NATIONAL PARK SERVICE

Conserve O Gram

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Re-Use Of Ethanol In Processing Biological Specimens

It is never desirable to re-use ethanol solutions in the processing of fluid-preserved specimens. The ethanol that is used with any specimen becomes contaminated with fats, fatty acids, and amino acids. Other possible contaminants include traces of formaldehyde or other fixatives, pigments, and the decomposition products from labels and containers. When collections resources do not permit the use of fresh ethanol solutions, the following guidelines for re-use should be applied.

Note: The method given here is designed for situations when ethanol must be re-used during initial processing. It should not be applied to ethanol solutions used for long-term storage.

Ethanol

Ethanol (ethyl alcohol) has been used as a preservative for specimens in fluid for centuries. The alcohol slows the rate of decay of biological material by killing bacteria.

There are two forms of ethanol available commercially in North America:

- **Denatured ethanol.** Various concentrations of ethanol and water, with additives designed to make it non-potable. These denaturants include: aviation fuel, purgatives, various organic solvents, mercury salts, thymol, etc.
- **Potable alcohol.** Various concentrations of undenatured ethanol and water, usually 95% or 99% ethanol by volume.

Note: The sale of undenatured (potable) alcohol is regulated in North America. Special permits are required to purchase large quantities. Small quantities of 95% ethanol may be purchased from liquor stores as pure grain alcohol.

Ethanol Concentration and Specimen Processing

Only solutions of undenatured ethanol in deionized or distilled water should be used for specimen processing.

The concentration of an ethanol solution is usually expressed as either proof or volume percent. The proof system is used for taxation of potable alcohol. Proof is approximately twice the volume percent measured at a specific temperature. Volume percent (v/v) is the percent of anhydrous ethanol (theoretically 100% ethanol) in a given volume of solution at a given temperature.

The concentration of ethanol used to store biological specimens may vary with the type of specimen, the method of fixation or other treatments the specimen has undergone. Solutions of 70-80% v/v ethanol in water are most common. Concentrations from 50-80% v/v are the most effective bactericides. Effectiveness generally decreases above or below that range.

Processing specimens prior to long-term storage involves the use of solutions that gradually increase in ethanol concentration. This helps

remove water from the tissues and/or excess fixative. Starting with a solution of lower ethanol concentration helps minimize shrinkage that can result from the loss of water. The concentrations generally begin at 1/3 the strength of the final storage solution depending on the type of specimen. For example: 25% and 50% volume percent solutions might be used to stage the transfer to 75% ethanol. See *Conserve O Gram* 11/3 for a description of staging specimens through alcohol.

The re-use of ethanol in the staging process can be adopted as a cost-saving measure. While this is never ideal, the following protocol can be used to help remove some contaminants from ethanol prior to re-use.

Procedure

For your safety:

- All steps in the procedure should be performed in a fume hood or in a well-ventilated outdoor area.
- Wear a rubber apron, heavy nitrile gloves, and safety glasses designed for protection against splashes (no side vents).
- Work with small quantities of solution (less than 4 liters at a time).
- Do not decant alcohol from large containers unless the container is appropriately grounded to prevent an explosion.
- Keep an appropriate spill kit nearby for cleanup of any spills.
- See *Conserve O Gram* 2/18 for more on the safe handling of fluid preserved specimens.

Steps for Preparing Used Ethanol for Re-Use:

1. Pour the used alcohol into a straight-sided container, seal well, and place in a refrigerator or standard freezer overnight. This will solidify some fats, cause some contaminants to precipitate, and will polymerize residual formaldehyde into milky strings.
2. Filter the cold alcohol through Whatman #1 filter paper into a clean container.
3. Freeze or refrigerate overnight again and filter a second time.
4. Remove fluid from cold storage and allow it to return to room temperature.
5. Mix well.
6. Determine the volume percent concentration of the alcohol:
    - A *digital density meter* provides the most accurate measurements. Use the Ethanol Computer Program from the Canadian Conservation Institute to convert density meter measurements to concentrations.
    - An *alcohol hydrometer* also measures concentrations approximately. Make sure the ambient and fluid temperatures are approximately 20°C (68°F).

7. Correct the concentration to the desired level using the formula developed by Sendall and Hughes:

$$y = \frac{(z-a)x}{(b-z)}$$

z = desired concentration
a = measured concentration (volume percent) of the filtered fluid
x = height of fluid in container (cm or in)
b = concentration (volume percent) of the fresh ethanol to be added
y = amount of ethanol to add (cm or in)

8. Verify the new concentration following steps 5 and 6.

9. Label all specimens that have been subject to re-used alcohol in processing so that researchers and other collection users are aware of potential contamination.

Sources of Supplies and Equipment

Ethanol Computer Program:

Canadian Conservation Institute
1030 Innes Road
Ottawa, ON K1A 0M5
Canada
(613) 998-3721
(613) 998-4721 (Fax)
www.cci-icc.gc.ca

Whatman No. 1 Filter Paper, Digital Density Meters and Alcohol Hydrometers:

Fisher Scientific
(800) 766-7000
www.fisherscientific.com

Safety Goggles, Spill Kits, Rubber Aprons and Heavy Nitrile Gloves:

Lab Safety Supply, Inc.
401 S. Wright Road
PO Box 1368
Janesville, WI 53546
(800) 356-0783
(800) 543-9910 (Fax)
www.labsafety.com

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