

AFB Stain, Kinyoun - Technical Memo

SOLUTIONS:

	250 ml	500 ml	1 Liter	1 Gallon
Carbol Fuchsin Stain Solution, Kinyoun	Part 1031A	Part 1031B		
Acid Alcohol, 1% Solution		Part 10011A	Part 10011B	Part 10011C
Methylene Blue Stain, 0.14% Working	Part 12401A			

Additionally Needed:

Acid Fast Bacteria (AFB) Control Slides	Part 4011
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply AFB Stain, Kinyoun is used to demonstrate the presence of acid-fast mycobacteria in tissue sections. Phenol is employed to render the cell walls of bacteria permeable to the fuchsin stain. The use of weak acid for differentiation allows excess stain to be removed from tissues, but will not remove stain from the acid-fast organisms.

METHOD:

Fixation: 10% Phosphate Buffered Formalin (Part 1090)

Technique: Paraffin sections cut at 5 microns

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

STAINING PROCEDURE:

1. Filter Carbol Fuchsin Stain Solution, Kinyoun before use.
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - a. See Procedure Notes #1 and #2.
3. Stain in freshly filtered Carbol Fuchsin Stain Solution for 60 minutes at room temperature. Keep solution covered.
4. Wash in running tap water for 2 to 3 minutes.
5. Differentiate in Acid Alcohol, 1% Solution until color no longer runs off the slide and sections are pale pink; 3 to 10 rapid dips.
6. Wash in running tap water 3 to 5 minutes; rinse in distilled water.
7. Counterstain slides individually in Methylene Blue Stain, 0.14% Working; 1 or 2 dips. Sections should be pale blue.
 - a. See Procedure Notes #3 and #4.
8. Rinse in distilled water.
9. Dehydrate quickly in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
 - a. See Procedure Note #5.

RESULTS:

Acid-fast bacteria	Bright red
Background	Light blue

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. Dip slides 1-2 times in Methylene Blue Stain, 0.14% Working; rinse in tap water, followed by a distilled water rinse and check microscopically. It is important not to over-counterstain, as the organisms may be masked. If section is over-stained, remove methylene blue with acid alcohol, rinse thoroughly, and repeat methylene blue step (Step #7).
4. If laboratory tap water is generally acidic, the methylene blue stain may be pale. Adjust staining time accordingly.
5. Dehydrate quickly to maintain Methylene Blue staining.
6. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 224-226.
2. Kinyoun, J.J. "A Note on Uhlenhuth's Method for Sputum Examination, for Tubercle Bacilli." *American Journal of Public Health* 5.9 (1915). 867-870.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 236-237.
4. Modifications developed by Newcomer Supply Laboratory.