

BACTERIAL STAINING (continue)

By:

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ACID-FAST STAINING: (Ziehl-Neelsen stain)

- To stain Mycobacterium species especially *M. tuberculosis* because have an unusual cell wall resulting in their inability to be gram stained.
- Have High lipid content this makes decolorisation very difficult –extraordinary property.
- Principle:
- ***Acid fast(resist)*** – Property of Mycobacterium species - once this bacteria stained with primary dye – difficult to decolorise with acid.
- This property due to fatty mycolic acids in their cell wall.
- *M. tuberculosis* – also ***Alcohol fast***

Staining reagents:

1. Strong Carbol fuchsin – primary stain
2. 20% sulphuric acid/3% Hcl – decoloriser – acid-fast property.
3. 95% alcohol- decoloriser- alcohol – fast property
4. Methylene blue/ Malachite green- counterstain.

Note:

- 5% sulphuric acid – for *M. leprae*.
1% sulphuric acid – for *Nocardia species*.

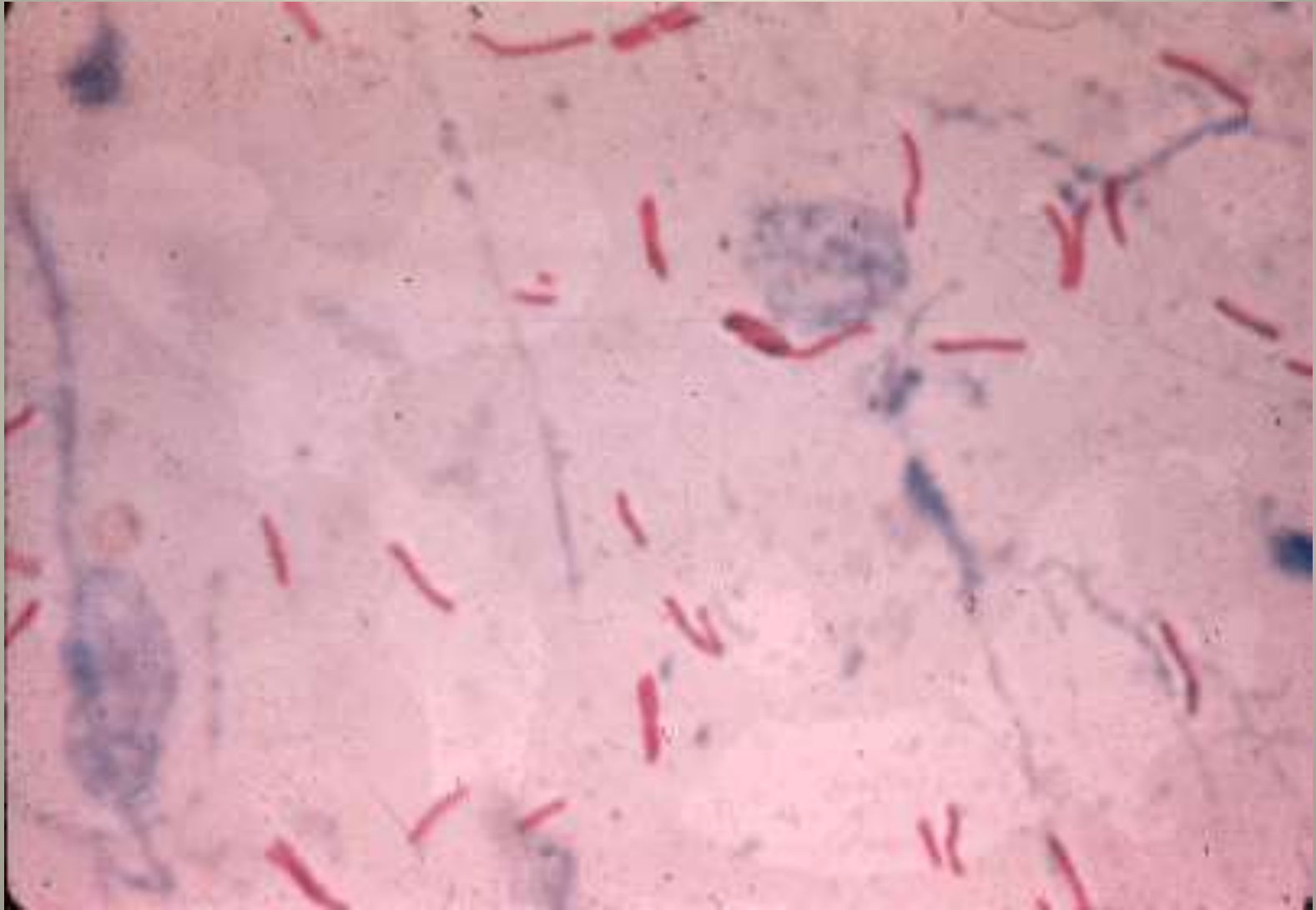
Procedure:

1. 1. Alcohol-fix the dried sputum smear by covering it with one or two drops of 70% v/v ethanol or methanol for 2-3 minutes. Heat fixation does not kill *M. tuberculosis*.
2. 2. Strong Carbol fuchsin-heat till steam rises – allow 5-10 min to act (alternately leave it 10-15 min – cold staining method) – wash with distilled water.
3. 3. Decolorise with acid-alcohol 3% v/v mixture till get a faint pink colour in the smear (take 3-5 min) –wash with distilled water.
4. 4. Methylene blue/Malachite green 0.5 % w/v – 2 min – wash with distilled water.
5. 5. Allow to dry and focuss under microscope.

Result:

- **Pink bacilli – Acid fast bacteria/bacilli**
e.g., M. tuberculosis – long slender bacilli straight or slightly curved rods, occurring singly or in small groups.
- *M. leprae* – short thick bacilli.
- **Blue or green colored bacteria – Non-acid fast** *e.g., Epithelial cells, pus cells, other bacteria.*

Acid fast stain:



- Reporting of sputum smears:
- More than 10 AFB/field  report +++
- 1-10 AFB/field  report ++
- 10-100 AFB/100 fields  report +
- 1-9 AFB/100 fields  report the exact number

SPECIAL STAIN:

**Used to stain special structures of bacteria—
capsule, spores, flagella, metachromatic granules.**

CAPSULE STAIN:

Negative staining:

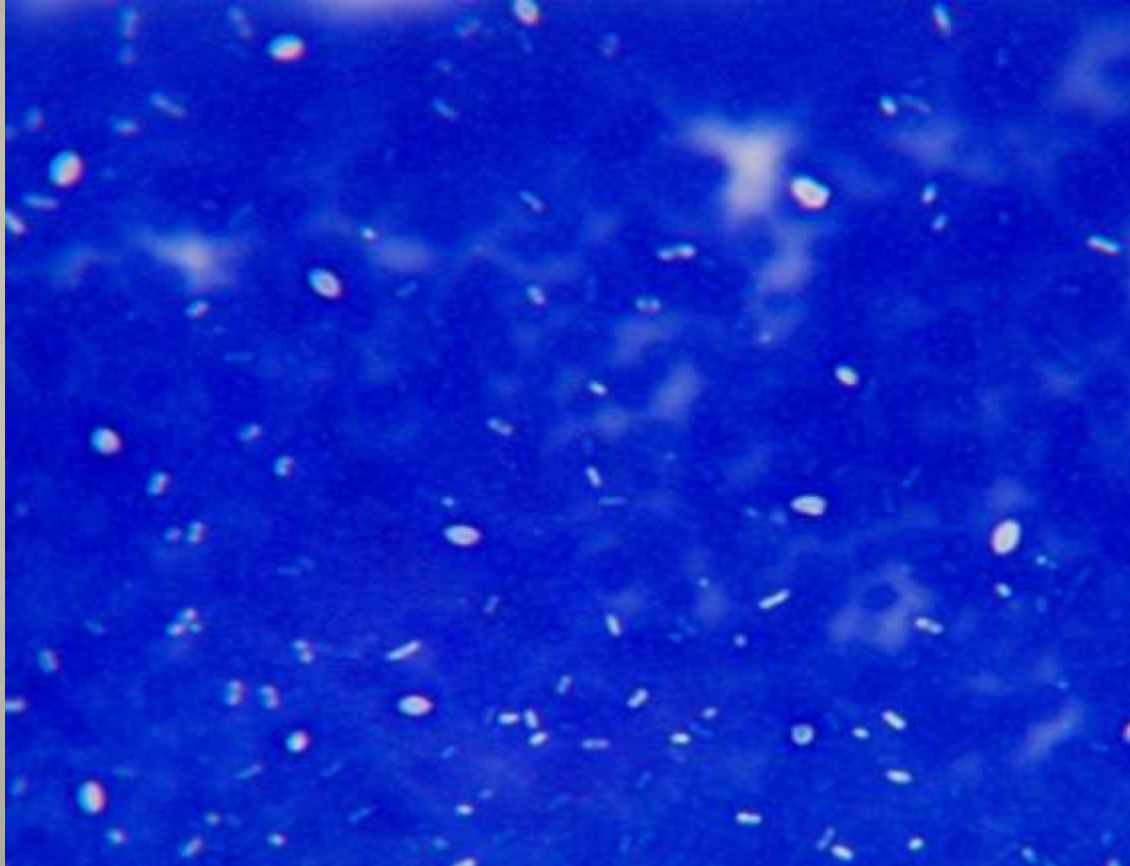
Dry method:

1. Place a loopful of 6% glucose
2. Drop of Nigrosin or indian ink
3. Bacterial culture (1-2 colonies)
4. Spread evenly and air-dry.
5. Look for unstained structures (halo zone) against stained background.

CAPSULE STAIN BY NIGROSIN INK (BLACK)



CAPSULE STAIN- INDIAN INK(BLUE)



SPORE STAIN: By using Malachite green method

1. Bacterial film is prepared, dried and fixed.
2. Malachite green 5%- 2 minutes- heat stain till steam rises – wait for 2 minutes - wash by tap water.
3. Counterstain with safranin 0.5% or 0.05% Carbol fuchsin –1 min- wash.
4. Dry the slide and examine microscopically.

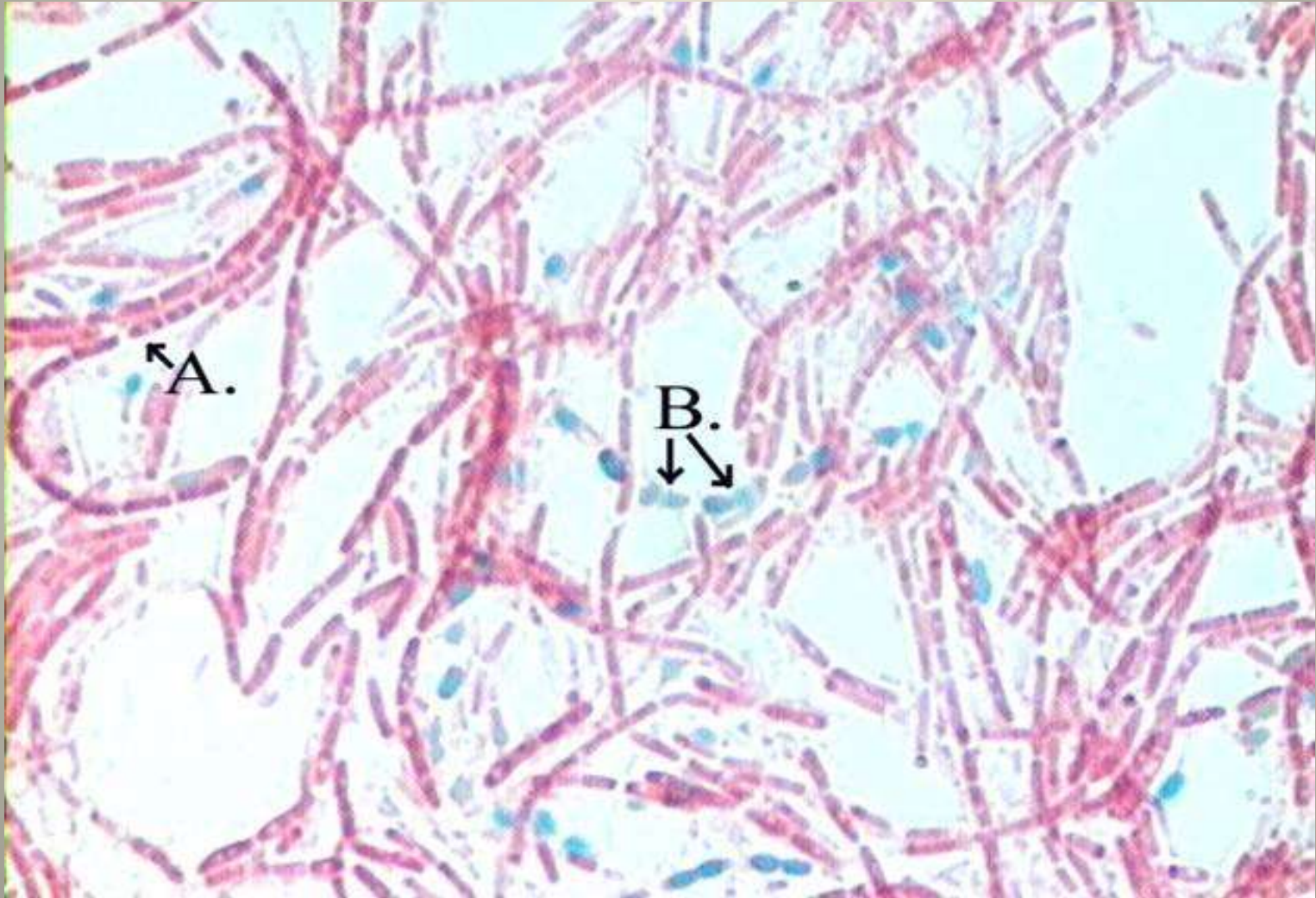
The spore will stain green where as the vegetative parts of the cells will be red as the counter stain.

Spore forming bacteria:

Eg., *Clostridium species*.

Bacillus species – Eg. *B. anthracis*

Spore stain

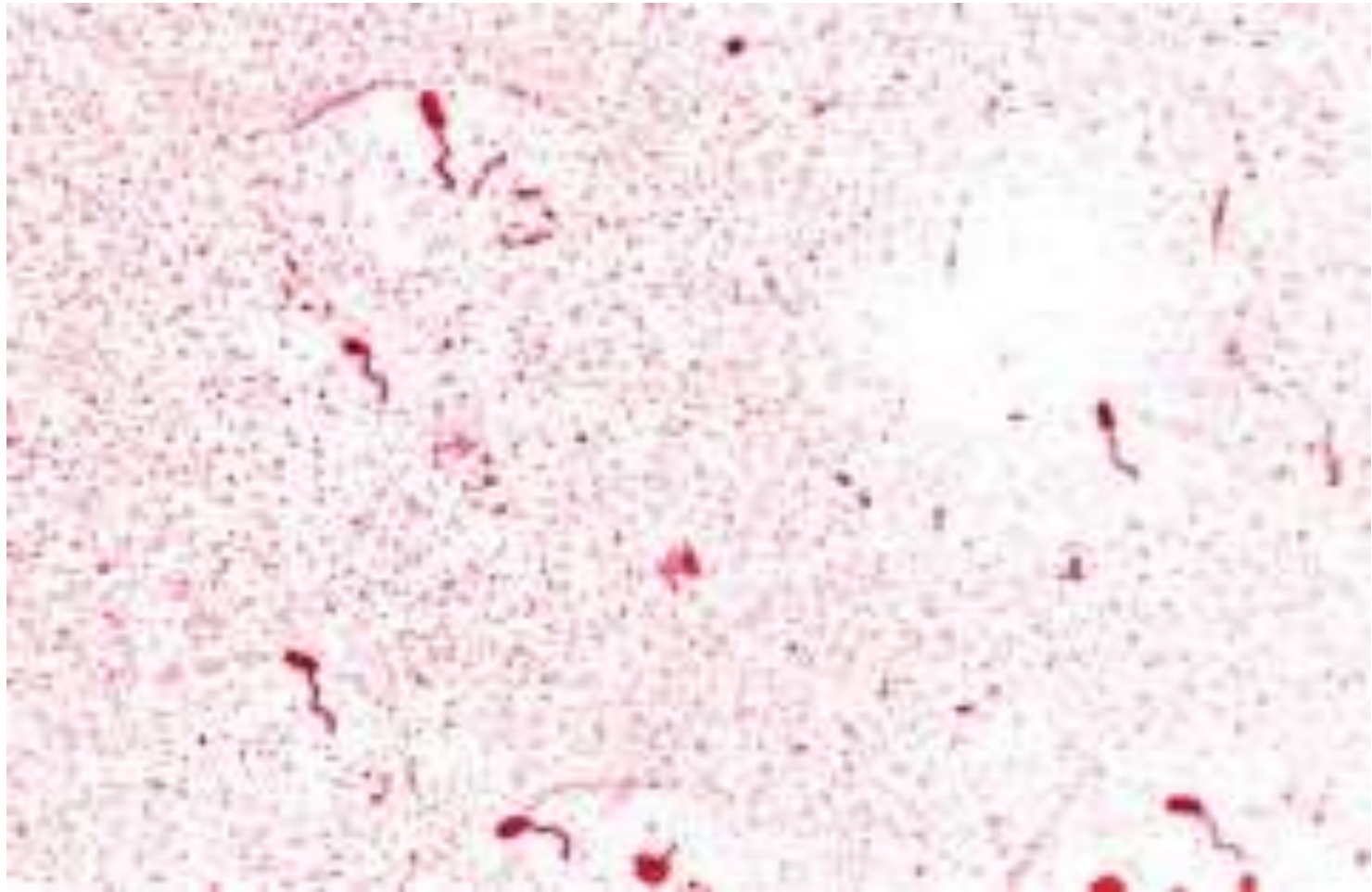


FLAGELLAR STAIN – SILVER STAIN:

- This stain increases the thickness of flagella – thus easy to see under light microscope.
- **Components**
 1. 1% Osmic acid
 2. Mordant
10% Tannic acid
Sat.potassium alum
10% Ferric chloride
 3. Fontana's silver solution

- **Use:**

This is used to demonstrate the flagella and the organisms stain black and flagella appear light brown.



- **Metachromatic granule staining:**
- To demonstrate polar granules of **Corynebacterium diphtheriae**.
- Take up the stain of methylene blue – but appears bluish black – hence granules called metachromatic granules.
- Bacilli stains blue not bluish black.