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Part 9101
Revised June 2015

AFB, Ziehl-Neelsen Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9101A**
Solution A: Carbol Fuchsin Stain, Ziehl-Neelsen	250 ml
Solution B: Acid Alcohol 1%	250 ml
Solution C: Methylene Blue Stain 0.14%, Alcoholic	250 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

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, Ziehl-Neelsen Stain Kit procedure 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: 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 Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

STAINING PROCEDURE:

1. Filter Solution A: Carbol Fuchsin Stain, Ziehl-Neelsen 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 Solution A: Carbol Fuchsin Stain, Ziehl-Neelsen for 60 minutes at room temperature. Keep solution covered.
4. Rinse in running tap water for 2 to 3 minutes.
5. Differentiate in Solution B: 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 in Solution C: Methylene Blue Stain 0.14%, Alcoholic.
    a. *Dip slides a few 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. Wash in running tap water for 1 minute; rinse in distilled water.
9. 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	Pale 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, 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. 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. 226-227.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 236-237.
3. Modifications developed by Newcomer Supply Laboratory.
