

Ziehl-Neelson Stain

For In-Vitro Diagnostics

For Professional Use Only

The Ziehl-Neelsen (Zn) technique is used to stain Mycobacterium species including *M. tuberculosis*, *M. ulcerans*, and *M. leprae*. Mycobacteria, unlike most other bacteria, do not stain well by the Gram technique. They can however be stained with carbol fuchsin combined with phenol.

Sample Preparation

Any well fixed tissue (excluding tissue fixed in Carnoy's Solution)

Mode of Action

The stain binds to the mycolic acid in the mycobacterial cell wall. After staining, an acid decolorizing solution is applied. This removes the red dye from the background cells, tissue fibres, and any organisms in the smear except mycobacteria which retain (hold fast to) the dye and are therefore referred to as acid fast bacilli, or simply AFB. Following decolorization, the smear is counterstained with malachite green or methylene blue which stains the background material, providing a contrast colour against which the red AFB can be seen.

Warnings and Precautions

Carbol Fuchsin

Carbol Fuchsin concentrate is toxic if inhaled or swallowed; causes severe skin burns and is suspected of causing cancer and genetic defects. Refer to the Safety Data Sheet.



Acid Alcohol

Acid alcohol is highly flammable harmful if inhaled; it is corrosive and will cause burns to skin, and eye damage. Refer to the Safety Data Sheet.



Ingredients

Carbol Fuchsin Concentrate

Substance	CAS	Conc
Carbol Fuchsin	4197-24-4	1-2%
Ethanol	64-17-5	<10%
Glycerol		<10%
Phenol	108-95-2	<10%
Water	7732-18-5	Balance

Acid Alcohol

Substance	CAS	Conc
HCl	7647-01-0	0 – 1%
Ethanol	64-17-5	Balance

Methylene Blue

Substance	CAS	Conc
Methylene Blue	61-73-4	0 – 1%
Water	7732-18-5	Balance

Specifications

Carbol Fuchsin

Appearance	Dark red liquid
pH @ 20°C	5.5 – 7.5
Density @ 20°C	1.000 to 1.040 g/cm ³

Acid Alcohol

Appearance	Clear, colourless solution
pH @ 20°C	< 1

Methylene Blue

Appearance	Dark Blue Liquid
Density @ 20°C	1.000 to 1.005 g/cm ³

Stability

The expiry date of each reagent is printed on the label. Store each reagent away in sealed bottle away from heat and light.

This product should not be used if 1) the appearance has changed; 2) the expiration date has passed; or 3) there are other signs of deterioration.

Technical Procedure

1. Prepare working methylene blue solution by diluting 3.5mL of the 1% Methylene Blue;
2. Deparaffinise and hydrate sections to deionised water;

3. Stain the sections with freshly filtered carbol-fuchsin solution for 30 minutes;
4. Wash sections well in running water;
5. Decolourise with 1% Acid Alcohol solution until sections are pale pink;
6. Wash thoroughly with tap water, then with deionised water;
7. Counterstain in the methylene blue working solution. Sections should be pale blue;
8. Wash with tap water, then with deionised water;
9. Dehydrate quickly in 95% and absolute alcohols, 2 changes each;
10. Mount with synthetic resin

Results and Interpretation

Only experienced and suitably qualified persons should carry out interpretation of stained slides.

Acid Fast Bacteria	Red
Cells	Green/Blue
Background	Green/Blue

Notes

- Do not use tap or distilled water before the carbol fuchsin; use only deionised water. Acid fast bacteria may be present in tap/distilled water
- If section is overstained, take it back to acid alcohol to remove the methylene blue, wash with water, and then repeat the counterstaining step;
- If the acid is not washed out of the tissue before counterstaining, the tissue will not stain;
- If section is allowed to dry after the carbol-fuchsin step, a compound resistant to decolorisation will be formed.
- This method should not be used to demonstrate *Mycobacterium leprae*
- Fixation in Carnoy's solution will make acid-fast organisms non-acid fast

References

Survana KS, Layton C, and Bancroft JD. *Bancroft's Theory and Practice of Histological Techniques*, 7th Ed. London, Churchill Livingstone, 2012

Sheehan, D.C. and Hrapchak B.B, *Theory and Practice of Histotechnology*, 2nd Edition; 1987, Battelle Press

Carson, Freida; Hladick, Christa; *Histotechnology – A Self Instructional Text*; 3rd Edition; American Society for Clinical Pathology Press 2009

Cheesbrough M. District Laboratory Practice in Tropical Countries, 2nd Ed. New York, Cambridge University Press, 2006

Ordering

Product	Size	Code
Carbol Fuchsin 1%	500mL	FNNHH003
Carbol Fuchsin 1%	2.5L	FNNHH004
Acid Alcohol 1%	5L	FNNACIDAL15
Methylene Blue 1%	100mL	FNNZIEHL1D
Methylene Blue 1%	500mL	FNNHH027