# Cryopreservation Lab Dry Lab

## OREGON NATIONAL PRIMATE RESEARCH CENTER/OHSU

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# Cryopreservation Equipment

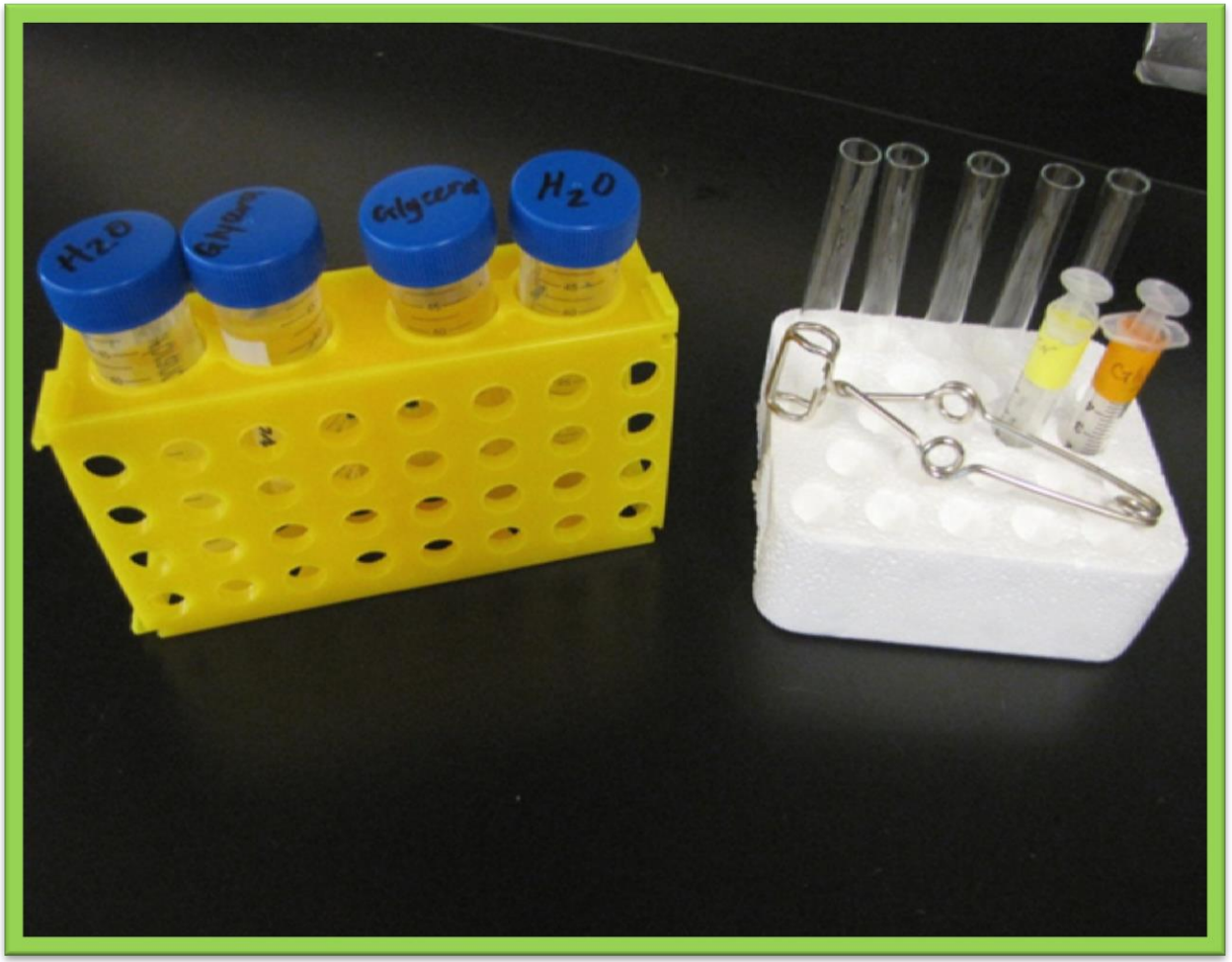


Photo: Diana Gordon, MAT, MPH, ONPRC

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# Step 1: Calculate the volume of glycerol and water needed for each solution and then label the test tubes.

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# Step 2: Using a syringe, make up solutions of 0, 20, 40, 60, and 80% glycerol in water for a total volume of 3 ml.

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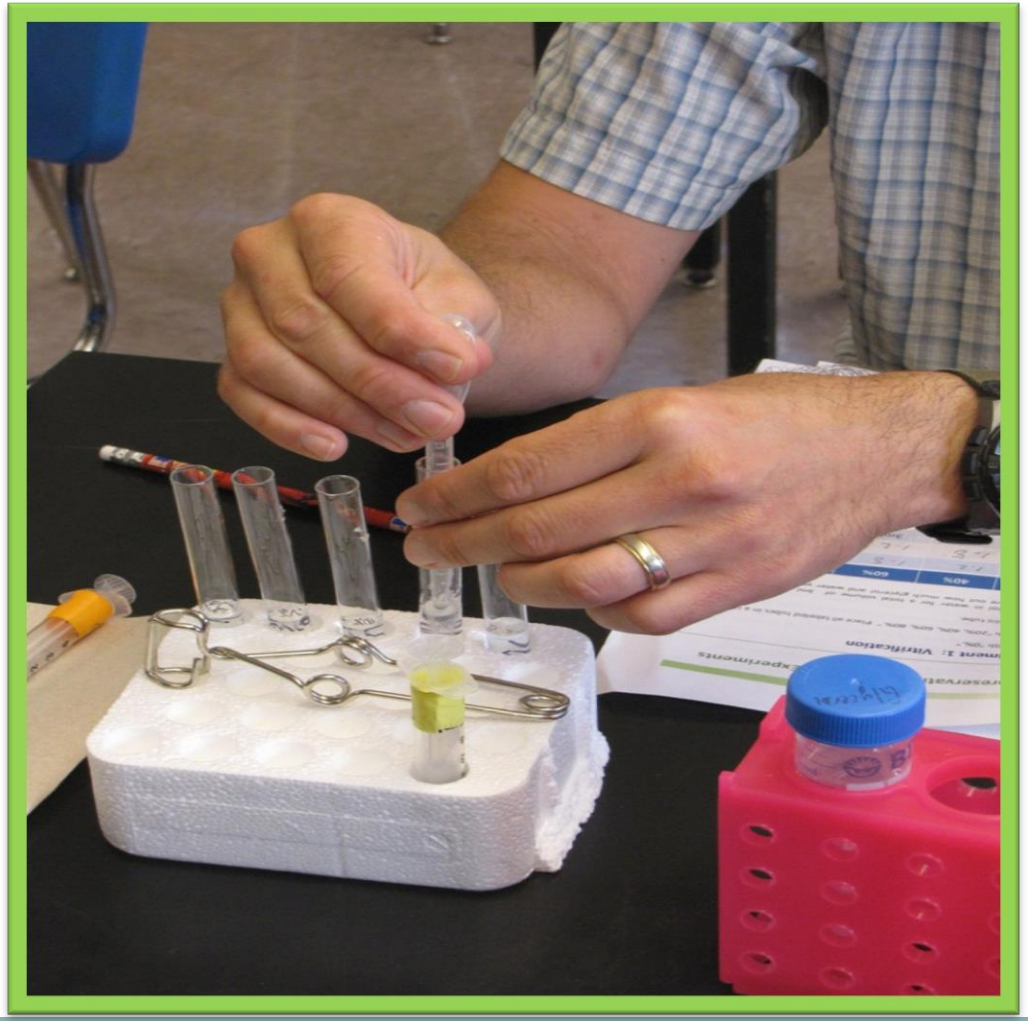
# Step 3: Mix each solution with a transfer pipette.



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# Step 4: Wear safety goggles & thermal gloves; start with the lowest % glycerol & submerge one tube at a time in liquid nitrogen for 30 seconds.


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# Step 5: At 30 seconds, carefully take the tube out of the liquid nitrogen and observe the solution.

Record your observations and results in your data table.

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

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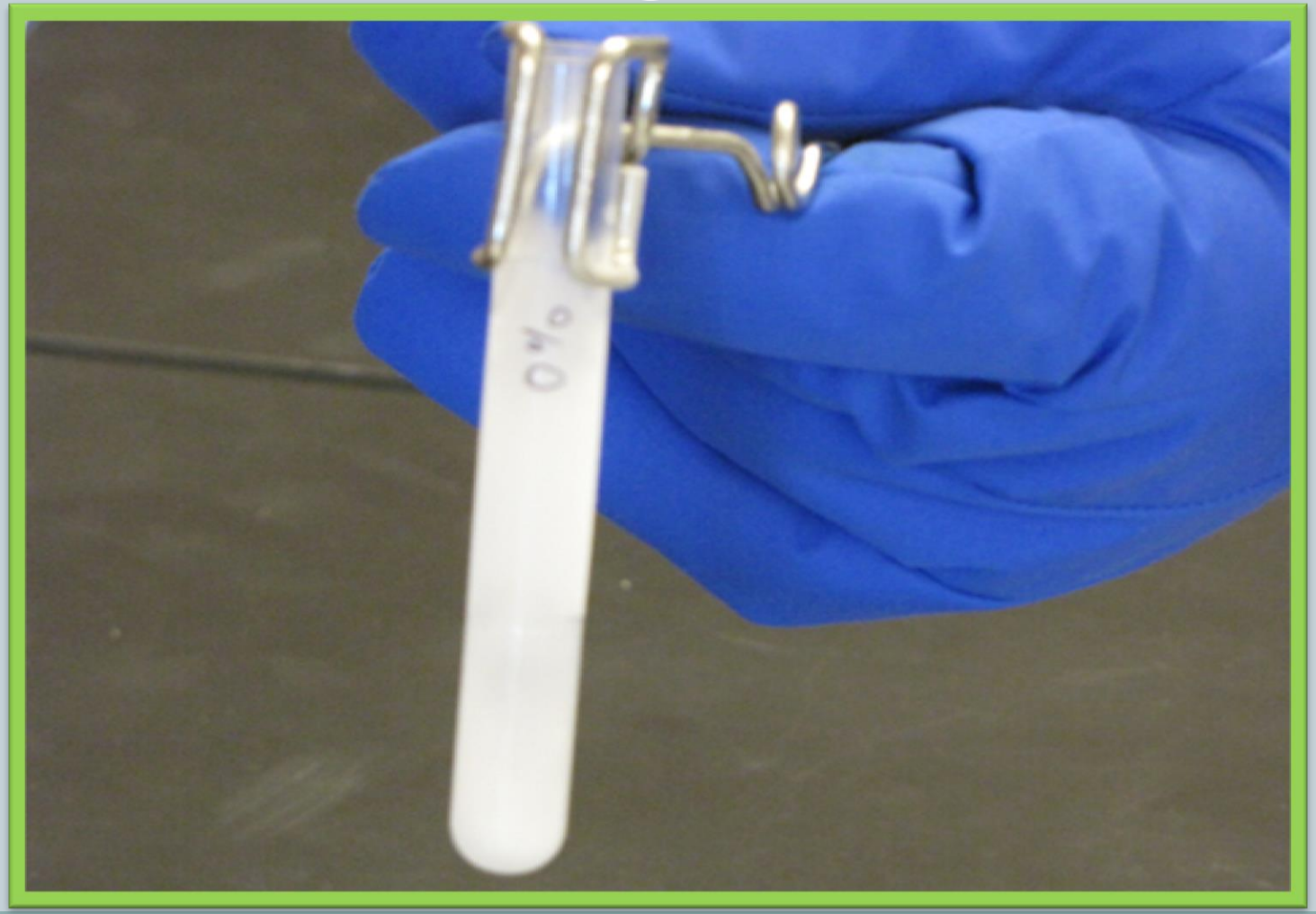
# 0% Glycerol

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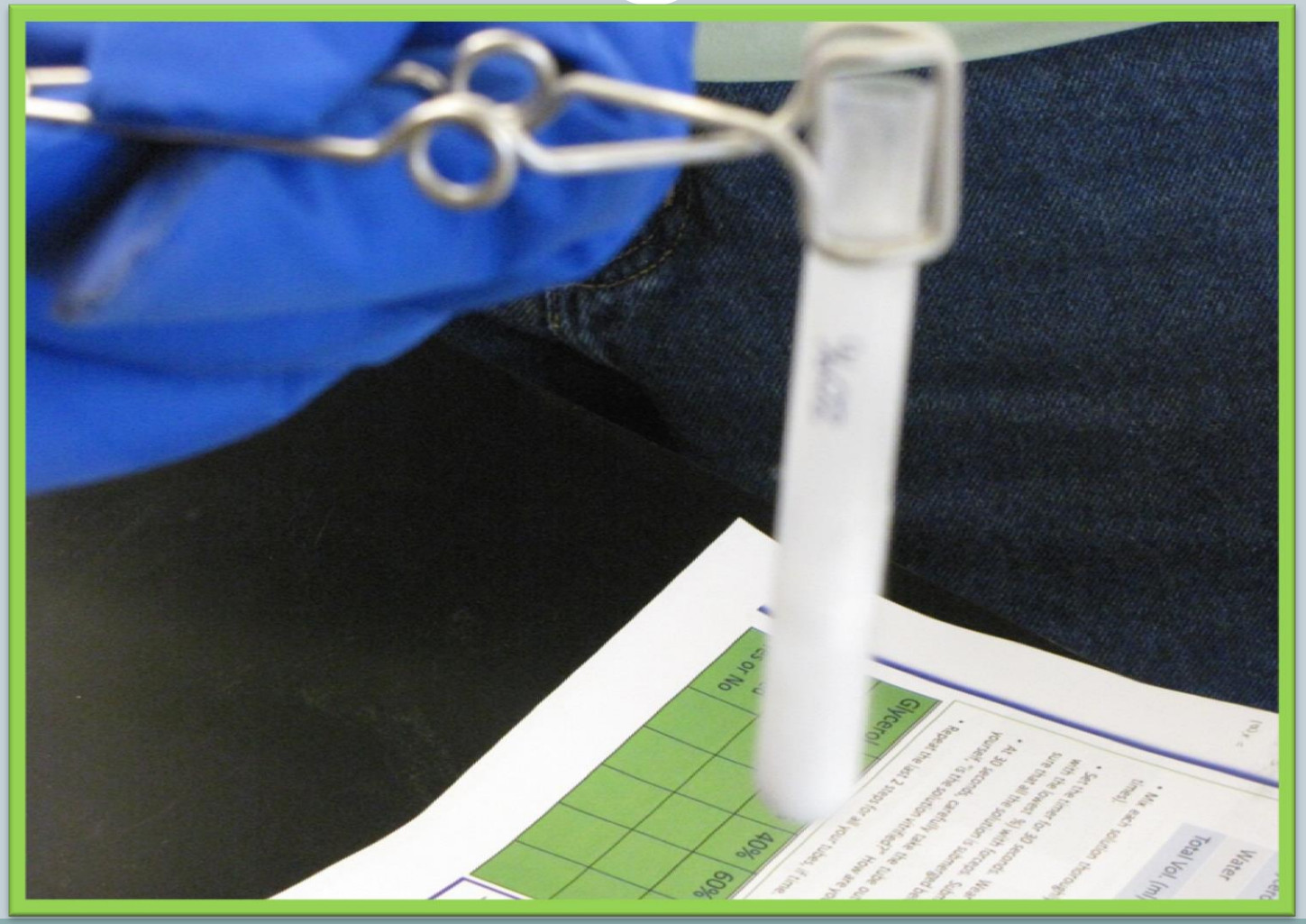
# 20% Glycerol

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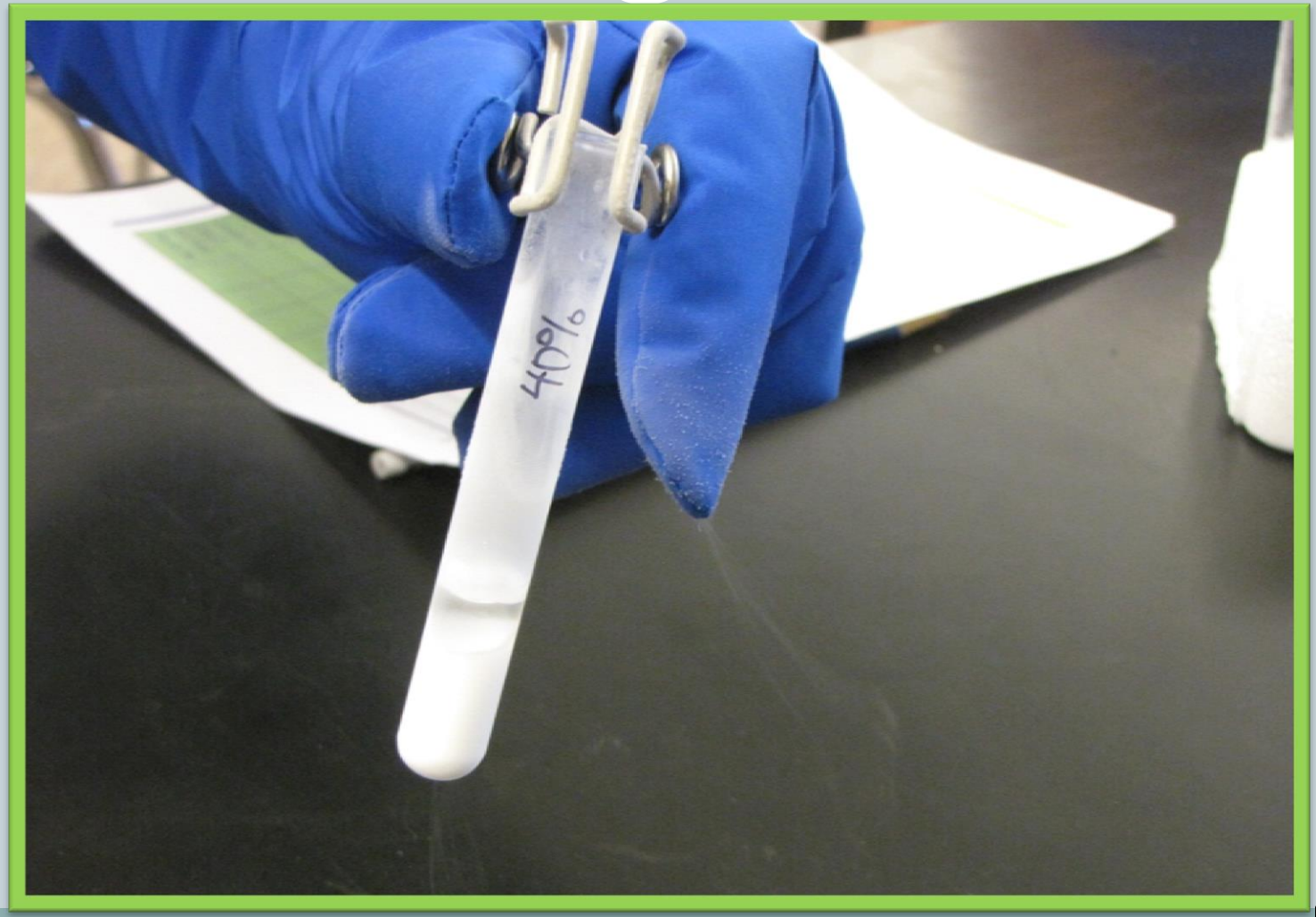
# 40% Glycerol

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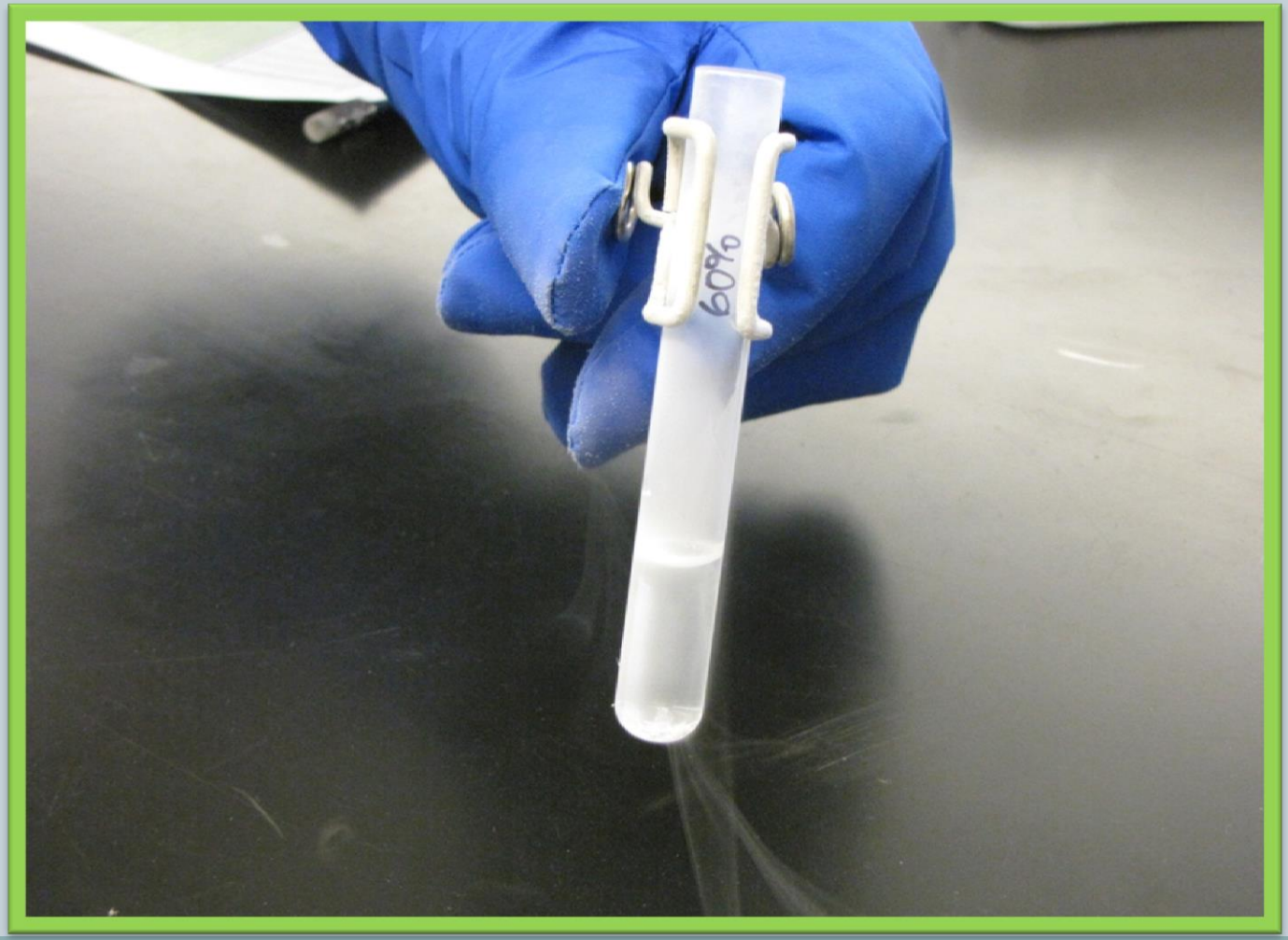
# 60% Glycerol

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# 80% Glycerol

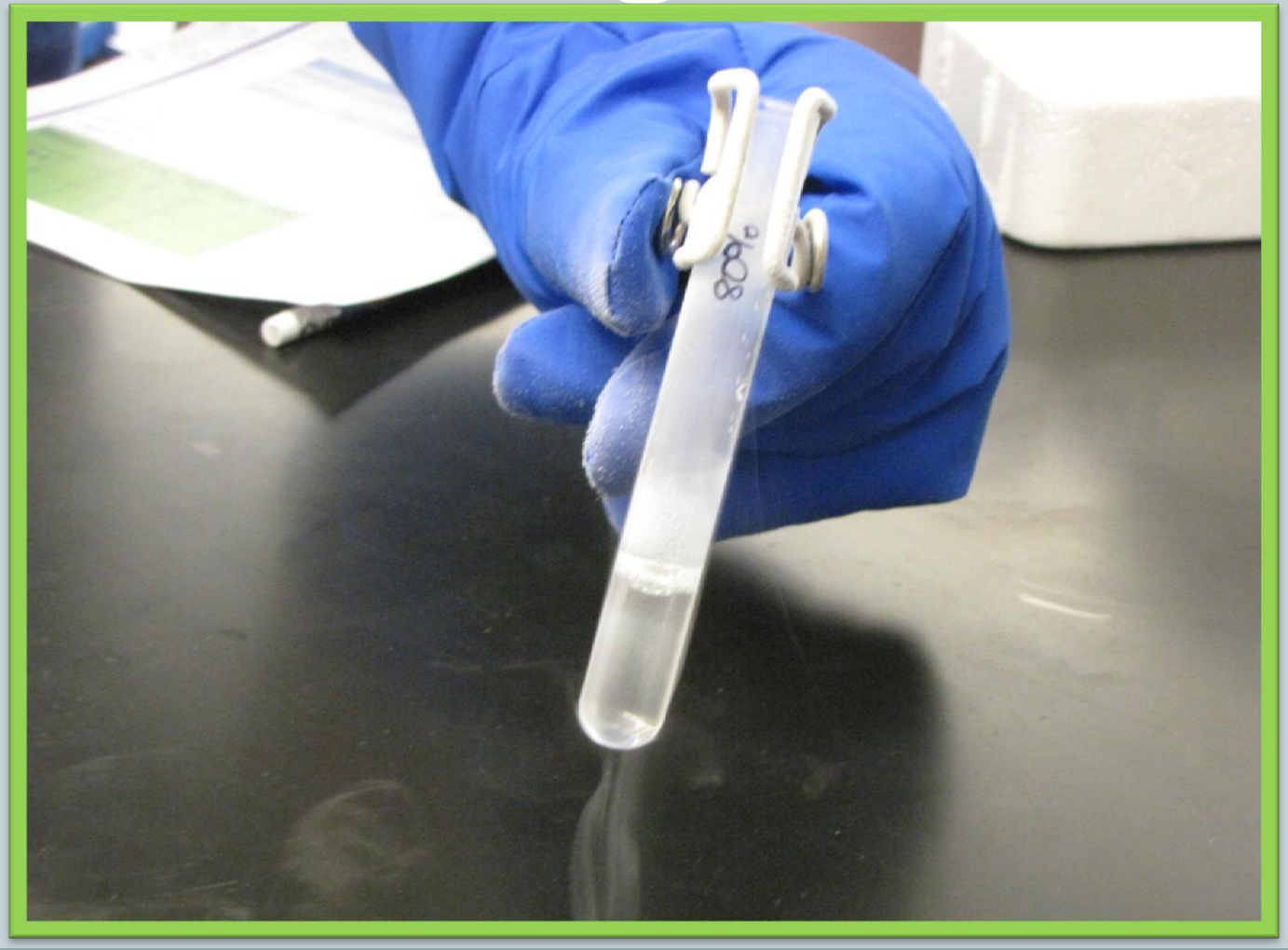


Photo: Diana Gordon, MAT, MPH, ONPRC

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# Step 6: Answer Questions

1. How can you tell that a solution is vitrified successfully?

2. What is the percentage of glycerol needed for successful vitrification?

3. Give an example of cryopreservation in nature.

4. Why is cryopreservation important in fertility preservation?

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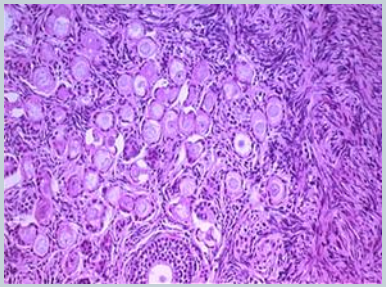
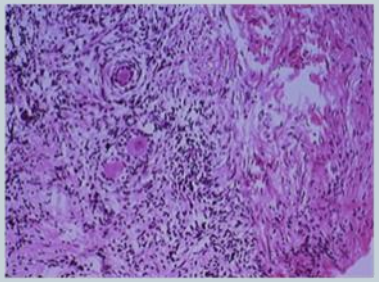
# H & E Staining of Ovarian Tissue

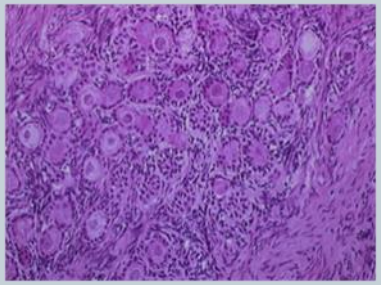
5. Describe the difference in the morphology of cryopreserved ovarian cortex compared to fresh ovarian cortex – what do you notice about follicles, oocytes, stromal tissue?



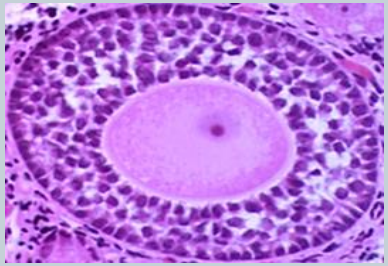
Fresh Tissue

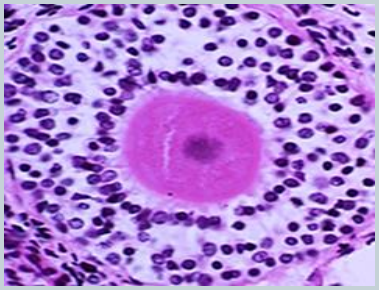


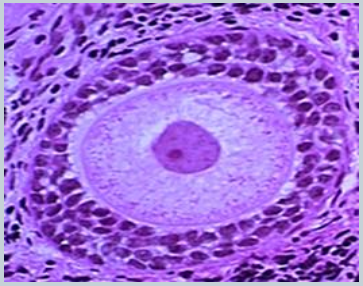
Tissue After Slow Freeze



Tissue After Vitrification







Top photos – low magnification of ovarian tissue;
Lower photos – high magnification of follicles

Photos: Mary Zelinski, PhD, ONPRC

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