

Macroanalytical Procedures Manual (MPM)

III. Reagents for Macroanalytical Methods

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This chapter lists some of the reagents which are of special significance to the methods contained in Chapter V: Macroanalytical Methods. For each listed reagent, special notations on applications and/or preparation are highlighted. Except where noted, technical grade reagents are acceptable.

1. **Alcohol** -- Use 95% commercial ethanol unless otherwise specified. Make all dilutions by volume.
2. **Chloral Hydrate** -- Used for clearing plant and insect materials. Prepare solution from equal parts of chloral hydrate and water or use Hertwig's solution (see below).
3. **Crystal Violet** -- Used to detect mold in pickle relish and stain vinegar eels. Either a water-based or alcohol-based solution may be used. To prepare the water-based solution, dissolve 5 g dye (Color Index 681) in 100 mL water. To prepare the alcohol-based solution, dissolve 10 g dye (Color Index 42555) in 100 mL alcohol.
4. **Detergent Solution** -- Prepare aqueous sodium lauryl sulfate solution as required.
5. **EDTA (Ethylenediaminetetraacetic acid)** -- Used to clean sieves.
6. **FAA (Formalin – Alcohol – Acetic Acid)** -- Used in fixation of parasites in fish flesh. Consists of a mixture of formalin, alcohol, and acetic acid. Combine 10

parts 37-40% formaldehyde, 70 parts 95% ethanol, 15 parts water, and 5 parts acetic acid.

7. **Glycerin Alcohol** -- 1:1 (v/v) glycerin and ethyl alcohol are added as needed to the petri dish before the filter paper is placed in the dish to keep the sample material moist and free of mold.
8. **Glycerin Jelly** -- Used as a mounting medium because fragment specimens can be transferred directly from aqueous solutions (e.g., glycerin alcohol) to the medium. The formulation for glycerin jelly is 10 g of gelatin, 70 mL of glycerin, 60 mL of water and 1 g of phenol. Pour the gelatin in cold water to soak, then heat over a steam bath to completely dissolve the gelatin. Mix in the glycerin and phenol while still hot and then cool to room temperature. The cooled mixture should have the consistency of jelly and may be stored at room temperature in a tightly capped container for years. (USFDA, 1981) Commercial varieties are sold by biological supply houses. Other varieties, such as phenol-free glycerin jelly, may be considered.
9. **Heptane** -- Use commercial *n*-heptane containing less than 8% toluene.
10. **Hertwig's Solution** -- Use only for temporary mounts, not for permanent slides. Also known as acidified chloral hydrate-glycerol solution. Dissolve 45 g chloral hydrate crystals in 25 mL HCl (1 + 8) and 10 mL glycerol. Useful for clearing plant and insect materials. Example of a method that uses this reagent: examination for rot in potato chips.
11. **Hoyer's Medium** -- Especially useful in mounting mites. Dissolve 30 g of gum arabic (crystals) in 50 mL distilled H₂O at room temperature. Add 200 g Chloral hydrate and leave the mixture for a day or two until all solids have dissolved. Add 20 mL glycerin. Filter through glass wool. Store in a bottle with a glass stopper (Krantz, 1978).
12. **Hydrogen Peroxide** -- Used to demonstrate catalase activity in raisins. Use 5% solution.
13. **Iodine in Potassium Iodide (I-KI)** -- Used to detect foreign starch grains in peanut butter. Dissolve 0.5 g I and 1.5 g KI in 25 mL water.
14. **Isopropanol** -- Use 100% isopropanol.
15. **Lactophenol Cotton Blue Solution** -- Used to stain mold and yeast in samples.
16. **Lugol's Iodine Solution** -- Refer to Bacteriological Analytical Manual (BAM) ([Link](#)).

17. **Pancreatin Solution** -- Used to clean sieves. Use USP or soluble pancreatin kept refrigerated at 10°C. Use fresh solution. Mix at rate of 5 g/100 mL water at less than or equal to 40°C. Stir with malted milk unit or blender 10 min, or let stand 30 min with frequent shaking. Centrifuge at 1500 rpm and filter supernatant through Whatman No. 8 filter paper, or equivalent. Alternatively, filter through cotton pads 10-13 cm thick and then through rapid No. 8 filter paper in Hirsch funnel with vacuum filtration.
18. **Petroleum Ether** -- Used to defat peanut butter and potato chips.
19. **Sodium Phosphate Solution** -- Used to wash olive oil away from sample. Use technical grade Na₃PO₄. Prepare 5% solution.
20. **Stabilizer Solutions** -- 0.5% Na carboxymethylcellulose preferred (Hercules Inc., Cellulose and Protein Products Dept. 1313 Market St., Wilmington, DE 19899). Place 500 mL boiling H₂O in high-speed blender. With blender running, add 2.5 g Cellulose Gum and 10 mL 37% formaldehyde solution w/w, and blend about 1 min. Alternatives: 3-5% pectin or 1% algin. Add required amount of stabilizer directly to H₂O while agitating in high-speed blender. Treat solution with vacuum or heat to remove air bubbles. Add 2 mL formaldehyde solution/100 mL solution as preservative. (If blender is not available, mix dry stabilizer with alcohol to facilitate incorporation with H₂O.) Adjust to pH 7.0-7.5. Filter solution through 8 µm pore size membrane filter (Millipore No. SCWP-047-00, or equivalent) using suitable vacuum filtration apparatus (Millipore No. XX15-047-00, or equivalent).
21. **Tween 80 (Polysorbate 80)** -- A solution of Tween 80 mixed with 60% ethanol can be used to make a temporary (ca. 24 hr.) slide mount of hairs. Refer to LIB 2016 (See Appendix C).
22. **Water** -- Unless otherwise specified, tap water is to be used in all procedures.

References Cited in Section:

Krantz, G. W. (1978). *A Manual of Acarology*, 2nd ed. (p. 509) Oregon University Press: Corvallis, Oregon.

USFDA (1981). *FDA Technical Bulletin No. 1 Principles of Food Analysis for Filth, Decomposition, and Foreign Matter*, 2nd ed.

USFDA (1998). *BAM R40: Lugol's Iodine Solution*. Retrieved from <https://www.fda.gov/food/laboratory-methods-food/bam-r40-lugols-iodine-solution>

Additional Information:

Barbosa, P., D.L. Berry & C.S. Kary (2015). *Insect Histology. Practical laboratory Techniques*. Wiley Blackwell, Oxford, UK. Pp. 348.

Martin, J. E. H. (1977). *The Insects and Arachnids of Canada, Part 1: Collecting, Preparing, and Preserving Insects, Mites, and Spiders*. Publication # 1643. Canadian Government: Quebec, Canada. Pp.182.

Revision History

Version No.	Purpose of change	Date
V0	New process	1984
V1	Electronic version	1998
V2	1. Spelled out EDTA and FAA. 2. Added the following reagents: glycerin alcohol, glycerin jelly, Hoyer's medium, lactophenol cotton blue solution, and Lugol's iodine solution. 3. Added references and additional information source.	2021