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Part 1031
Revised November 2015

Carbol Fuchsin Stain, Kinyoun for AFB Stain, Kinyoun - Technical Memo

SOLUTION:	**250 ml**		**500 ml**	
Carbol Fuchsin Stain, Kinyoun	Part 1031A		Part 1031B	
Additionally Needed For AFB Stain, Kinyoun:				
Acid Fast Bacteria (AFB) Control Slides	Part 4011			
Acid Alcohol 1%	Part 10011			
Methylene Blue Stain 1.4%, Alcoholic	Part 1240A	or	Methylene Blue Stain 0.14%, Alcoholic	Part 12401A
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 Carbol Fuchsin Stain, Kinyoun a crucial element in the AFB Stain, Kinyoun procedure is used to demonstrate the presence of acid-fast mycobacteria in tissue sections. Phenol is employed in this solution 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: Formalin 10%, Phosphate Buffered (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, 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, Kinyoun 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% 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%, Alcoholic (12401). Or dilute 5 ml Methylene Blue Stain 1.4%, Alcoholic (1240) with 45 ml tap water to a 0.14% solution.
    a. *Dip slides 1-2 times in counterstain; rinse in tap water, followed by a distilled water rinse and check microscopically. Sections should be pale blue.*
    b. *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. It is important not to over-counterstain, as the organisms may be masked. If section is over-stained, remove methylene blue with Acid Alcohol 1% (10011), rinse thoroughly, and repeat methylene blue counterstain 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 Uhlenhuths 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.

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