achieved during testing	 If it exceeds the time provided in original report
Increase in control pressure					
Increase in batch volume without a scaled increase in filter surface area	 If batch volume was a criterion met in the original study				
Change in sterilization conditions generally increase in time,temperature,or number of cycles					 If the new sterilization conditions exceed those provided in the original report
Change in filtration temperature	 Match test to process temp.when organisms can survive in that temp			 If temperature is increased.	 If temperature is increased.
Change in sterilization method					

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