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Part 1030
Revised July 2019

Carbol Fuchsin Stain, Ziehl-Neelsen for AFB Stain - Technical Memo

SOLUTION:	**250 ml**		**500 ml**	**1 Liter**
Carbol Fuchsin Stain, Ziehl-Neelsen	Part 1030A		Part 1030B	Part 1030C

Additionally Needed For AFB Stain, Ziehl-Neelsen:

Acid Fast Bacteria (AFB) Control Slides	Part 4011			
Acid Alcohol 1%	Part 10011			
Light Green SF Yellowish Stain 0.1%, Aqueous	Part 12203	or	Methylene Blue Stain 0.14%, Alcoholic	Part 12401
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 product labels.

APPLICATION:

Newcomer Supply Carbol Fuchsin Stain, Ziehl-Neelsen, a crucial element in the AFB Stain, Ziehl-Neelsen is used to demonstrate the presence of acid-fast mycobacteria in tissue sections. Acid-fastness is a physical property of certain bacteria and cellular structures. Carbol Fuchsin Stain, Ziehl-Neelsen, combines phenol and basic fuchsin that works to permeate the lipoid capsule of acid-fast organisms and renders them resistant to acid alcohol decolorization.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 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 provided staining procedure.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Filter Carbol Fuchsin Stain, Ziehl-Neelsen with filter paper whenever a thick sheen develops on solution surface.

STAINING PROCEDURE:

3. 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.*
4. Stain in Carbol Fuchsin Stain, Ziehl-Neelsen for 15 minutes at room temperature. Keep solution covered.
    a. *See Procedure Note #3.*
5. Rinse in running tap water for 2 to 3 minutes.
6. Differentiate in Acid Alcohol 1% (10011) until color no longer runs off the slide and sections are pale pink; 3 to 10 rapid dips.
7. Wash in running tap water 3 to 5 minutes; rinse in distilled water.
8. Counterstain with 3-6 dips in counterstain of choice;
    a. *Light Green SF Yellowish Stain 0.1%, Aqueous (12203)*
    b. *Methylene Blue Stain 0.14%, Alcoholic (12401). Do not overstain; sections should be pale blue.*
9. Rinse slides:
    a. *Light Green SF Yellowish counterstain; rinse with one quick dip in distilled water or proceed directly to Step #10 without a distilled water rinse.*
    b. *Methylene Blue counterstain; rinse in running tap water for 1 minute; rinse in distilled water.*
10. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Acid-fast bacilli	Bright red
Background	Green (with Light Green SF Yellowish counterstain)
Background	Pale blue (with Methylene Blue counterstain)

PROCEDURE NOTES:

1. Drain slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. Sections can remain in Carbol Fuchsin Stain, Ziehl-Neelsen for up to 60 minutes without adverse effect. Additional differentiation may be required in Step #6.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 218-220.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 237.
3. Modifications developed by Newcomer Supply Laboratory.

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Part 1030
Revised July 2019

Carbol Fuchsin Stain, Ziehl-Neelsen for AFB, Fite Stain - Technical Memo

SOLUTION:	**250 ml**		**500 ml**	**1 Liter**
Carbol Fuchsin Stain, Ziehl-Neelsen	Part 1030A		Part 1030B	Part 1030C

Additionally Needed For AFB Stain, Fite:				
Fite Stain, *Nocardia Sp*. Control Slides	Part 4215	or	Abnormal Animal Spleen Custom Tissue Slides	Part CT28730A
Xylene/Peanut Oil, 2:1	Part 1449			
Acid Alcohol 1%	Part 10011			
Light Green SF Yellowish Stain 0.1%, Aqueous	Part 12203	or	Methylene Blue Stain 0.5%, Aqueous	Part 12402
Xylene, ACS	Part 1445			

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

APPLICATION:

Newcomer Supply Carbol Fuchsin Stain, Ziehl-Neelsen, a crucial element in the AFB Stain, Fite is used to detect the presence of either *Nocardia sp.* or *Mycobacterium leprae sp.* (causative agent of leprosy) in tissue sections. Minor procedural variations are included for detection of either organism.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin Sections cut at 4 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 provided staining procedure.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Filter Carbol Fuchsin Stain, Ziehl-Neelsen with high quality filter paper.
3. If staining for Nocardia sp., prepare Diluted Acid Alcohol Solution:
    a. *Acid Alcohol 1% (10011)* *20 ml*
    b. *Distilled water* *20 ml*

STAINING PROCEDURE:

4. Deparaffinize slides in Xylene/Peanut Oil, 2:1 (1449), two changes for 10 minutes each.
    a. *See Procedure Note #1*
5. Drain slides, wipe off excess oil, and blot to opacity taking care to remove residual oil.
    a. *See Procedure Note #2.*
6. Stain in freshly filtered Carbol Fuchsin Stain, Ziehl-Neelsen for 15 minutes at room temperature.
7. Rinse well in distilled water.
8. Differentiation:
    a. *For Nocardia sp.: Differentiate slides individually in Diluted Acid Alcohol Solution (Step #3) until background is pale pink; 10-20 dips. Quickly rinse in distilled water and check microscopically for correct differentiation.*
    b. *For Mycobacterium leprae sp.: Differentiate slides individually in Acid Alcohol 1% (10011) until sections are light pink; 5-10 dips.*
9. Rinse well in distilled water.
10. Counterstain with 5-10 dips in counterstain of choice;
    a. *Light Green SF Yellowish Stain 0.1%, Aqueous (12203).*
    b. *Methylene Blue Stain 0.5%, Aqueous (12402). Do not overstain; sections should be pale blue.*
11. Rinse slides:
    a. *Light Green SF Yellowish counterstain; rinse in distilled water.*
    b. *Methylene Blue counterstain; wash in running tap water, rinse in distilled.*
12. Blot excess water from slide and air-dry or oven-dry completely.
13. Dip dried slides in xylene and coverslip with compatible mounting medium.

RESULTS:

Acid-fast bacilli and *Mycobacterium leprae sp.*	Red
Nocardia sp.	Red
Background	Green (with Light Green SF Yellowish counterstain)
Background	Pale blue (with Methylene Blue counterstain)

PROCEDURE NOTES:

1. Acid-fastness of leprosy organisms is enhanced when the waxy capsule is protected by the mixture of xylene-peanut oil and avoidance of dehydrating solutions.
2. It is important to blot well; residual oil may produce staining artifact.
3. A small percentage of *Nocardia sp*. organisms may resist taking the red stain and remain green (or blue, depending upon counterstain used) due to growth phase of the individual organism.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for coverslipping step.

REFERENCES:

1. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 220-221.
2. Fite, George, P.J. Cambre and M.H. Turner. "Procedure for Demonstrating Lepra Bacilli in Paraffin Sections". *Archives of Pathology* 43 (1947). 624-625.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 237.
4. Modifications developed by Newcomer Supply Laboratory.