Designation: E1200 – 87 (Reapproved 2012)

Standard Practice for Preserving Zooplankton Samples[1]

This standard is issued under the fixed designation E1200; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice describes the proper procedures for preserving zooplankton samples with either formaldehyde, ethanol, glutaraldehyde, Lugol's iodine solution, or vinegar (acetic acid).

1.2 *This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

3. Summary of Practice

3.1 A zooplankton sample collected with either a qualitative or quantitative sampler is preserved with formaldehyde, ethanol, glutaraldehyde, Lugol's iodine solution, or 25 % vinegar or 3 % acetic acid, as dictated by needs of the study. The preservatives are listed in order of preference.

4. Significance and Use

4.1 Calcium Carbonate ($CaCO_3$) buffered formalin (3 to 5 %) can be used as a permanent preservative for zooplankton. Lugol's iodine solution can be used to preserve zooplankton for up to one year. Thirty percent ethanol, 30 % glutaraldehyde, or 25 % vinegar (can use 3 % acidic acid solution) can be used for more temporary storage and preservation of zooplankton samples. A 25 % vinegar solution is preferred to preserve soft-bodied planktonic coelenterates.

5. Reagents

5.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[3]

5.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type II of Specification D1193.

5.3 *Formaldehyde Solution*—37 to 40 % aqueous.

5.4 *Ethanol* (95 %)—Dilute with water 30 mL of ethanol to 100 mL.

5.5 *Glutaraldehyde*—Dilute with water 30 mL of glutaraldehyde to 100 mL.

5.6 *Lugol's Iodine Solution*—Dissolve 60 g of potassium iodide and 40 g of iodine crystals in 1000 mL of water.

5.7 *Vinegar Solution*—Dilute 25 or 250 mL of vinegar to 100 or 1000 mL of water, respectively. An alternative is to dilute with water 3 mL of 100 % acetic acid to 100 mL.

5.8 *Detergent Solution* (20 %) —Dilute with water 20 mL of household liquid detergent to 100 mL.

6. Procedure

6.1 If the sample is to be examined within 2 to 3 h after collection, no special treatment is necessary. A zooplankton sample may be maintained longer if refrigerated or iced at 2 to 3°C. For extended storage, beyond 72 h, preservation is required. There are numerous preservatives for zooplankton. The formaldehyde solution is the most commonly used.

6.2 To use the formaldehyde solution, to each 1000 mL of sample add 30 mL of 37 to 40 % aqueous formaldehyde solution (100 % formalin) and 5 mL of 20 % liquid detergent solution.[4]

6.3 For soft-bodied forms like planktonic coelenterates, 25 % vinegar solution or 3 % acetic acid is a more suitable preservative.

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[1] This practice is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.24 on Water Microbiology.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, D.C. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Analar Standards for Laboratory U.K. Chemicals," BDH Ltd., Poole, Dorset, and the "United States Pharmacopeia."

[4] Greeson, P. E., Ehlke, T. A., Irwin, G. A., Lium, B. W., and Slack, K. V., "Methods for Collection and Analysis of Aquatic Biological and Microbiological Sampler," *U.S. Geological Survey, Technology of Water-Resources Investigations*, Book 5, Chapter A4, p. 332.

6.4 Since many chemicals are oxidized by excess light, store zooplankton samples in the dark or closed boxes.