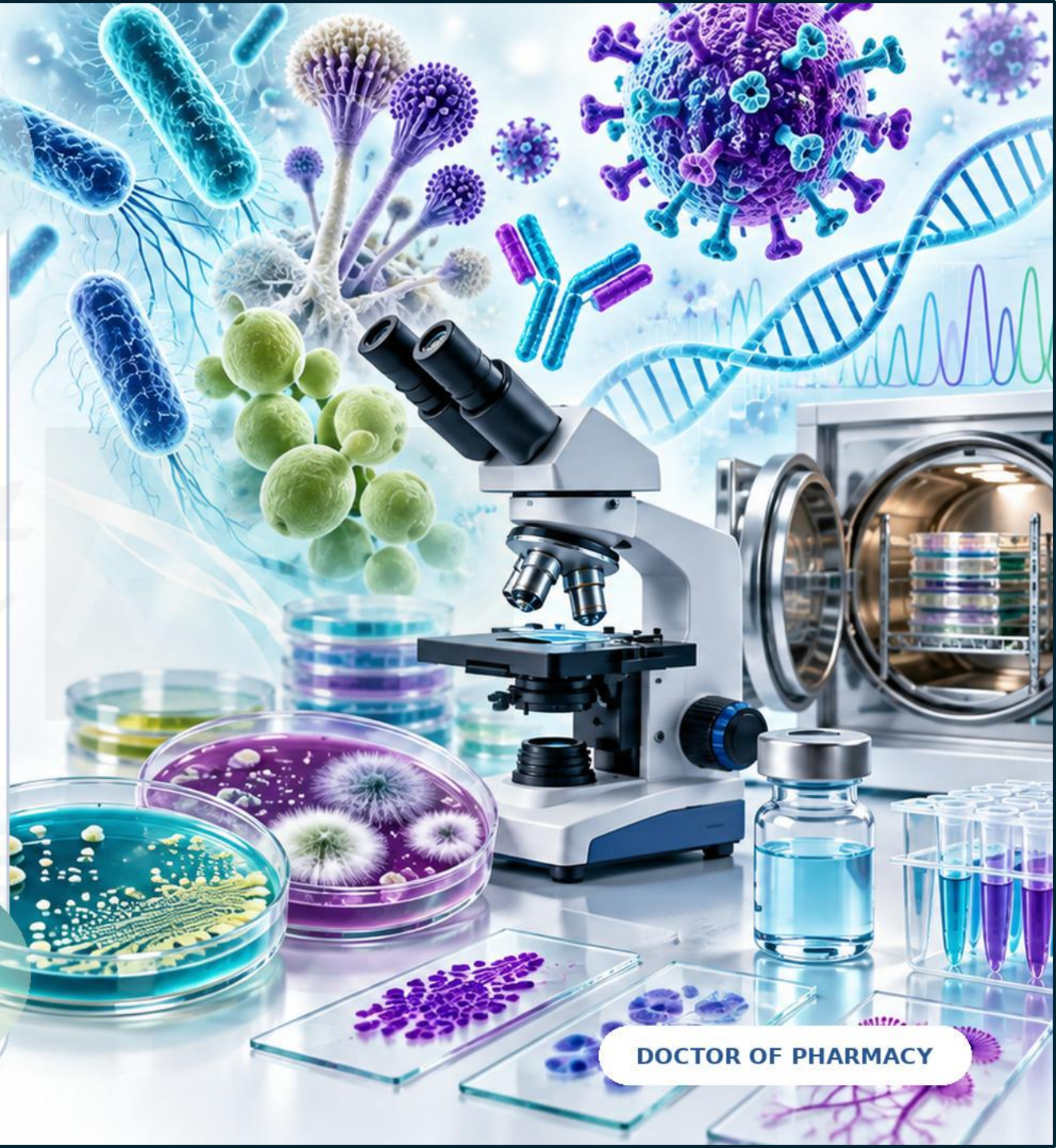
PHARM.D • YEAR 2

Pharmaceutical Microbiology

CHAPTER 4

Isolation and Identification of Bacteria: Staining and Biochemical Reactions

PPT CHAPTER COVER



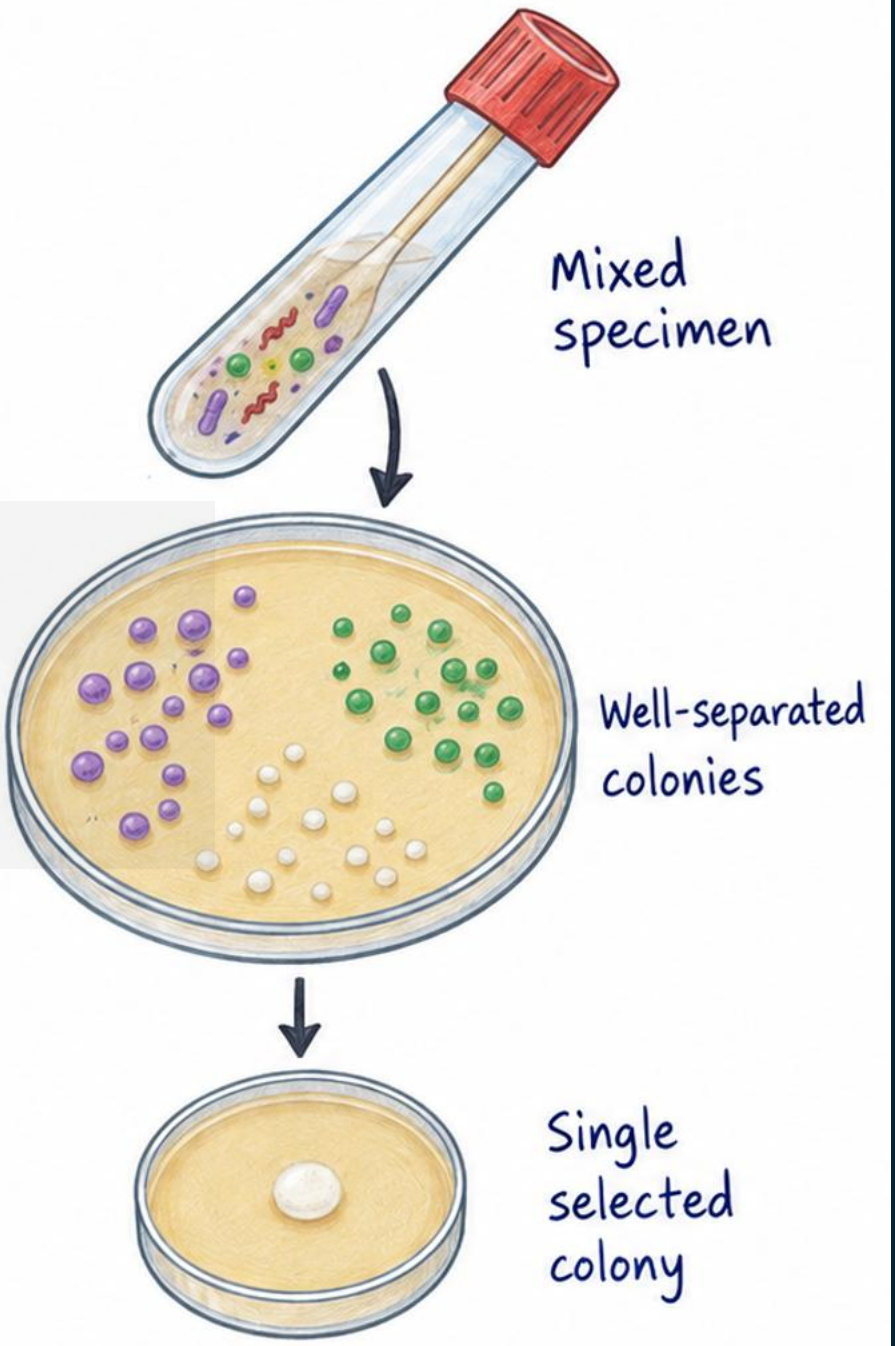
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Why Isolate a Pure Culture?

A clinical or environmental specimen may contain several microbes. **Isolation** separates individual cells or groups so a colony can be examined as a presumptive pure culture.

Purity matters before identification or susceptibility testing. Record the specimen source, collection conditions and any prior antimicrobial exposure; contamination and poor transport can change the result.

One isolated colony can arise from a cell cluster, so visual separation is only a first step. Re-streak and confirm purity when required.



Streak, Spread and Pour Plates

Streak plating dilutes cells across the agar surface to obtain isolated colonies. Spread plating distributes a measured diluted sample on the surface and can support viable counting. Pour plating mixes a diluted sample with molten agar, so colonies may grow within and on the surface. Select the method according to isolation, enumeration and organism requirements.

Streak Plate



Purpose: obtain isolated colonies.

Spread Plate



Purpose: support viable counting.

Pour Plate



Purpose: colonies may grow within and on the surface.

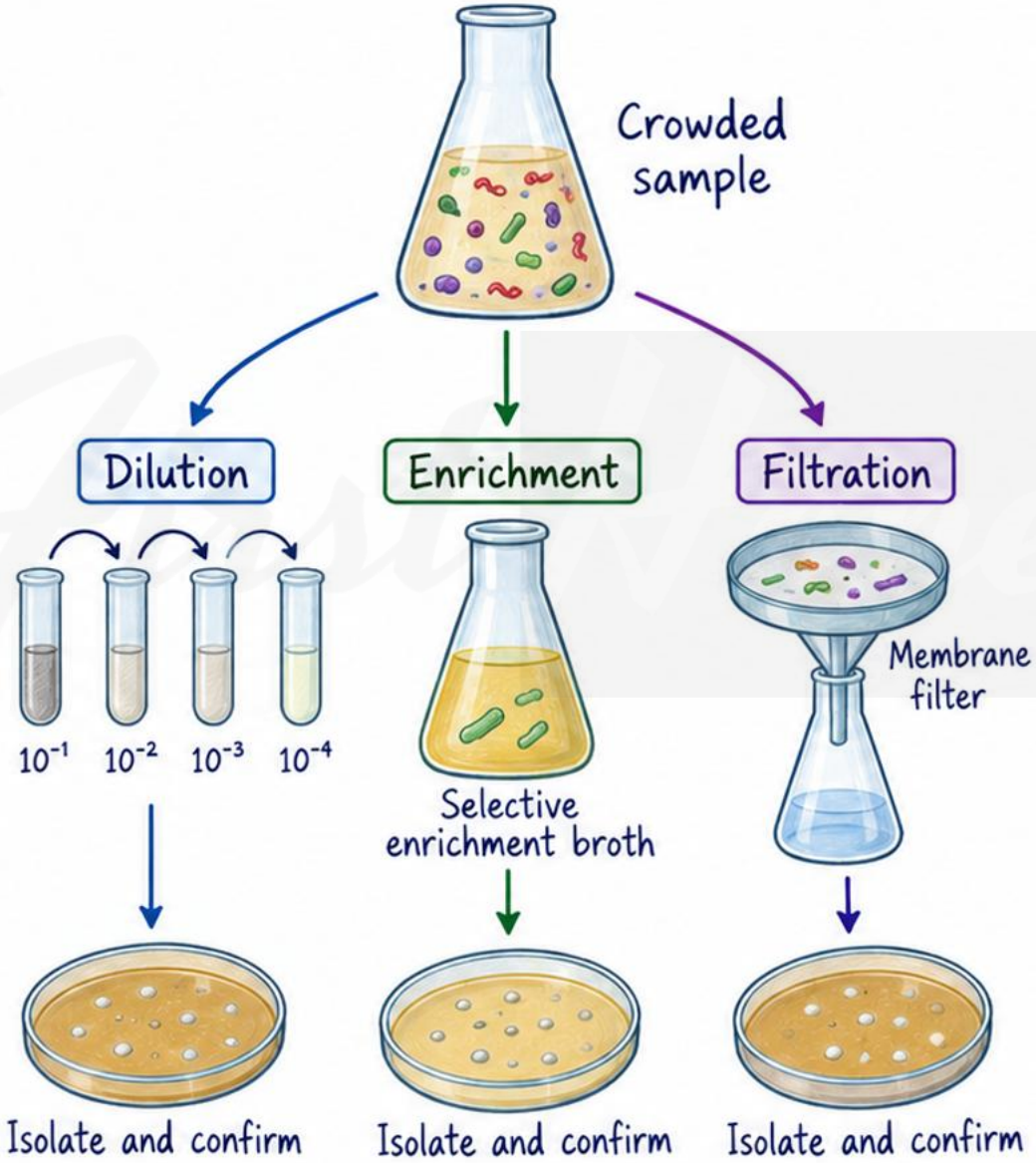
Spread and pour methods require a known plated volume for counting.
Molten agar temperature must not injure the organism in a pour plate.

Dilution, Enrichment and Filtration

Serial dilution lowers a dense microbial load before plating.

Enrichment conditions favor a sought organism when it is outnumbered, but the resulting growth still needs isolation and confirmation.

Selective media suppress competitors; differential media show visible reactions.



Membrane filtration can concentrate microbes from a measured liquid volume before culture.

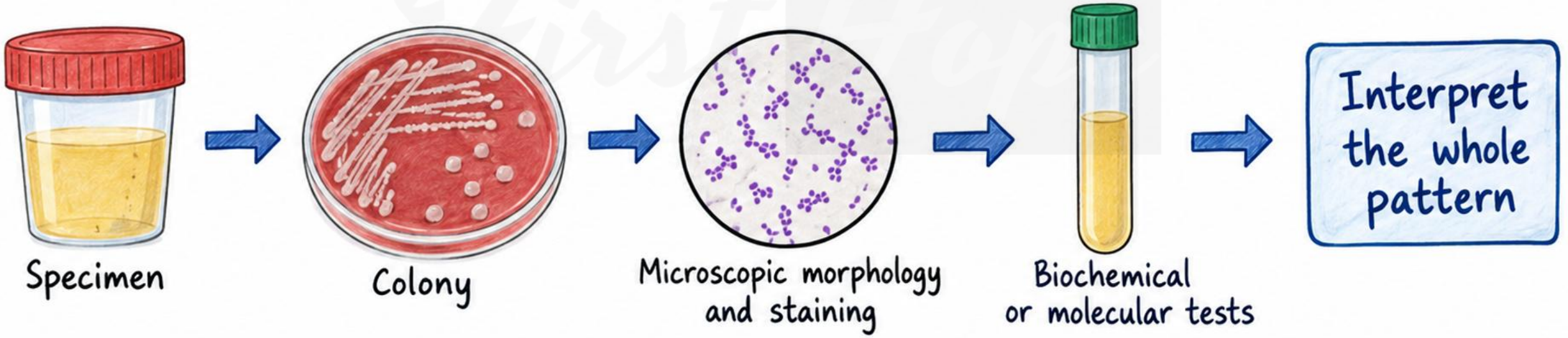
Specialized single-cell methods serve narrower research questions.

Enrichment increases the chance of recovery but may distort relative abundance.

A filter captures organisms from large liquid volumes before transfer to medium.

Identification Is a Chain of Evidence

Begin with specimen quality and a well-isolated colony. Observe colony appearance, microscopic morphology and staining. Add biochemical or molecular tests appropriate to the question, then interpret the whole pattern. A single colony color or one positive reaction is seldom enough for definitive species identification. Quality controls and reference methods support reliable reporting.



The logic is cumulative: specimen context narrows possibilities, morphology directs tests, and confirmatory evidence supports the final name. Flag discordant findings.

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