CARBOL FUCHSIN STAIN

(ZIEHL-NEELSEN)

- For in vitro use only - Catalogue No. SC24K

Our Carbol Fuchsin (Ziehl-Neelsen) Stain is used in the microscopic detection of acid-fast microorganisms such as *Mycobacterium.*

Acid-fast organisms such as *Mycobacterium* have cell walls that are resistant to conventional staining by aniline dyes such as the Gram stain. However methods that promote the uptake of dyes are available; once stained these organisms are not easily decolorized even with acid-alcohol or acid-acetone solutions therefore they are described as acid-fast. Their resistance to destaining is a useful characteristic in differentiating these organisms from contaminating organisms and host cells.

The Ziehl-Neelsen staining procedure is often referred to as hot carbolfuchsin because of the need to apply heat during the staining process. The primary stain for the Ziehl-Zeelsen procedure is the aniline dye, basic fuchsin, that stains all the cells present red. The unique ability of mycobacteria to resist decolorization by acid-alcohol is why they are termed acid-fast, and will keep their red coloration throughout the staining process. The decolorizer used is a hydrochloric acid-ethanol mixture that will decolorize non-acid-fast material present. The last step in the staining procedure is the application of the counterstain, methylene blue, which colors other cells and background material present on the slide blue.

The acid-fast smear plays an important role in early diagnosis of mycobacterial infection because of the lengthy incubation times required to culture mycobacteria. Nonmycobacterial organisms with various degrees of acid-fastness include *Rhodococcus* species, *Nocardia* species, *Legionella micdadei*, and the cysts of *Cryptosporidium*, *Isospora*, *Cyclospora* and microsporidia. Detection of these organisms is possible using some of the staining reagents provided but in most instances requires a modified staining procedure and additional reagents not provided.

Formulation per 100 mL

SC25 Carbol Fuchsin Stain (Ziehl-Zeelsen)

Basic Fuchsin	0.3 g
Phenol	5.0 g
Ethanol	10 mL
De-ionized Water	90 mL

SC26 Carbol Fuchsin Decolorizer

Hydrochloric Acid	3.0 mL
Ethanol	97.0 mL

SC27 Carbol Fuchsin Counterstain (Methylene Blue)

Methylene Blue	0.3 g
De-ionized Water	100 mL

Recommended Procedure

1. Prepare slides by applying sample in a thin smear and heat fixing the sample to the slide.
2. Flood the entire slide with Carbol Fuchsin Stain (Ziehl-Zeelsen).
3. Heat the slide using a bunsen burner or electric staining rack until it is steaming.
4. Maintain steaming for 5 minutes by using low or intermittent heat.
5. Allow the slide to cool briefly and rinse the slide with water.
6. Flood the slide with Carbol Fuchsin Decolorizer and allow smear to decolorize for 2 minutes; flood until no more color drains from the slide.
7. Rinse the slide thoroughly with distilled water and shake off any excess moisture.
8. Flood the slide with Carbol Fuchsin Counterstain (Methylene Blue) and allow the slides to stain for 30 to 45 seconds.
9. Rinse thoroughly with water and allow to air dry. Do not blot.
10. Examine the smear microscopically under a 100x oil immersion objective.

Interpretation of Results

Acid-fast mycobacteria will appear as dark-pink to red bacilli against a blue background when examined microscopically. Mycobacteria are typically slender, 1 to 10-µm long rods that may appear curved or bent. Individual bacilli may display heavily stained areas and area of alternating stain, producing a beaded appearance. Some nontuberculous mycobacteria may appear pleomorphic, appearing as long filaments or coccoid forms, with uniform staining. *Mycobacterium kansaii* are often recognized by their large size and cross-banding appearance.

When a carbol fuchsin smear is read a minimum of 300 fields should be examined before the smear is reported as negative. To verify the staining procedure and staining intensity of the acid-fast organisms it is recommended that a positive and negative control slide be included with each run of stains.

Non-acid-fast organisms and background material will stain blue.

- *Rapidly growing mycobacteria may vary in their ability to retain acid-fast dyes and may fail to stain*
- *Be aware of adequate safety precautions and procedures required when handling specimens that are submitted for mycobacterial evaluation*
- *Mycobacterial staining should always be used as a adjunct to culture methods since culture techniques are much more sensitive than all acid-fast staining procedures*

Quality Control

Organism	Expected Result
Mycobacterium tuberculosis ATCC 25177 (H37Ra)	Dark pink to red bacilli

Storage and Shelf life

Our Carbol Fuchsin Stain, Decolorizer and Counterstain should be stored at room temperature and protected from light. Under these conditions they have a shelf life of 52 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
SC24K-250	Carbol Fuchsin Stain Kit (Ziehl-Neelson) [Includes stain, decolorizer & counterstain]	3 x 250mL
SC25-250	Carbol Fuchsin Stain (Ziehl-Neelson)	250-mL
SC26-250	Carbol Fuchsin Decolorizer (Ziehl-Neelson & Kinyoun)	250-mL
SC27-250	Carbol Fuchsin Counterstain (Ziehl-Neelson)	250-mL

References

1. Baron EJ, Finegold SM. Bailey and Scott's diagnostic microbiology. 8th ed. St. Louis: Mosby, 1990.
2. Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol 1. Washington, DC: ASM, 1992.
3. Bloom BR, Ed. Tuberculosis: pathogenesis, protection, and control. Washington, DC: ASM 1994.
4. Murray PR, Baron E, Pfaller M, Tenover F, Yolken. Manual of clinical microbiology. 7th ed. Washington, DC: ASM, 1999.

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