KINYOUN CARBOL FUCHSIN STAIN

- For in vitro use only - Catalogue No. SK50

Our Kinyoun Carbol Fuchsin 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 Kinyoun staining procedure is often referred to as cold carbolfuchsin because no heat is applied during the staining process unlike the Ziehl-Neelsen procedure. The primary stain for the Kinyoun 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 a counterstain. If methylene blue is used other cells and background material present on the slide stain blue; if brilliant green is used other cells and background material present on the slide stain green.

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

SK50 Kinyoun Carbol Fuchsin Stain
Basic Fuchsin ..... 3.33 g
Phenol ..... 6.67 g
Ethanol ..... 16.7 mL
Deionized Water ..... 83.3 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
Deionized Water .....100 mL

SC35 Carbol Fuchsin Counterstain (Brilliant Green)
Brilliant Green ..... 1.0 g
Deionized Water .....100 mL

Recommended Procedure

1. Flood the entire slide with Kinyoun Carbol Fuchsin Stain.
2. Allow the smear to stain for 2 minutes.
3. Rinse the slide with water.
4. Flood the slide with Carbol Fuchsin Decolorizer and decolorize until no more color drains from the slide (approx 3 to 5 seconds).
5. Rinse the slide thoroughly with water and shake off any excess moisture.
6. Flood the slide with Carbol Fuchsin Counterstain (Methylene Blue or Brilliant Green) and allow the slide to stain for 30 seconds.

7. Rinse thoroughly with water and allow to air dry. Do not blot.
8. 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 (methylene blue) or green (brilliant green) 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 or green depending on the counterstain used.

- *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

Control slides should be reviewed before the patient smears are read to confirm staining performance and intensity.

Organism	Expected Result
Mycobacterium tuberculosis ATCC 25177 (H37Ra)	Dark pink to red bacilli
Nocardia asteroides ATCC 3308	Blue or green
Escherichia coli ATCC 25922	Blue or green

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
SK50-250	Kinyoun Carbol Fuchsin Stain	250-mL (each)
SC26-250	Carbol Fuchsin Decolorizer (Ziehl-Neelson & Kinyoun)	250-mL (each)
SC27-250	Carbol Fuchsin Counterstain (Methylene Blue)	250-mL (each)
SC35-250	Carbol Fuchsin Counterstain (Brilliant Green)	250-mL (each)

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, Eds. Manual of clinical microbiology. 7th ed. Washington, DC: ASM, 1999.

Original: December 2005
Revised / Revisited: October 2014