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Part 1461
Revised August 2015

Zenker Fixative, Modified, Zinc Chloride – Technical Memo

SOLUTION:	**1 Liter**	**1 Gallon**
Zenker Fixative, Modified, Zinc Chloride	Part 1461A	Part 1461B

Additionally Needed:
Acetic Acid, Glacial, ACS — Part 10010

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

APPLICATION:

Newcomer Supply Zenker Fixative, Modified, Zinc Chloride provides an alternative to the classic Zenker fixative made with mercuric chloride. Environmental concerns and costly requirements for disposal of mercury-containing fixatives have led to the modification of the original Zenker formulation by substituting zinc chloride for mercuric chloride, maintaining the benefits of Zenker fixation with no concern for mercury pigment.

Zenker Fixative, Modified, Zinc Chloride, a specialty fixative that provides crisp nuclear detail, is frequently used for bone marrow clots and biopsies and also serves as a mordant for tissues to be stained with phosphotungstic acid hematoxylin (PTAH) procedures.

METHOD:

Fixation: Recommended fixation time is a minimum of 2 to 4 hours, depending upon tissue size and type.
   a. *See Procedure Note #1.*

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

FIXATION PROCEDURE:

1. Zenker Fixative, Modified, Zinc Chloride Working Solution:
   a. *Zenker Fixative, Modified, Zinc Chloride 38 ml*
   b. *Acetic Acid, Glacial, ACS 2 ml*
2. Combine and mix solutions directly before use. Fix tissue in this fresh working solution for 2 to 4 hours depending upon tissue size and type.
   a. *See Procedure Note #2.*
3. Hold tissue specimens in Zenker Fixative, Modified, Zinc Chloride Working Solution until ready to process or a maximum of 24 hours.
   a. *See Procedure Note #3.*
4. Wash fixed tissue thoroughly in running tap water for a minimum of 10 minutes to remove residual zinc chloride.
5. Place on tissue processor, starting with Formalin 10%, Phosphate Buffered (Part 1090) fixation step.
6. Post-fixation applications of Zenker Fixative, Modified, Zinc Chloride include the use of this working fixative as a mordant to intensify color reactions in phosphotungstic acid hematoxylin (PTAH) staining procedures. Refer to PTAH stain protocol for additional information.

PROCEDURE NOTES:

1. The acidic nature of Zenker Fixative, Modified, Zinc Chloride Working Solution is generally enough to sufficiently decalcify bone marrow cores without additional decalcification steps.
2. Zenker Fixative, Modified, Zinc Chloride should not be used for preservation of red blood cells.
3. Extended storage of tissue in Zenker Fixative, Modified, Zinc Chloride Working Solution is not recommended. After a maximum fixation time of 24 hours, rinse tissue in running tap water for a minimum of 10 minutes; transfer Zenker fixed wet tissue to Alcohol, Ethyl Denatured, 70% (Part 10844) or Formalin 10%, Phosphate Buffered for long-term storage purposes.
4. Due to the corrosive nature of zinc chloride do not discard Zenker Fixative, Modified, Zinc Chloride solutions down the drain.
5. Neutralize Zenker Fixative, Modified, Zinc Chloride solutions with magnesium hydroxide/oxide, sodium carbonate or sodium bicarbonate to precipitate zinc at pH 7.0-8.0. Separate solids from liquid and dispose of according to local and state environmental regulations.
   a. *Approximately 100 grams of sodium bicarbonate will neutralize/precipitate zinc from 1 liter of Zenker Fixative, Modified, Zinc Chloride.*

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 69, 148.
2. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 16-17, 20-21, 207-208.
3. Dapson, Janet Crookham, and Richard Dapson. *Hazardous Materials in the Histopathology Laboratory: Regulations, Risks, Handling, and Disposal*. 4th ed. Battle Creek, MI: Anatech, 2005. 148, 279.
4. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 49.
5. Modifications developed by Newcomer Supply Laboratory.
