MICROBIOLOGY STAINING PROCEDURES – QUICK NOTES

1. Simple Staining
• Purpose: To observe shape, size, arrangement of bacteria.
• Dyes used: Methylene blue, Crystal violet, Safranin.
• Steps:
1. Prepare smear → air dry → heat fix.
2. Add basic dye for 1 minute.
3. Rinse with water & blot dry.

2. Gram Staining (Differential Stain)
• Purpose: To differentiate Gram-positive & Gram-negative bacteria.
• Principle: Difference in cell wall peptidoglycan thickness.
• Steps:
1. Primary stain: Crystal violet (1 min).
2. Mordant: Iodine (1 min).
3. Decolorizer: Alcohol/Acetone (few seconds).
4. Counterstain: Safranin (1 min).

• Results:
Gram + = Purple
Gram – = Pink

3. Acid-Fast Staining (Ziehl–Neelsen)
• Purpose: To identify Mycobacteria (waxy cell wall with mycolic acid).
• Steps:
1. Primary stain: Carbol fuchsin with heat.
2. Decolorizer: Acid alcohol.
3. Counterstain: Methylene blue.
• Results:
Acid-fast = Red
Non–acid-fast = Blue

4. Endospore Staining (Schaeffer–Fulton)
• Purpose: To detect bacterial spores (e.g., Bacillus, Clostridium).
• Steps:
1. Primary stain: Malachite green with heat.
2. Rinse with water.
3. Counterstain: Safranin.
• Results:
Spores = Green
Vegetative cells = Red

5. Capsule Staining (Negative Staining)
• Purpose: To visualize bacterial capsules (non-ionic & non-staining).
• Steps:
1. Mix organism in India ink / Nigrosin.
2. Air dry (no heat fixing).
3. Counterstain: Crystal violet.
• Results:
Capsule = Clear halo
Background = Dark
Cells = Purple

6. Flagella Staining (Hanging Drop)
• Purpose: To observe motility.
• Principle: Use of mordant to thicken flagella for visibility.

End of Notes