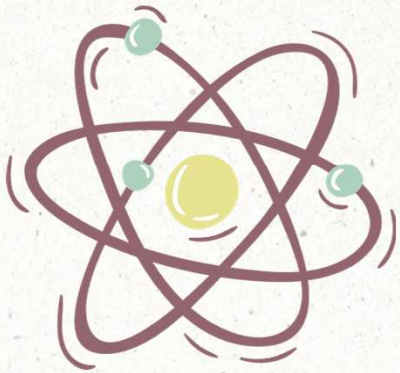
ZIEHL-NEELSEN STAIN

JIA QIAN, MAGHA, AMOS

Pathweb, Department of Pathology, NUHS

LEARNING OBJECTIVES

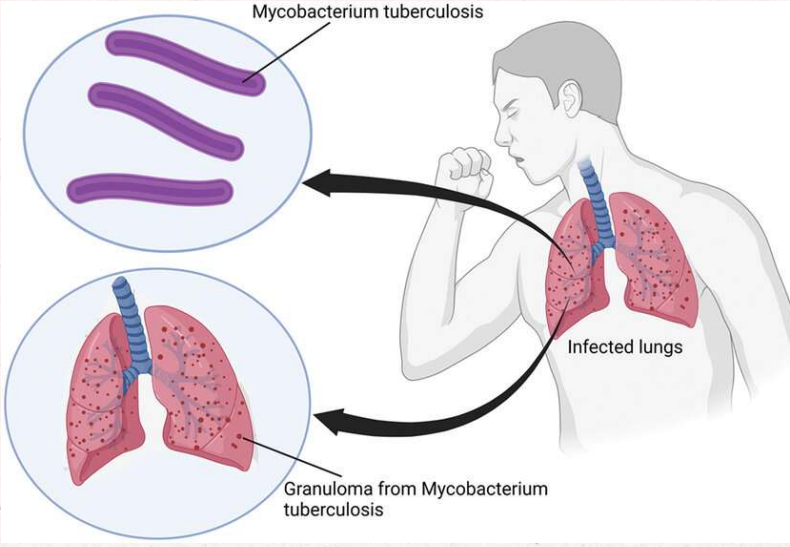
- Understand the principle of acid-fastness
- Identify acid-fast bacilli and differentiate from non-acid-fast cells
- Recognize technical pitfalls affecting false results



ZN STAIN

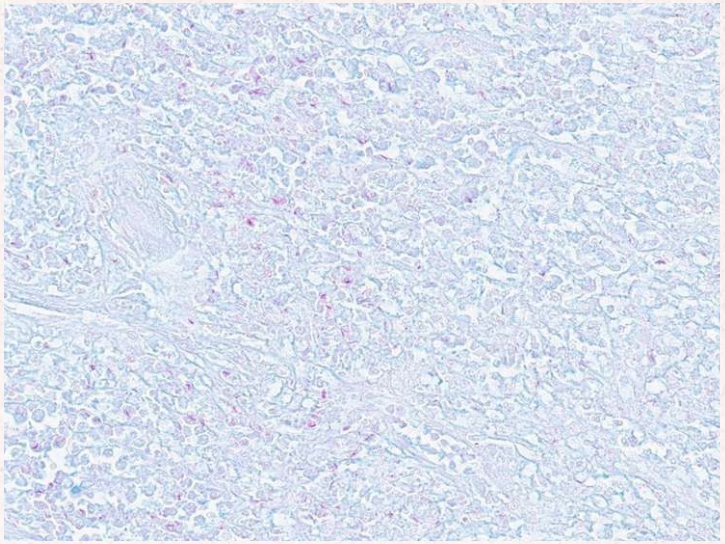
ZN stain is used to identify acid-fast bacilli (AFB), particularly Mycobacterium species (*e.g., M. tuberculosis, M. leprae*)

- AFB possess lipid-rich cell walls containing mycolic acids, making them resistant to decolorization.
- Carbol Fuchsin penetrates the waxy cell wall with the help of heat and phenol.
- AFB retain the red/pink stain even after acid-alcohol treatment , while non-acid fast cells are decolorized.
- Methylene blue counterstain stains non-acid fast cells blue.



ZN STAIN

- **Positive:** Red/Pink
- **Negative:** Blue



BSS ZN

Manual ZN

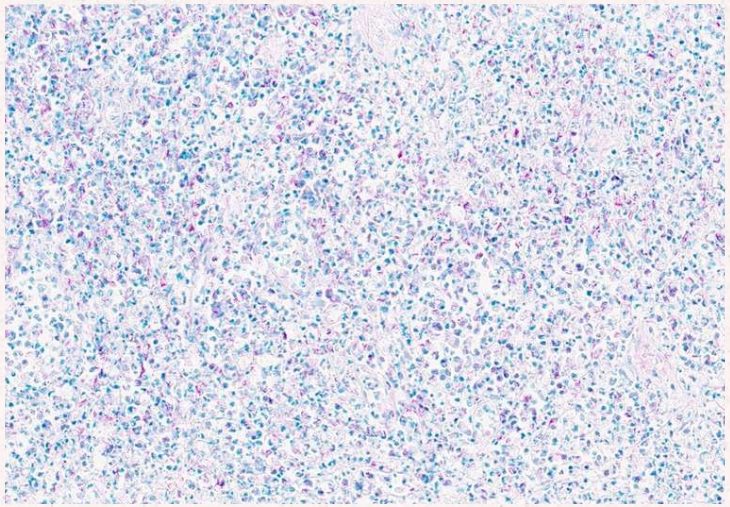
- BSS (autostainer) ZN shows more uniform and consistent staining.
- Manual ZN may show a variability in staining intensity.
- BSS ZN provides a faster, consistent and accurate results as compared to manual ZN.

PROCEDURE STEPS

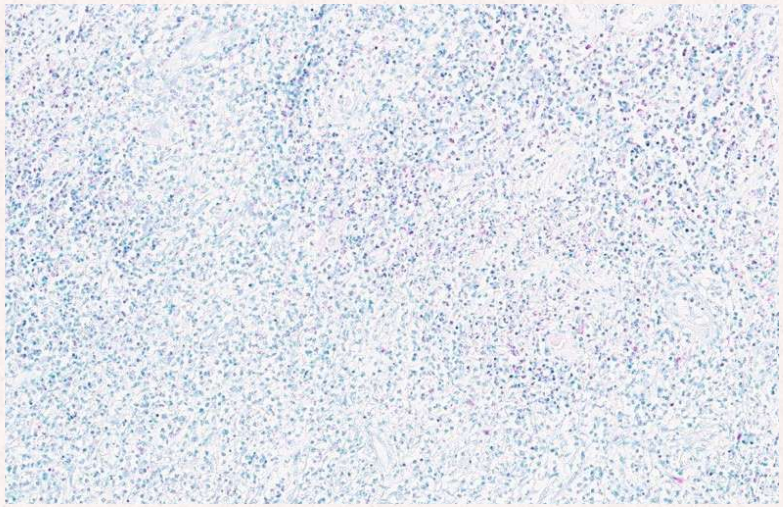
1. DEWAX SLIDES
2. IMMERSE SLIDES WITH CARBOL FUCHSIN -15 MINS
3. WASH WITH TAP WATER
4. DIFFERENTIATE IN 0.5% ACID ALCOHOL RINSE UNTIL LIGHT PINK
5. WASH WITH TAP WATER
6. DIFFERENTIATE IN 70% ALCOHOL
7. COUNTERSTAIN IN 1 % ACIDIFIED METHYLENE BLUE - 10 SECS
8. RINSE WITH WATER
9. DEHYDRATE, CLEAR AND MOUNT

TROUBLESHOOTING

30 MIN OF CARBON FUCHSIN VS NORMAL



Carbon Fuchsin

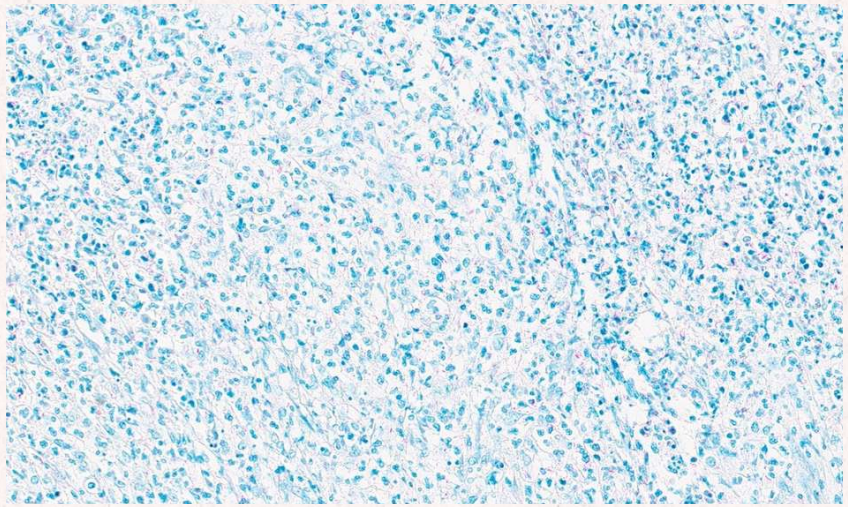


Normal

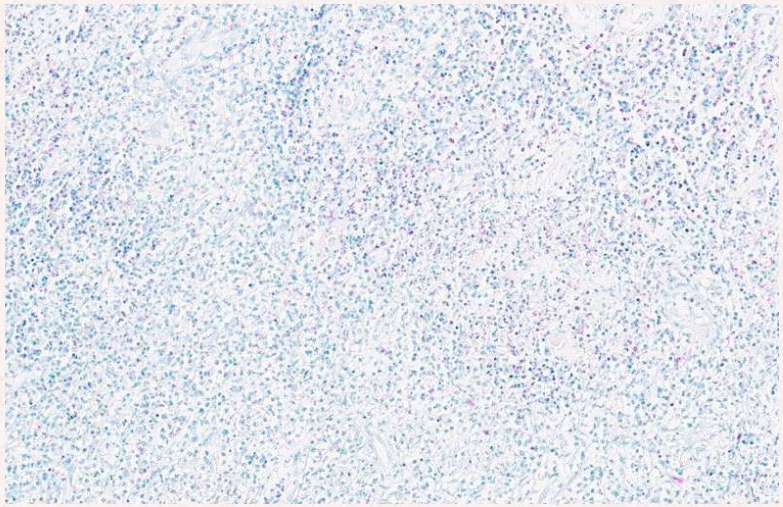
- Background staining ↑
- Non-acid fast cells may have retained excess pink dye

TROUBLESHOOTING

OVERDECOLORIZATION VS NORMAL



Overdecolorization

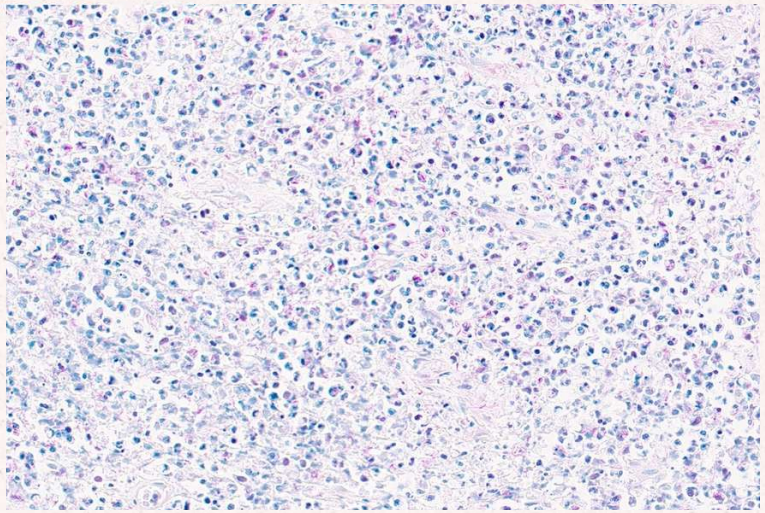


Normal

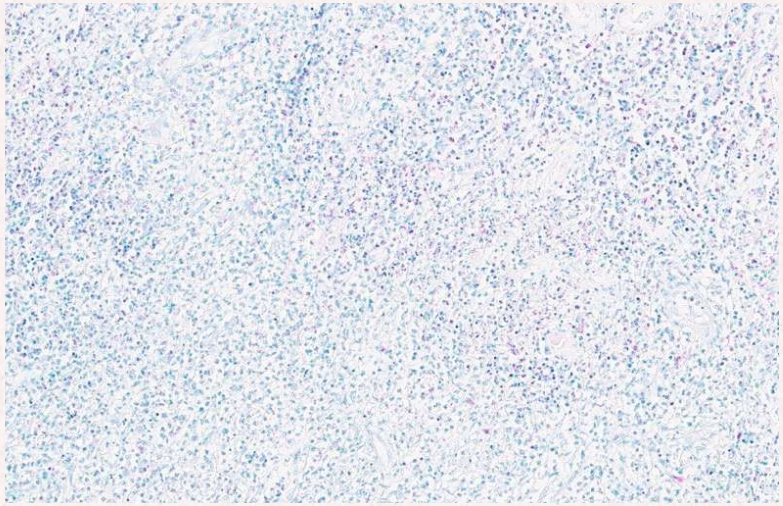
- Excess acid-alcohol removes stain even from acid-fast bacteria.
- Acid-fast bacilli may appear faint or blue.
- May lead to **false negative** results

TROUBLESHOOTING

UNDERDECOLORIZATION VS NORMAL



Underdecolorization

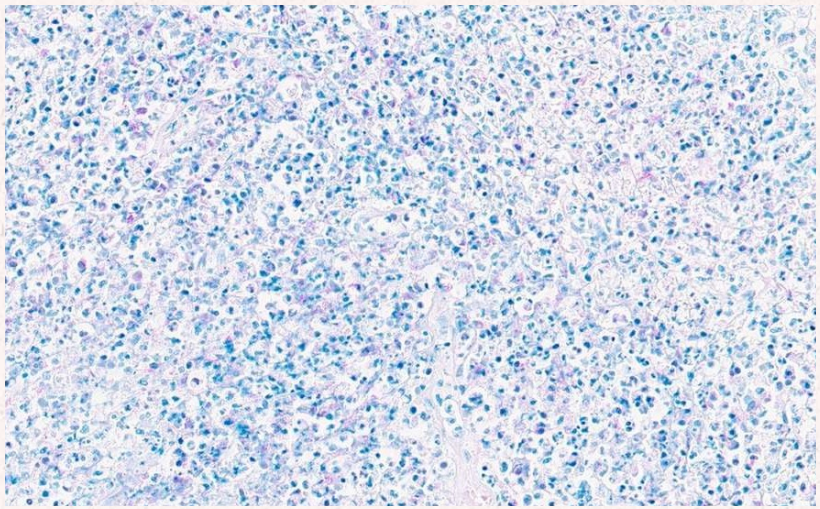


Normal

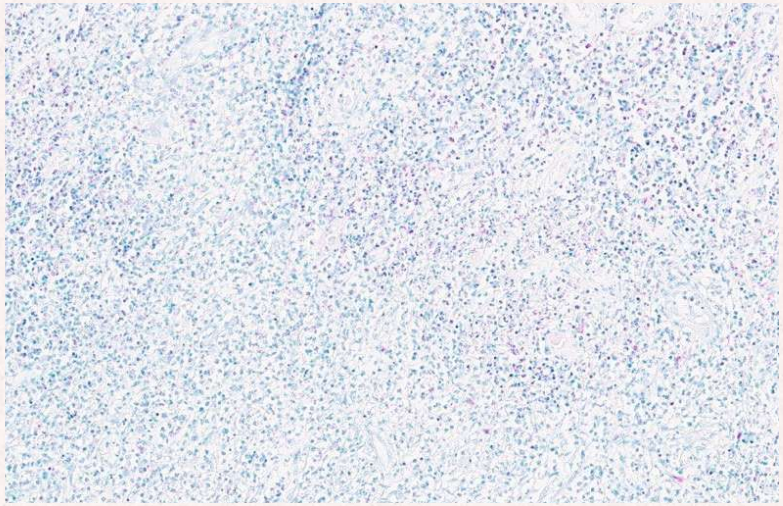
- Non-acid-fast bacteria retain pink/red stain.
- Leads to false positive result.

TROUBLESHOOTING

ABSENCE OF 70% ALCOHOL VS NORMAL



No 70% Alcohol

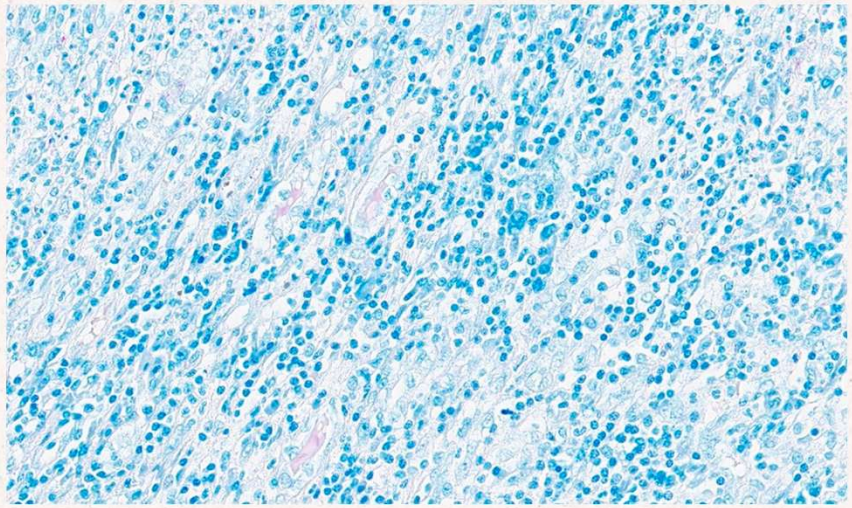


Normal

- Proper removal of staining reagent may not occur
- Uneven staining and reduced contrast
- Acid-fast bacilli may appear faint and more difficult to distinguish

TROUBLESHOOTING

30 MIN OF METHYLENE BLUE VS NORMAL



30 mins of Methylene Blue

Normal

- Excessive counterstain with methylene blue increase background intensity
- Background becomes **very dark blue.**
- Acid-fast bacilli becomes **less distinct** due to strong counterstaining.

DISCUSSION

- The Ziehl–Neelsen stain relies heavily on **proper decolorization and correct staining time** to differentiate acid-fast bacteria from non–acid-fast cells.

- Deviations from the standard Ziehl–Neelsen staining protocol can affect stain contrast and clarity, leading to **misinterpretation and potential false positive or false negative results**.

- Overall, **accurate timing and proper differentiation steps** are essential to ensure reliable identification of acid-fast bacilli in Ziehl–Neelsen staining.

TAKE HOME MESSAGES

- Acid-fast organisms retain carbol fuchsin due to mycolic acids
- The decolorization step is crucial for accurate interpretation
- Both manual and autostainer-stained ZN are effective when timing and technique are correct

REFERENCES

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