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It's Important to Know!

BP303T — Pharmaceutical Microbiology

B.Pharm Semester 3 · Unit 2 — Bacterial identification and sterilisation

Unit II: Bacterial identification and sterilisation

Staining and biochemical identification

Simple staining improves cell shape and arrangement. Gram staining separates many bacteria into Gram-positive and Gram-negative groups according to cell-envelope behaviour during decolourisation and counterstaining. Acid-fast staining detects organisms with lipid-rich cell walls that resist ordinary decolourisation. Staining quality depends on smear thickness, fixation, reagent condition and timing.

Biochemical tests identify metabolic traits. The IMViC group includes indole, methyl red, Voges-Proskauer and citrate tests. A biochemical result must be interpreted with controls, culture purity and the complete identification scheme rather than one reaction alone.

Sterilisation principles

Sterilisation means removal or destruction of all forms of viable microbial life as defined by the method and validation system. Moist heat damages essential components through denaturation and hydrolysis. Dry heat acts through oxidation and requires different time-temperature conditions. Filtration removes organisms from heat-sensitive liquids or gases but does not destroy them.

Chemical sterilants act through oxidation, alkylation or other chemical injury. Gaseous methods can penetrate packaged materials but require exposure control and residue assessment. Radiation can damage nucleic acids. Mechanical cleaning reduces bioburden and supports, but is not by itself, sterilisation.

Indicators and validation

Physical records, chemical indicators and biological indicators provide different evidence. Biological indicators use resistant spores appropriate to the process. Validation must consider load, packaging, cold spots, exposure, equipment performance and routine monitoring. The organism, cycle parameters and acceptance criteria are set out in the validated procedure for each process.

Unit II: Frequently asked questions

Q: What is the main basis of Gram staining?

A: It is the differential retention of the primary stain after decolourisation, related to cell-envelope structure.

Q: Why is filtration not the same as sterilisation by heat?

A: Filtration physically removes retained organisms and requires filter integrity, while heat inactivates organisms by thermal damage.

Q: Why are biological indicators used?

A: They challenge a process with resistant spores and provide direct evidence that the process can achieve the required microbial inactivation.

Unit II: Key takeaways

- Staining is useful only when smear preparation and controls are adequate.
- IMViC tests are interpreted as a pattern, not as isolated facts.
- Sterilisation method depends on material, load and process compatibility.
- Indicators support monitoring and validation.
- Cleaning, process control and documentation work together.

Unit II: Practical correlation

Practical correlation: autoclave cycle review. A release decision should consider cycle records, load configuration, indicator results and deviations. Exact cycle parameters and biological indicator selection are set out in the validated procedure.