

Cryobiology Lab Modules 4A, 4B, 4C on Fertility Preservation – Teacher Notes

Equipment:

Small styrofoam coolers – A central monitored area should be set up away from the student lab desks where their water and glycerol solutions are prepared. When ready for the liquid nitrogen, student lab group should bring their handouts, pencils/pens, small test tube holder with the 5 labeled solutions of water and glycerol, test tube holders, and timer to that area. When all 5 solutions have been held in the liquid nitrogen for 30 seconds, the group should return to their desks – to avoid congestion around the liquid nitrogen.

Liquid nitrogen – Obtain from local college, university, or research lab – Since gaseous nitrogen turns to liquid nitrogen at -196°C , use Extreme Caution to avoid contact with skin and eyes.

Cryo gloves, elbow length, water resistant - Obtain from Fisher Scientific at:

<https://www.fishersci.com/us/en/catalog/search/products?keyword=cryo+gloves&nav=>

Caution: Students should be cautioned not to let the cryo glove fingertips touch the liquid nitrogen as they hold the test tube holder with the test tube lowered in the liquid nitrogen. The gloves are meant to act as a barrier; however, the fingers in the cryo gloves can become very cold if held in the liquid nitrogen for even a short time. The test tube holder should be held by the end (to avoid unclamping the test tube as the student holds it) and the test tube should be lowered with the gloved hand gently supported by the rim of the styrofoam cooler (rather than the gloved hand being held directly over the liquid nitrogen as the test tube is lowered).

Central clock with a second hand or individual timers.

At each station of 2-4 students:

In a small test tube rack, 5 small 10 mL test tubes,

1-5mL needleless syringe labeled “water” and 1- 5 mL needleless syringe labeled “glycerol”,

2 sharpies,

1-3 mL graduated plastic dropper with bulb, with which to mix solutions.

In a large test tube rack, 1-50 mL capped vial labeled and containing “water” and 1-50 mL capped vial labeled and containing “glycerol”



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At each station of 2-4 students, continued:

2 metal small test tube holders

Pencil or pen for each student

Goggles and nitrile gloves for each student

Paper towels on which to lay glycerol laden syringes, etc. (Glycerol is slippery and very messy to clean off of surfaces.)

Data and Analysis:

Percent Glycerol	0%	20%	40%	60%	80%
Glycerol (ml)	0.0	0.6	1.2	1.8	2.4
Water (ml)	3.0	2.4	1.8	1.2	0.6
Total Vol (ml)	3.0	3.0	3.0	3.0	3.0

Note: Some students have math anxiety when completing this chart, so a sample calculation is often helpful. $20\% = 20/100=0.20$

$$0.20 \times 3.0 \text{ ml total volume} = 0.6 \text{ ml}$$

$$\text{Total volume } 3.0 \text{ ml} - 0.6 \text{ ml} = 2.4 \text{ ml}$$

Glycerol	0%	20%	40%	60%	80%
Evidence of Ice? Yes or No	Yes	Yes	Yes	No	No
Vitrified? Yes or No	No	No	No	Yes	Yes

Answers to questions:

1. You can tell that a solution is vitrified successfully if there is no presence of ice.
2. The percentage of glycerol needed for successful vitrification in the lab has been found to be 53%. Higher concentrations work equally well in terms of vitrification but the idea is to use the least amount of glycerol to avoid toxicity issues.
3. An example of cryopreservation in nature includes the Arctic beetle and the Wood Frog.
4. The follicles, oocytes, and stromal tissues after vitrification look very similar to the freshly obtained tissues and function in a very similar way. The tissues after a slow freeze show more disruption of the tissue structure due to ice formation.



5. Cryopreservation is important for fertility preservation especially in cancer survivors under 40 years of age. If their reproductive tissues can be vitrified before beginning cancer treatments, including radiation treatment and some types of chemotherapy, the cancer survivor's chance of having an offspring would be greatly increased. If the cancer treatments occur prior to tissue preservation, the cancer survivor will have a high likelihood of experiencing the unfortunate side effect of being infertile.

