



Standard Practice for Preserving Phytoplankton Samples¹

This standard is issued under the fixed designation D 4137; 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 procedure for preserving phytoplankton samples with either formaldehyde or Lugol's solution

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 Document

2.1 *ASTM Standards:*²

D 1193 Specification for Reagent Water

3. Summary of Practice

3.1 A phytoplankton sample collected with either a qualitative or quantitative sampler is preserved with either formaldehyde solution or Lugol's solution.

4. Significance and Use

4.1 Formaldehyde solution can be used as a permanent preservative of algae. Lugol's solution can be used to preserve algae for up to one year. Lugol's is a more suitable preservative for delicate algae such as naked flagellates.

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 conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.³ Other grades may be used,

provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

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 D 1193.

5.3 *Cupric Sulfate Solution* (saturated)—Dissolve 21 g of CuSO₄ in 100 mL of water.

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

5.5 *Formaldehyde Solution*, 37 to 40 % aqueous.

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

6. Procedure

6.1 If the sample is to be examined within 2 to 3 h after collection, no special treatment is necessary. A phytoplankton sample may be maintained for 24 h at 2 to 3°C, but for extended storage, preservation is required. There are numerous preservatives for phytoplankton of which formaldehyde solution and Lugol's solution are the most commonly used.

6.2 To use formaldehyde solution, to each 1000 mL of sample, add 30 mL of 37 to 40 % aqueous formaldehyde solution (100 % formalin), 5 mL of 20 % liquid detergent solution, and 1 mL of saturated cupric sulfate solution. This preservative maintains cell coloration and is effective indefinitely.⁴

6.3 For delicate algal forms such as naked flagellates, Lugol's solution is a more suitable preservative. Add 1 mL Lugol's solution to each 100 mL of sample and store in the dark. Lugol's solution has been found to be effective for at least 1 year.⁵ It facilitates sedimentation of cells and maintains fragile cell structures.

¹ This practice is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.01 on Aquatic Assessment and Toxicology.

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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, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, NY, 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 Samples," *U.S. Geology Survey, Technology of Water-Resources Investigations*, Book 5, Chapter A4, 1977, 332 pp.

⁵ Weber, C. I., "The Preservation of Phytoplankton Grab Samples," *American Microscopical Society Transactions*, Vol 87, pp. 70–81.

