

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

## SOLUTION:

Carbol Fuchsin Stain, Ziehl-Neelsen

250 ml

Part 1030A

500 ml

Part 1030B

1 Liter

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
Xylene, ACS	Part 1445		Part 12401
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 is used in the AFB Stain, Ziehl-Neelsen 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 lipid capsule of acid-fast organisms and renders them resistant to acid alcohol decolorization.

## METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (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 30 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, 2-5 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.
  - a. Additional differentiation may be required in Step #6.
4. If using a xylene substitute, follow manufacturer's recommendation 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, Dezná C. and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 237.
3. Modifications developed by Newcomer Supply Laboratory.

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

### SOLUTION:

Carbol Fuchsin Stain, Ziehl-Neelsen

250 ml

Part 1030A

500 ml

Part 1030B

1 Liter

Part 1030C

### Additionally Needed For AFB Stain, Fite:

Fite, *Nocardia*, Artificial Control Slides

Xylene/Peanut Oil, 2:1

Acid Alcohol 1%

Light Green SF Yellowish Stain 0.1%, Aqueous

Xylene, ACS

Part 4215

or

Fite, Leprosy, Animal Control Slides

Part 4212

Part 1449

Part 10011

Part 12203

or

Methylene Blue Stain 0.5%, Aqueous

Part 12402

Part 1445

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

### APPLICATION:

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

### METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (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:

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

### STAINING PROCEDURE:

- Deparaffinize slides in Xylene/Peanut Oil, 2:1 (1449), two changes for 10 minutes each.
  - See Procedure Note #1.
- Drain slides, wipe off excess oil, and blot to opacity, removing residual oil.
  - See Procedure Note #2.
- Stain in freshly filtered Carbol Fuchsin Stain, Ziehl-Neelsen for 15 minutes at room temperature.
- Rinse well in distilled water.
- Differentiation for *Nocardia*:
  - 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.
- Differentiation for *Mycobacterium leprae*:
  - Differentiate slides individually in Acid Alcohol 1% (10011) until sections are light pink; 5-10 dips.
- Rinse well in distilled water.
- Counterstain with 5-10 dips in counterstain of choice;
  - Light Green SF Yellowish Stain 0.1%, Aqueous (12203).
  - Methylene Blue Stain 0.5%, Aqueous (12402). Do not overstain; sections should be pale blue.

- Rinse slides:
  - Light Green SF Yellowish counterstain; rinse in distilled water.
  - Methylene Blue counterstain; wash in running tap water, rinse in distilled water.

- Blot excess water from slides and air-dry or oven-dry completely.
- Dip dried slides in xylene and coverslip with compatible mounting medium.

### RESULTS:

Acid-fast bacilli and <i>Mycobacterium leprae</i>	Red
<i>Nocardia</i>	Red
Background	Green (with Light Green SF Yellowish counterstain)
Background	Pale blue (with Methylene Blue counterstain)

### PROCEDURE NOTES:

- 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.
- It is important to blot well; residual oil may produce staining artifact.
- A small percentage of *Nocardia* organisms may resist taking the red stain and remain green (or blue, depending upon counterstain) due to growth phase of the individual organism.
- If using a xylene substitute, follow manufacturer's recommendation for coverslipping step.

### REFERENCES:

- Carson, Freida L. and Christa Cappellano. *Histotechnology: A Self-Instructional Text*. 5th ed. Chicago: ASCP Press, 2020. 215-216.
- Fite, George, P.J. Cambre and M.H. Turner. "Procedure for Demonstrating Leptra Bacilli in Paraffin Sections." *Archives of Pathology* 43 (1947). 624-625.
- Sheehan, Dezna C. and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 237.
- Modifications developed by Newcomer Supply Laboratory.