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SNS COLLEGE OF PHARMACY AND HEALTH SCIENCES

Affiliated To The Tamil Nadu Dr. MGR Medical University, Chennai

Approved by Pharmacy Council of India, New Delhi.

Coimbatore -641035

COURSE NAME : PHARMACEUTICAL MICROBIOLOGY - BP303 T

B.PHARM II YEAR / III SEM

UNIT 3

SUB TOPIC :STERILITY TESTING OF PHARMACEUTICALS

Sterility Testing Overview

Sterility testing is performed to detect viable contaminating microorganismcns in sterile pharmaceutical products.

IP / BP / USP Standards

Membrane Filtration

For Filterable Products

Direct Inoculation

For Non-Filterable Products

Culture Media

Fluid Thioglycollate Medium

For Bacteria (Aerobic & Anaerobic)

Soybean-Casein Digest Medium

For Fungi & Aerobic Bacteria

30–35°C
14 Days

For Bacteria

20–25°C
14 Days

For Fungi

Method Suitability Testing

+ Positive & Negative Controls

No Growth: Product Passes

Growth Present: Test Fails

Invalid Test: Environmental Contamination

Key Differences in Sterility Testing

IP, BP, and USP are largely harmonized, but minor differences exist in alignment, fungal incubation temperatures, diluting fluids, washing membranes, and neutralizing agents used in sterility testing.

Harmonized Alignment with EP

BP: Aligned with EP

European Pharmacopoenia

USP: Not specifically aligned with EP

Fungal Incubation Temperature

BP/ IP

BP / IP

BP uses 20–25°C for fungal medium

22.5 ± 2.5 °C

USP

BP uses 20–25°C for fungal medium

Specific Diluting Fluids (IP)

Fluid A

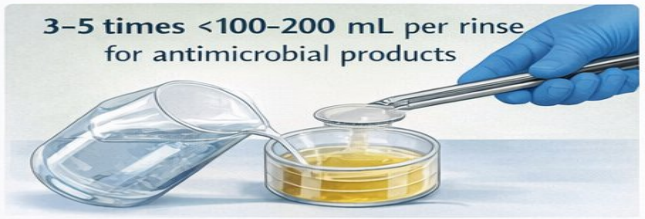
1 g/L Peptic Digest
pH 7.1 ± 0.2

+

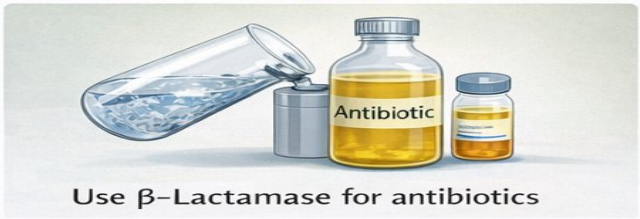
Fluid B

Fluid A + 1 mL/L
Polysorbate 80

Wash Membranes



Neutralizing Agents



Use β–Lactamase for antibiotics

Membrane Filtration

Direct Inoculation

Transfer Sample Directly *into* Culture Media

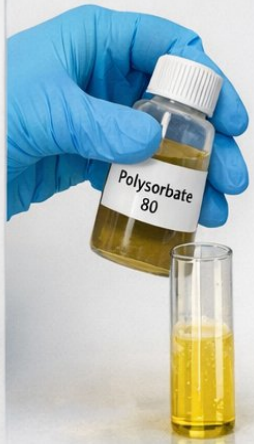
Sample ≤ **10**% of Medium Volume

Neutralize Antimicrobials

Dilution, Inactivating Agents, or Concentrated Media

Add Emulsifiers for Greasy Products

e.g., Polysorbate 80



Shake During Incubation

Incubate for ≥ 14 Days

Observe for Growth

Product-Specific Procedures and Quantities

Quantities are minimums per medium and are similar across IP, BP, and USP (based on container content or batch size). **Use whole contents where possible**; pool from multiple containers if needed.

Batch Sample Sizes

2–10% of Batch or Minimum 2–20 Items

Depending on Batch Size & Product Type

Example Sampling Quantities:

10 Petri Dishes

2 Broth Tubes

1 Fluid Thioglycollate Bottle

For Antimicrobials:

Add Inactivators like β-Lactamase.

Minimum Quantities per Medium

Harmonized Across IP, BP, USP

Product Type	Minimum Quanity per Medium
Liquids < 1 mL	Whole contents
Liquids 1–40 mL	Half contents (≥1 mL)
Liquids >40 mL but <100 mL	20 mL
Liquids >100 mL	10% of contents (≥20 mL)
Antibiotic liquids	1 mL
Solids < 50 mg	Whole contents
Solids 50–300 mg	Half contents (≥50 mg)
Solids 300 mg–5 g	150 mg
Solids > 5 g	500 mg
Ointments/Creams (non-emulsifiable)	≥200 mg

Liquids (Including Ophthalmic Solutions)

Preferred: Membrane filtration

Dilute with sterile diluent (e.g, Fluid A) to ~100 mL if needed; filter directly.

For Immiscible Liquids or Suspensions

Add emulsifiers or enzymes (e.g., lecithin, penicillinase).

Ophthalmic: Treat as liquids

Use whole container or pooled amounts for non-injectables.

For Aerosols (USP/IP)

Freeze & Transfer Contents

For Vacuum Packs (IP)

Admit Sterile Air

SOLIDS (Soluble and Insoluble)

SOLUBLE

Preferred: Membrane Filtration
after dissolving in sterlle solvent
(e.g. **Water for Injection** or **Fluid A**).

Filter through Membrane

INSOLUBLE

Suspend and Filter
or Use Direct Inoculation

Suspend & Filter

Direct Inoculation

ANTIBIOTICS/BULKS (USP/IP)

Composite samples (e.g. 6 g from multiple containers in **200 mL Fluid A**).

Incubate as Standard.

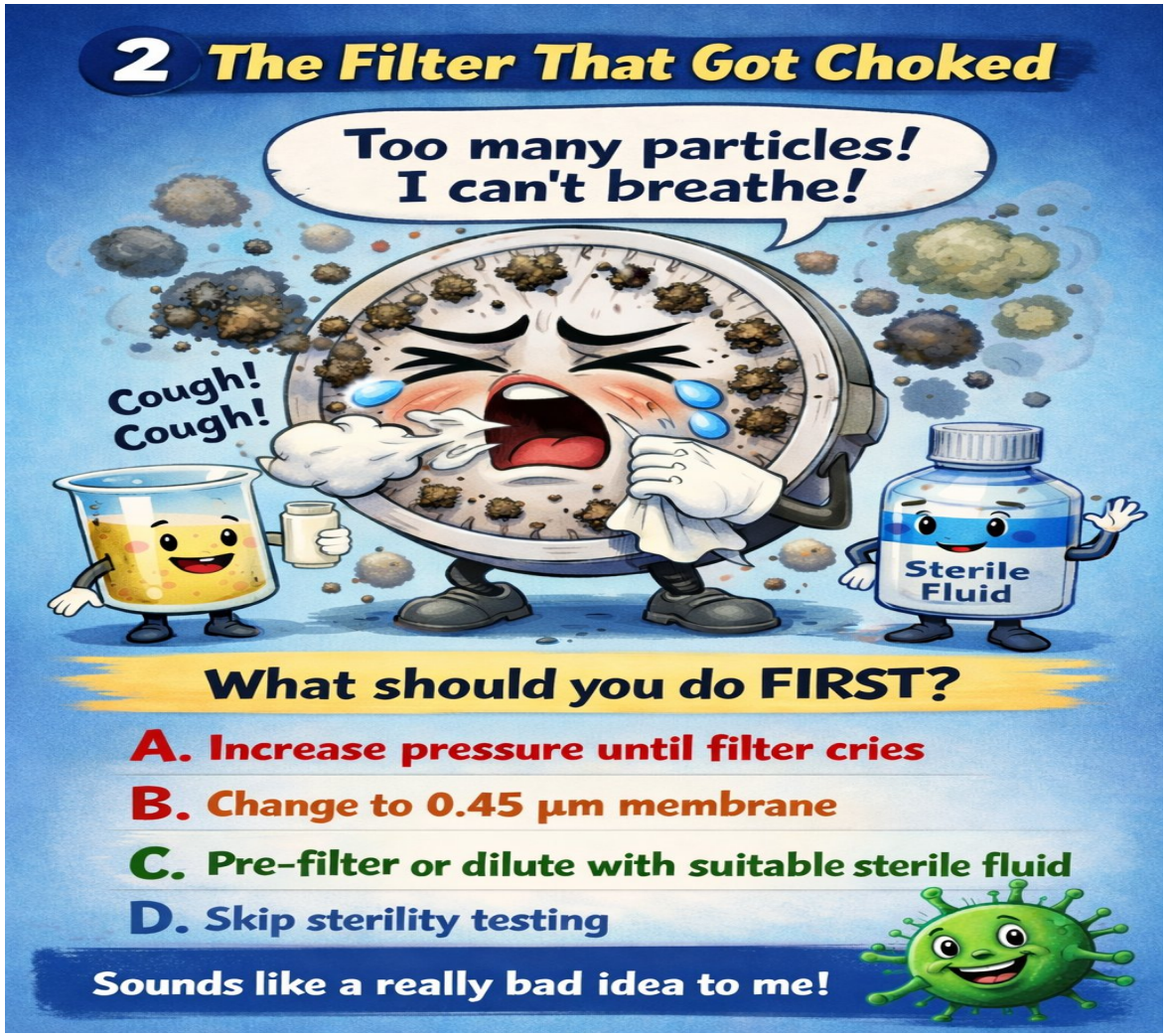

Ophthalmic and Other
Non-Injectable Preparations
Treat as liquids or ointments/creams;
membrane filtration or direct inoculation.
Dilute to ~100 mL
SAMPLE
Bacteria Test
Fungi Test
For Ophthalmic Ointments:
Dilute 1:10 with emulsifier
Ophthalmic Ointment
Emulsifier
1:10 Dilution
Batch: 5% or minimum 2 items for ≤200 containers (BP/USP)
≤ 200
Containers

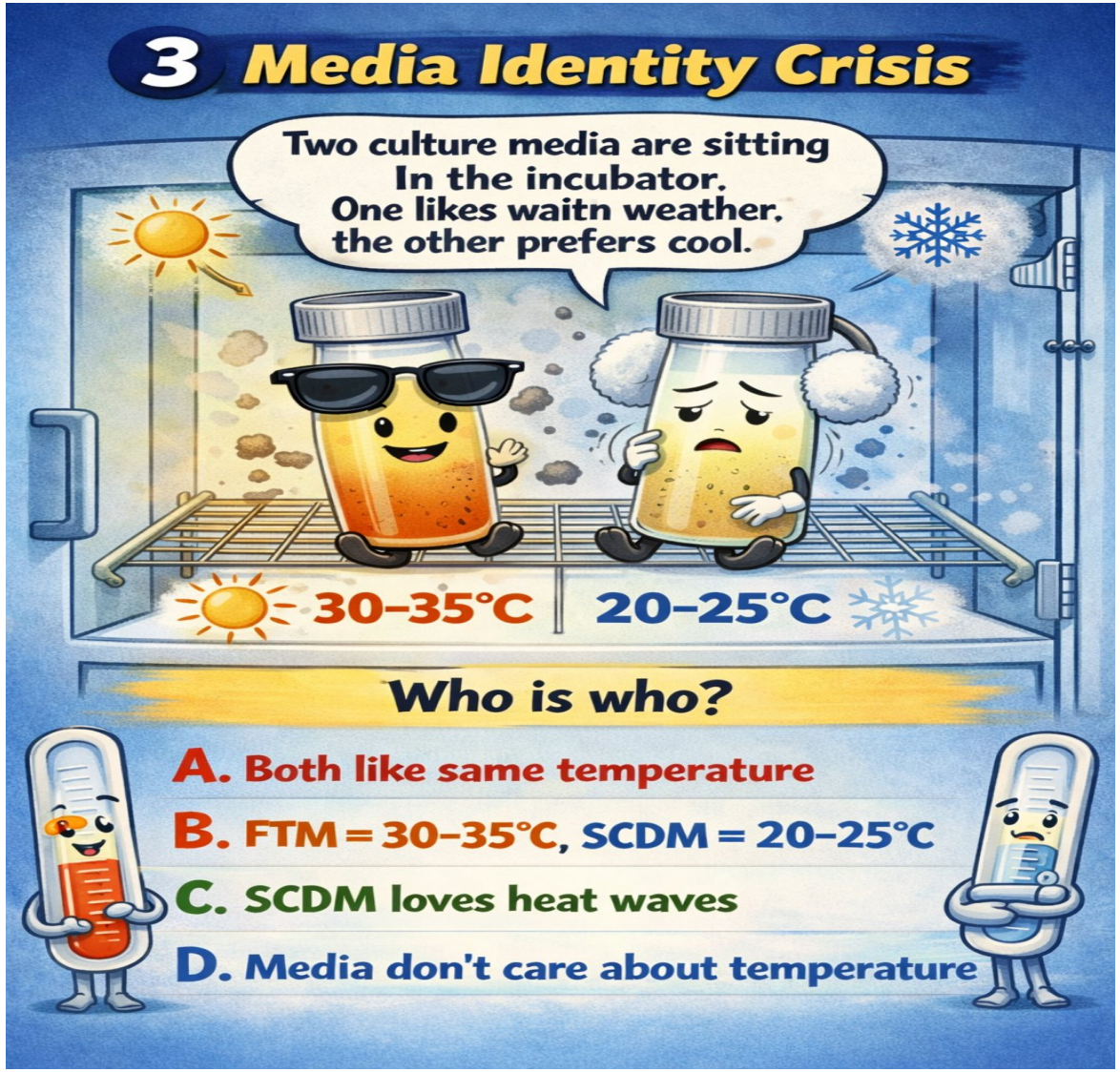
Other Sterile Products (e.g., Oils, Ointments, Creams, Devices, Sutures)

Devices (Syringes, Catheters)	Sutures / Catgut	Dressings / Gauze
		
Flush with ≥100 mL diluent (e.g, Fluid D/B with polysorbate); filter flushate. For lumens, fill and immerse.	Direct inoculation of 3 × 30 cm strands (beginning, middle, end) in 20–150 mL medium.	Immerse 100–500 mg from innermost part or whole item.

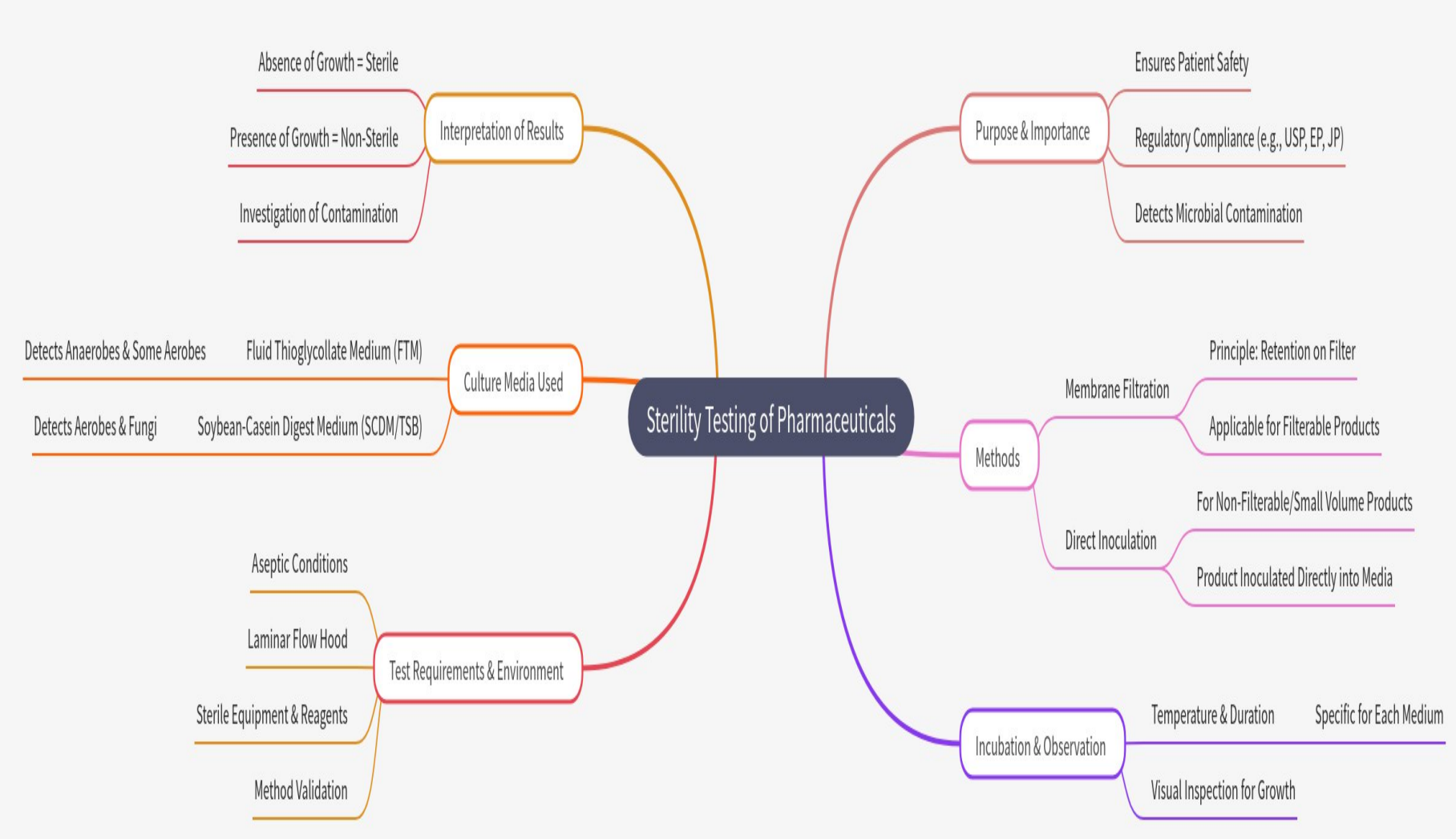
If insoluble in isopropyl myristate, use direct inoculation (IP).

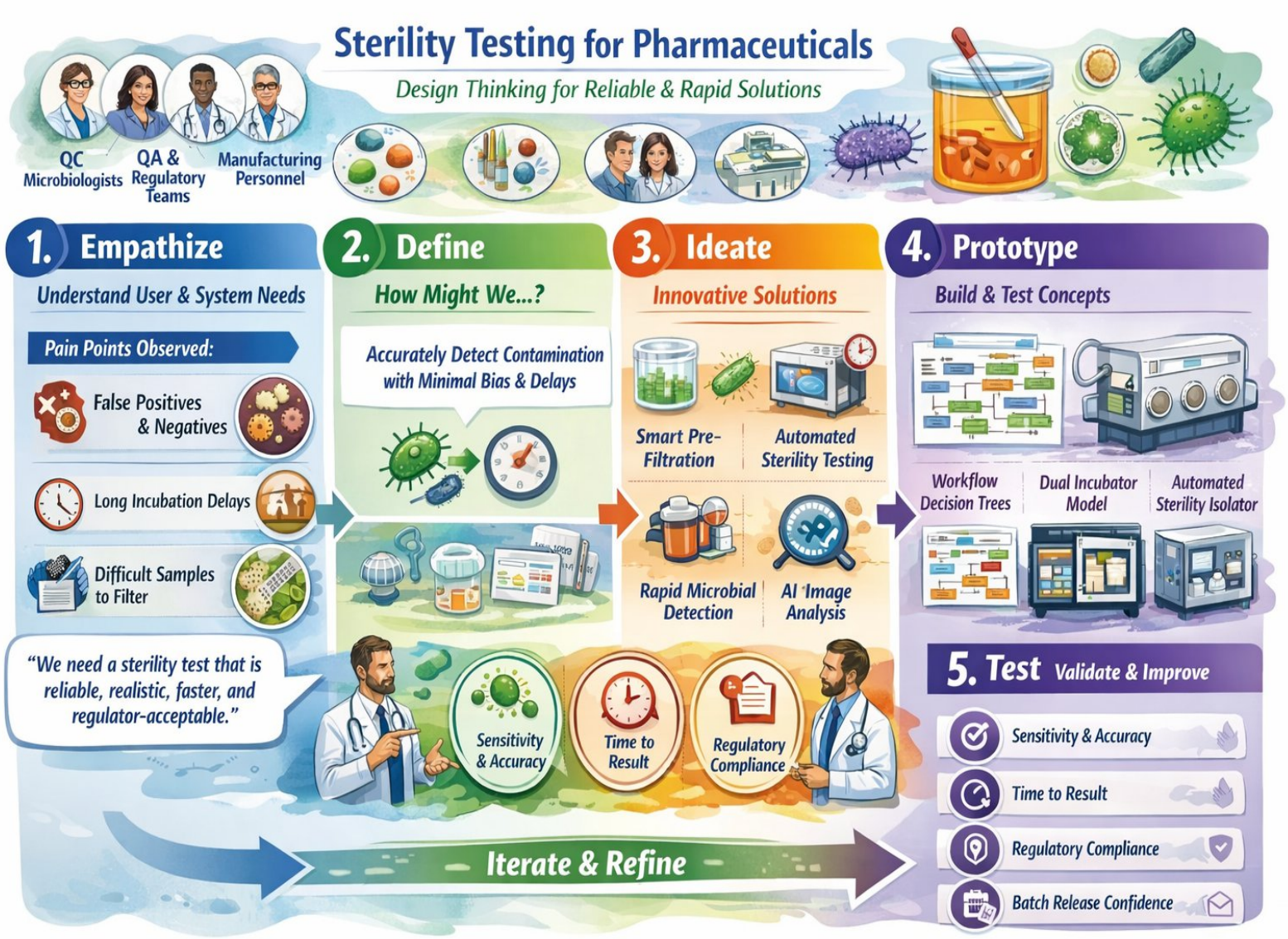
ASSESSMENT QUESTIONS


2 The Filter That Got Choked
Too many particles! I can't breathe!
Cough! Cough!
Sterile Fluid
What should you do FIRST?
A. Increase pressure until filter cries
B. Change to 0.45 µm membrane
C. Pre-filter or dilute with suitable sterile fluid
D. Skip sterility testing
Sounds like a really bad idea to me!


3 Media Identity Crisis
Two culture media are sitting
In the incubator.
One likes waitn weather.
the other prefers cool.
30–35°C
20–25°C
Who is who?
A. Both like same temperature
B. FTM = 30–35°C, SCDM = 20–25°C
C. SCDM loves heat waves
D. Media don't care about temperature


4 The Sneaky Microbe Party
After 7 days, the sterllity test shows turbidity. The product argues:
"Maybe the microbes are just visiting, not living."
7 DAYS STERILITY TEST
What is the correct scientific verdict?
A. Visitors are allowed
B. Wait and see till 14 days
C. FAIL – any growth = contamination
D. Give microbes warning letter


Sterility Testing of Pharmaceuticals
Purpose & Importance
Ensures Patient Safety
Regulatory Compliance (e.g., USP, EP, JP)
Detects Microbial Contamination
Methods
Membrane Filtration
Principle: Retention on Filter
Applicable for Filterable Products
Direct Inoculation
For Non-Filterable/Small Volume Products
Product Inoculated Directly into Media
Incubation & Observation
Temperature & Duration
Specific for Each Medium
Visual Inspection for Growth
Test Requirements & Environment
Aseptic Conditions
Laminar Flow Hood
Sterile Equipment & Reagents
Method Validation
Culture Media Used
Detects Anaerobes & Some Aerobes
Fluid Thioglycollate Medium (FTM)
Detects Aerobes & Fungi
Soybean-Casein Digest Medium (SCDM/TSB)
Interpretation of Results
Absence of Growth = Sterile
Presence of Growth = Non-Sterile
Investigation of Contamination


Sterility Testing for Pharmaceuticals
Design Thinking for Reliable & Rapid Solutions
QC Microbiologists
QA & Regulatory Teams
Manufacturing Personnel
1. Empathize
Understand User & System Needs
Pain Points Observed:
False Positives & Negatives
Long Incubation Delays
Difficult Samples to Filter
"We need a sterility test that is reliable, realistic, faster, and regulator-acceptable."
2. Define
How Might We...?
Accurately Detect Contamination with Minimal Bias & Delays
3. Ideate
Innovative Solutions
Smart Pre-Filtration
Automated Sterility Testing
Rapid Microbial Detection
AI Image Analysis
4. Prototype
Build & Test Concepts
Workflow Decision Trees
Dual Incubator Model
Automated Sterility Isolator
5. Test Validate & Improve
Sensitivity & Accuracy
Time to Result
Regulatory Compliance
Batch Release Confidence
Sensitivity & Accuracy
Time to Result
Regulatory Compliance
Iterate & Refine

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2. Prescott and Dunn., Industrial Microbiology, 4th edition, CBS Publishers & Distributors, Delhi.

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THANK YOU