

Ziehl Neelsen Staining.

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A.P. Chemical Pathology

KGMC.

Z.N. Staining.

- Named after a German bacteriologist, Franz Ziehl.
- Used to stain mycobacterium tuberculosis and mycobacterium leprae.
- Also called **AFB** stained with carbol fuschin, a red dye.
- They retain the dye when treated with acid, due to presence of mycolic acid in their cell wall.

Requirements.

- Glass slide with fixed smear.
- Bunsen Burner or spirit lamp.
- Staining reagents.
- Slide rack.
- Microscope.
- Specimen.

Sample.

- Sputum, fluids, tissue and urine etc.
- Sputum □ 3 consecutive samples to rule out P.T.B.
- Early morning 2mL sputum specimen is preferred.
- Advise sputum AFB in high risk patients i.e. immunocompromised, alcoholics or having recent exposure to TB patient.
- 2 mL bronchial washing.
- Pleural fluid.

Reagents.

1. Carbol fuschin (basic dye).
2. 20% sulphuric acid (decolorizer).
3. Methylene blue (counter stain) or Malachite green.
4. Mordant-Fixator (heat).

Procedure.

- Fix the smear of the specimen over the glass slide by heating it.
- Pour carbol fuschin over smear and again heat it gently until fumes appear.
- Do not overheat and allow it to stand for 5 minutes, then wash it off with water.
- Pour 20% sulphuric acid/ alcohol to decolorize it.

Cont...

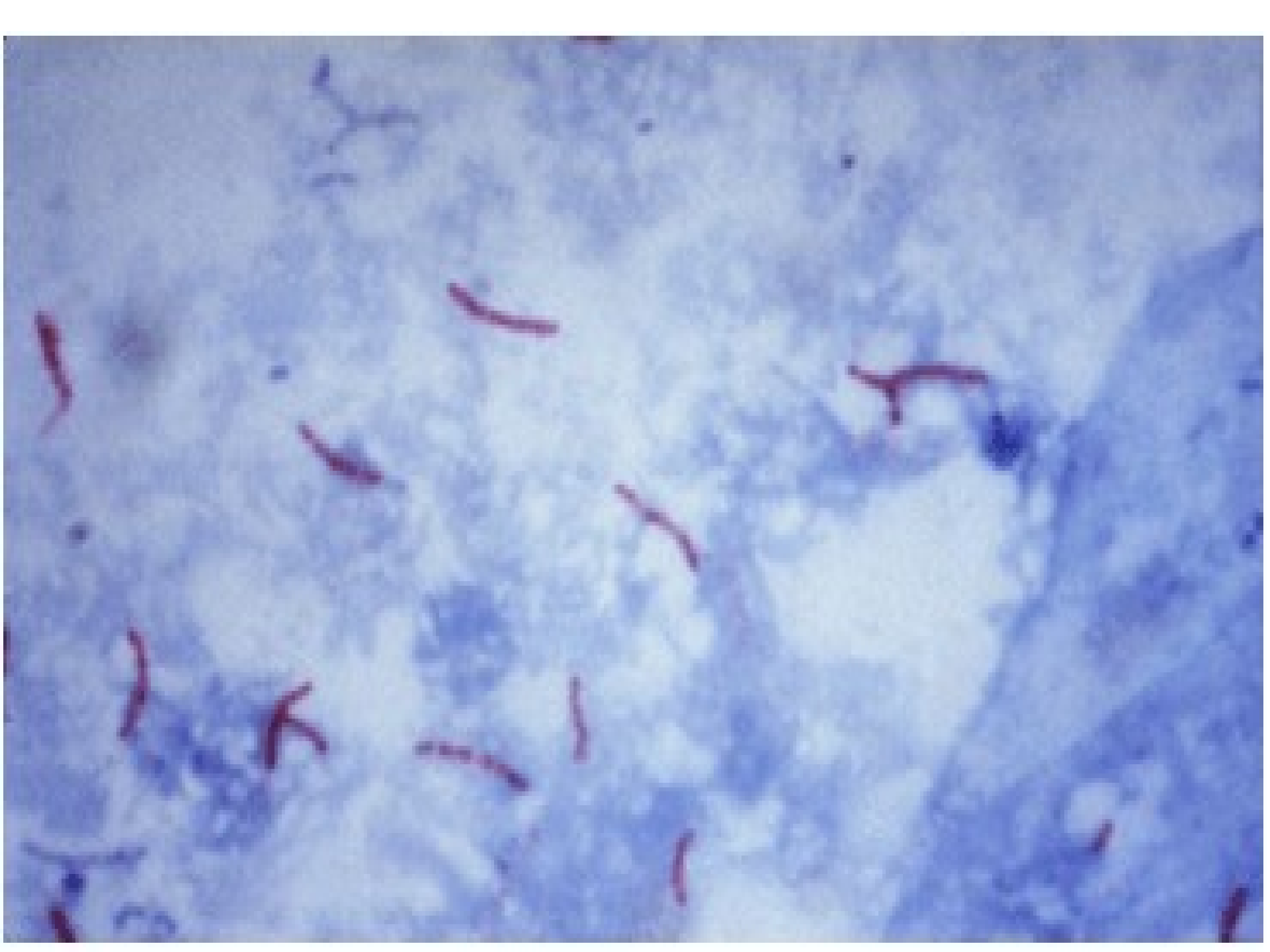
- Wash off with water.
- Pour counter stain Methylene Blue, wait for 1 minute.
- Again wash with water
- Allow it to air dry and examine under oil immersion lens.

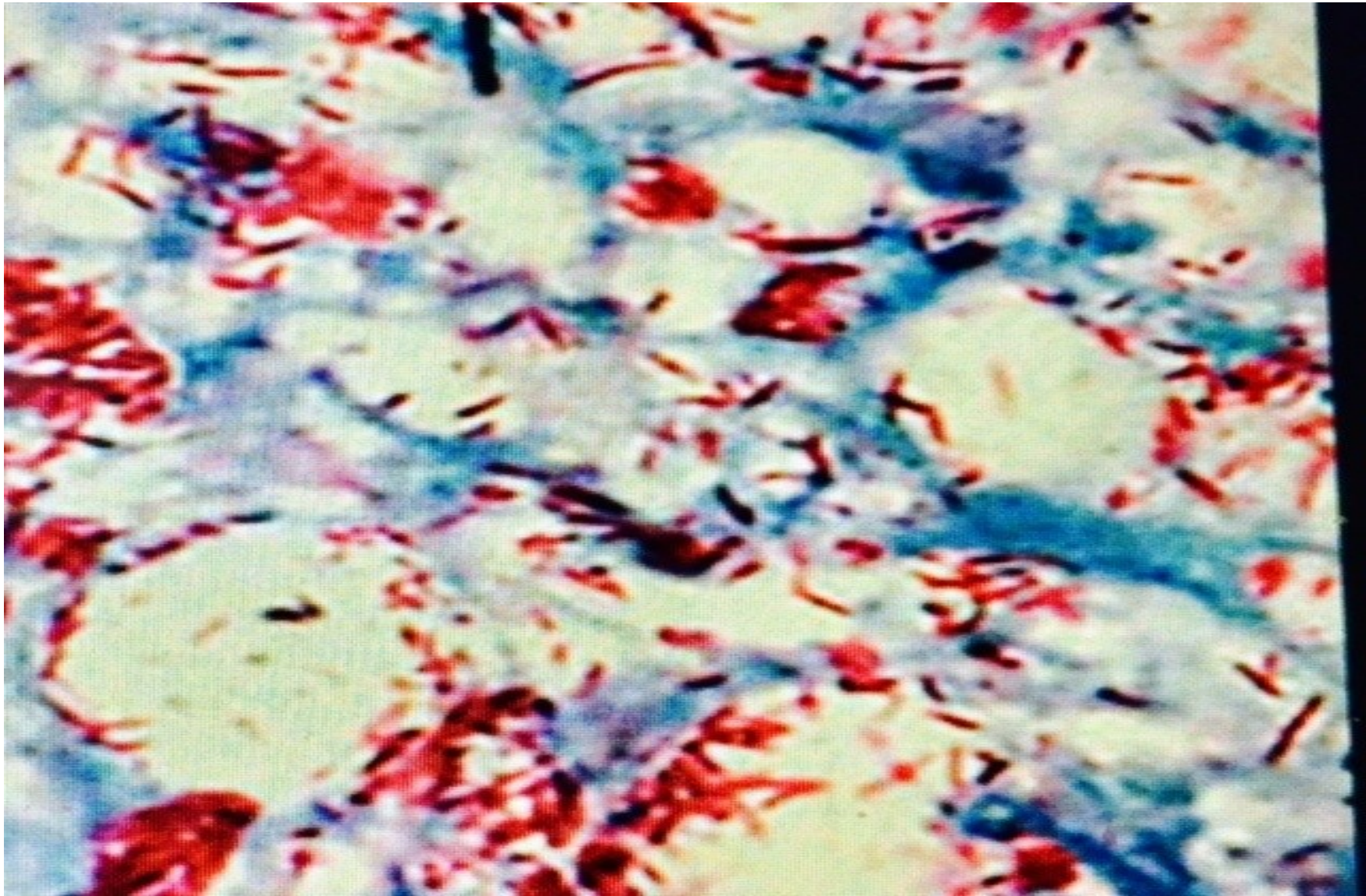
Result.

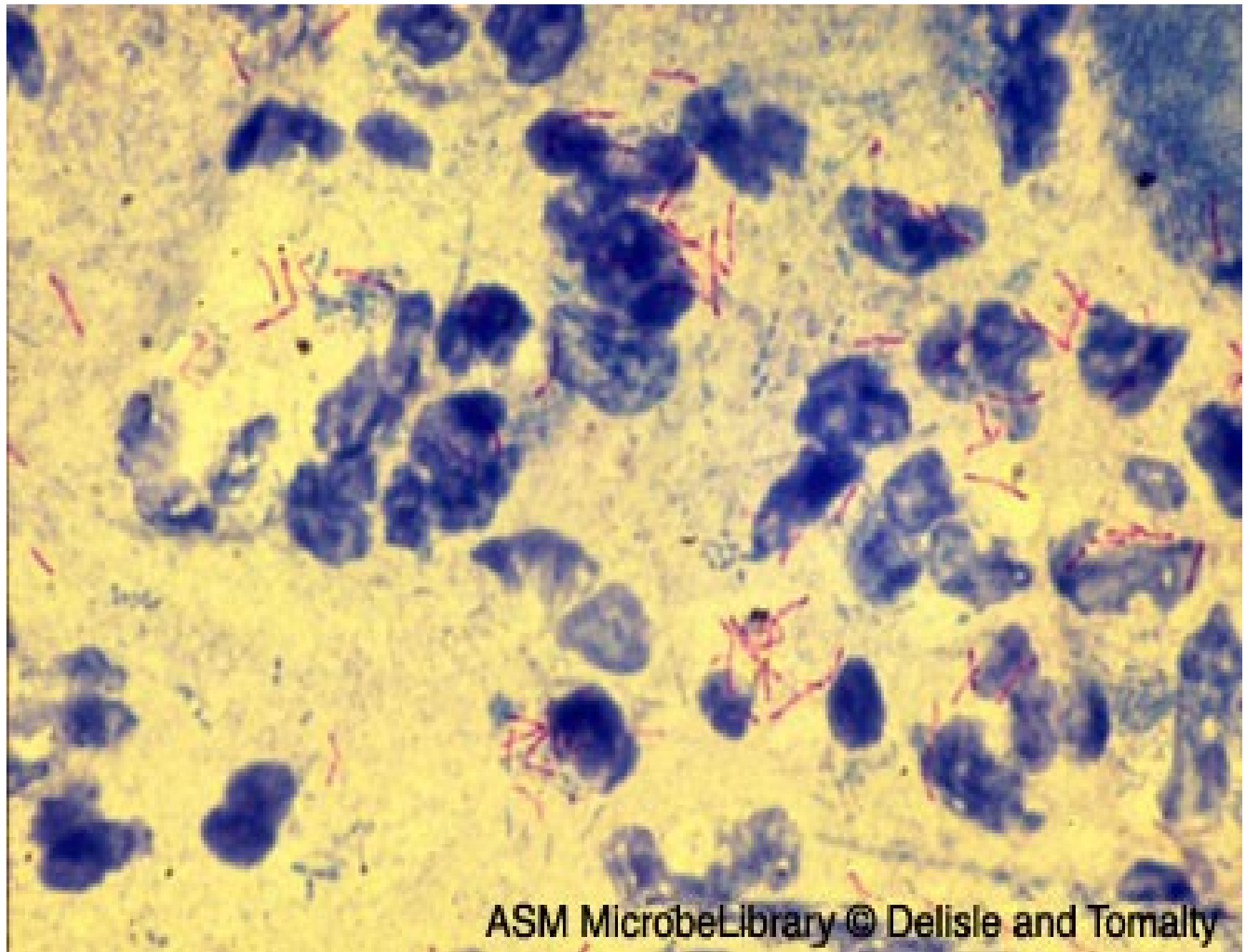
- Acid fast bacilli stain pink.
- Straight or slightly curved rods appear.
- Sometimes beaded appearance.
- The background appears blue due to methylene blue.

Interpretations.

- If definite bacilli are seen, report as AFB positive.
- Better to report result quantitatively:
 - A. > 10 AFB/1 high power field □+++
 - B. 1-10 AFB/1 high power field □ ++
 - C. 10-100 AFB/100 high power fields □ +
 - D. 1-9 AFB/100 high power fields □ Exact Number.
- However if no AFB is seen, write the result as “NO AFB SEEN” and “NEVER” write “NEGATIVE”.







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Video.

