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Part 1121
Revised July 2020

Wolbach Giemsa Stain - Technical Memo

SOLUTIONS:	**500 ml**	**1 Liter**
Giemsa Stock Stain, Wolbach	Part 1121A	Part 1121B

Additionally Needed:

Giemsa Control Slides	Part 4240
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Acid Alcohol 1%	Part 10011
Alcohol, Methanol Anhydrous, ACS	Part 12236
Rosin 10%, Alcoholic	Part 13398

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

APPLICATION:

Newcomer Supply Wolbach Giemsa Stain is used for differential staining of hematopoietic tissue and demonstration of bacteria and rickettsia that may be present in the sections

METHOD:

Fixation: Recommended for hematopoietic tissue:
- *a. Zenker Fixative, Modified, Zinc Chloride (Part 1461)*
- *b. B-5 Fixative Modified, Zinc Chloride (Part 1015)*
- *c. 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. Prepare fresh Working Wolbach Giemsa Stain Solution; combine and mix well.
   - *a. Distilled water — 50 ml*
   - *b. Giemsa Stock Stain, Wolbach — 2 ml*
   - *c. Methanol (12236) — 2 ml*

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. Treat slides with Acid Alcohol 1% (10011) Solution for 5 minutes.
   - *a. See Procedure Note #3.*
5. Wash in running tap water for 5 minutes.
6. Stain in fresh Working Wolbach Giemsa Stain Solution (Step #2) for 60 minutes at room temperature.
   - *a. See Procedure Note #4.*
7. Differentiate each slide individually in Rosin 10%, Alcoholic until sections are purplish-pink; 5-10 dips. Check microscopically.
   - *a. Rinse in 100% ethyl alcohol to stop differentiation.*
8. Dehydrate in two changes of 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
   - *a. Do not use a 95% ethyl alcohol dehydration step.*

RESULTS:

Nuclei	Blue/violet
Cytoplasm	Pink/rose to lighter blue shades
Bacteria	Blue
Rickettsia, inclusions	Reddish-purple

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. Acid Alcohol treatment ensures an acid pH and improved staining.
4. For increased staining, stain in Working Giemsa Stain Solution at 60°C for 60 minutes. Additional differentiation may be required.
5. The color range of cells may vary depending on fixative and degree of differentiation.
6. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 119-120.
2. Sheehan, Dezna C, and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 156-157.
3. Wolbach, S. Burt, and John Todd. *The Etiology and Pathology of Typhus*. S.l.: Harvard University Press, 1922. 13-14.
4. Modifications developed by Newcomer Supply Laboratory.