

ACID FAST BACTERIA (AFB) STAINS

KINYOUN METHOD AND ZIEHL NEELSEN METHOD

DESCRIPTION

Staining of bacteria from cultures or patient specimens suspected to contain Mycobacteria tuberculosis can be easily achieved using one of two methods: (1) hot method or (2) cold method. The traditional Ziehl Neelsen Method employs the use of heat, for which it is known as the hot method. Ziehl Neelsen staining method uses “heated” Carbol-fuchsin solution as a primary stain, followed by a decolorizer solution and a methylene blue solution as a counterstain. Whereas the Kinyoun Method provides a cold method alternative for staining without the need for heat with brilliant green or methylene blue counterstain option.

Acid-fast organisms are stained red, while non-acid-fast organisms and the background are stained green or blue. A major characteristic of mycobacteria is that they are “acid-fast” and once stained with aniline dye, basic fuchsin, they are difficult to decolorize, thus retaining the red color even when exposed to an acid alcohol rinse. Whether you’re testing surgical specimens, respiratory or sputum specimens, the two counterstains brilliant green and methylene blue will provide easy identification to the nonacid fast organisms.

PROCEDURE

1. Fix the slide.
2. Place fixed smear on a staining rack.
3. Stain slide using preferred hot or cold method:
Ziehl Neelsen (hot method): Flood slide with Ziehl Neelsen solution and heat: 3 minutes (avoid boiling)
Carbol Fuchsin (cold method): Flood slide with Carbol Fuchsin solution: 5 minutes
4. Rinse gently with water.
5. Flood slide with decolorizer for roughly 3 minutes. Rinse slide with water. If a thick specimen, decolorize again for 1 to 2 minutes until solution runs clear.
6. Rinse gently with water.
7. Flood slide with Counterstain (Methylene Blue or Brilliant Green) for 1-3 minutes.
8. Rinse gently with water until clear.
9. Examine dry under high magnification and verify under oil immersion.

RESULTS

The “acid fast” organisms will appear red.

Non-“acid fast” organisms will appear blue or green.

ORDERING INFORMATION

Cat. #	Description
6300	AFB Kinyoun Set
6301	AFB Kinyoun, Carbol Fuchsin
6302	AFB Kinyoun, Decolorizer
6303	AFB Kinyoun, Brilliant Green
6305	AFB Kinyoun, Methylene Blue
6309	AFB Ziehl-Neelsen Set
6310	AFB Ziehl-Neelsen, Carbol Fuchsin
6320	AFB Ziehl-Neelsen, Decolorizer
6330	AFB Ziehl-Neelsen, Methylene Blue

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