Muscle Biopsy - practical issues

- choose an affected, but not end stage, muscle for biopsy; biopsy **before** treating
- avoid EMG needle sites and other sites of trauma
- biopsy belly of muscle, not tendon insertion
- biopsy deep to the fascia unless fasciitis is a diagnostic consideration
- tissue requires special handling
  - biopsy should be taken in isometric clamp, but a clamp is not required
  - transport to lab fresh (moist, but not wet)
  - a portion will be **rapidly** frozen in isopentane (2-methyl-butane) cooled to -160 degrees C

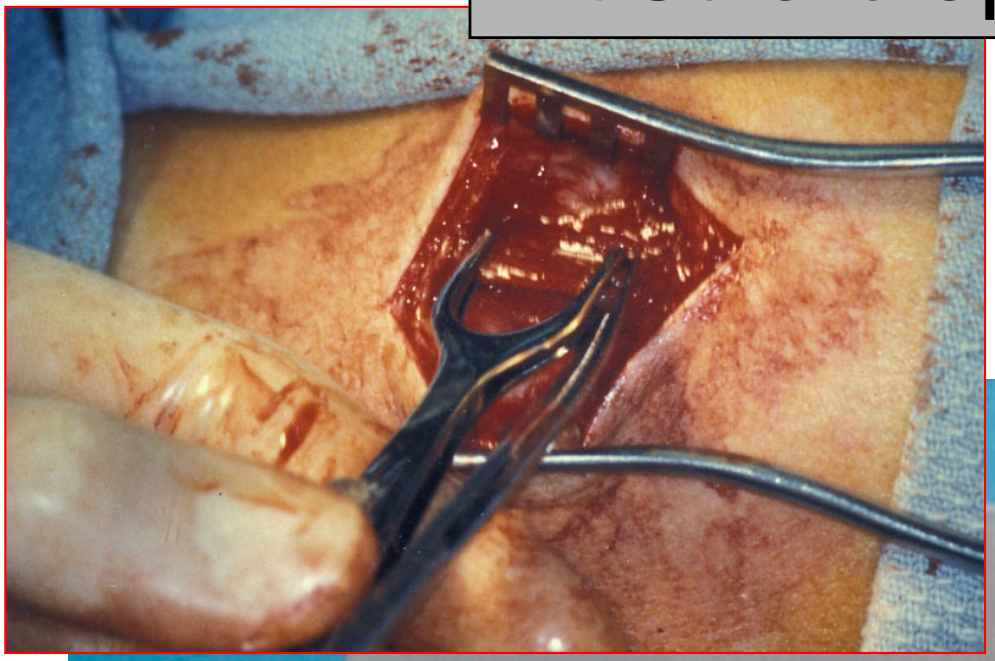
types of muscle biopsies

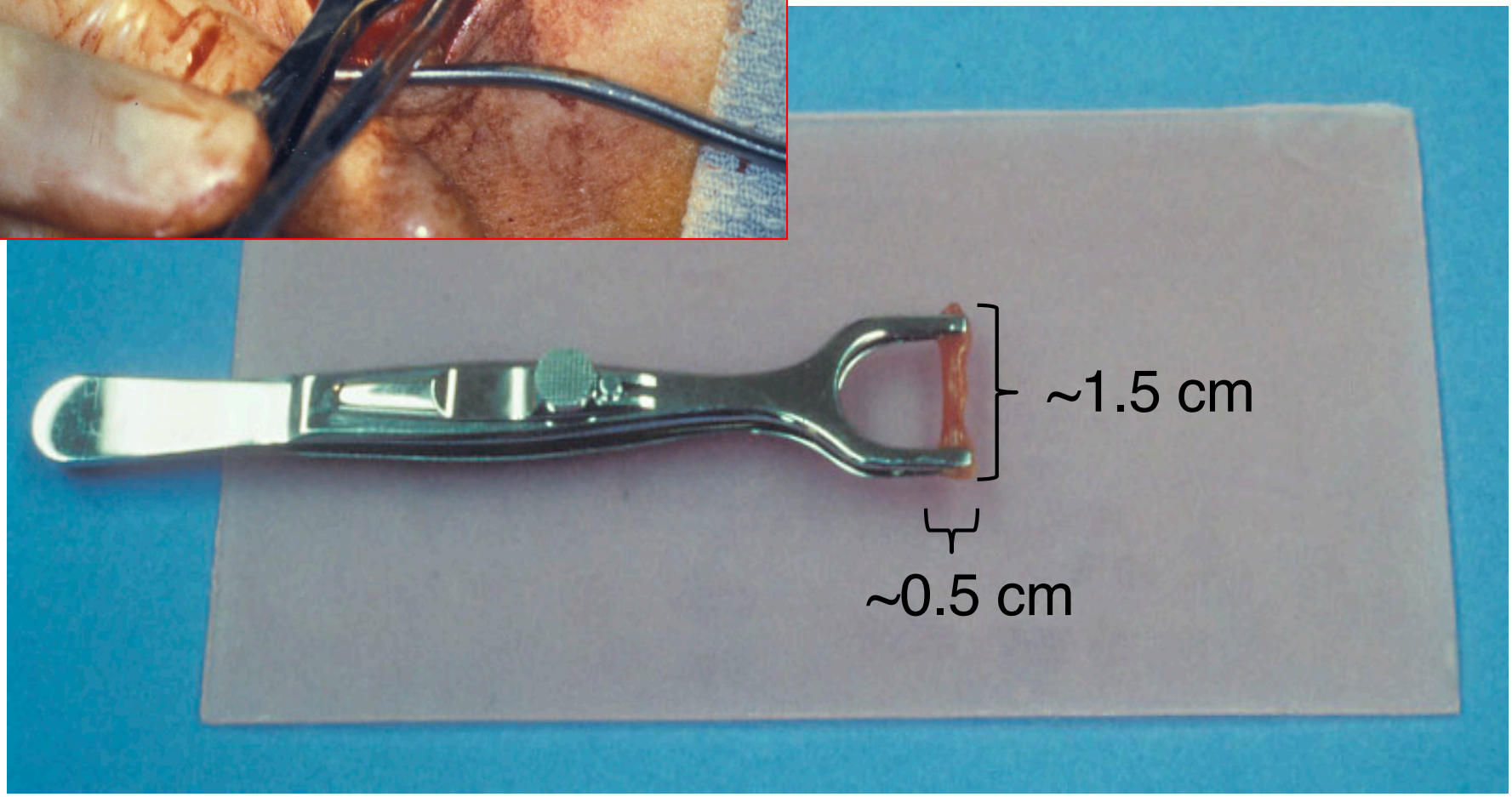
- needle biopsy
  - may be less painful
  - may not require general anesthesia
  - smaller amount of muscle may limit testing
  - requires more expertise to obtain good biopsy
- open biopsy
  - may be more painful
  - may require general anesthesia
  - larger amount of muscle allows for broader range of testing
  - muscle clamp technique easy to teach surgeons unfamiliar with muscle biopsy

distribute biopsy tissue

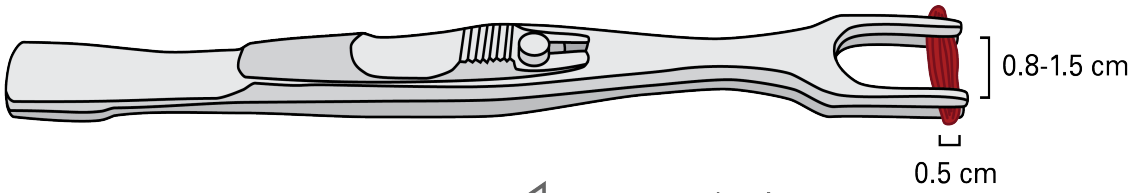
- frozen tissue
  - routine histology and enzyme histochemistry
  - immunostains
  - biochemistry (e.g. western blots)
  - DNA or mRNA studies
- formalin-fixed tissue
  - routine histology
  - special stains (e.g. Congo red and IHC)
- glutaraldehyde-fixed tissue
  - plastic section light microscopy (“thicks”)
  - electron microscopy (“thins”)

muscle biopsy using a clamp





mount muscle for cross sections and freeze in isopentane at approximately -160° C

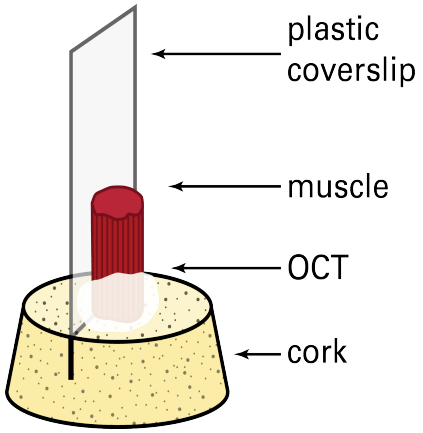


First priority:

1) Cut biopsy from between forks of clamp
2) Trim 0.1 cm diameter strip for glutaraldehyde
3) Mount remainder on cork
4) Freeze in isopentane at -160° C

If there is sufficient tissue:

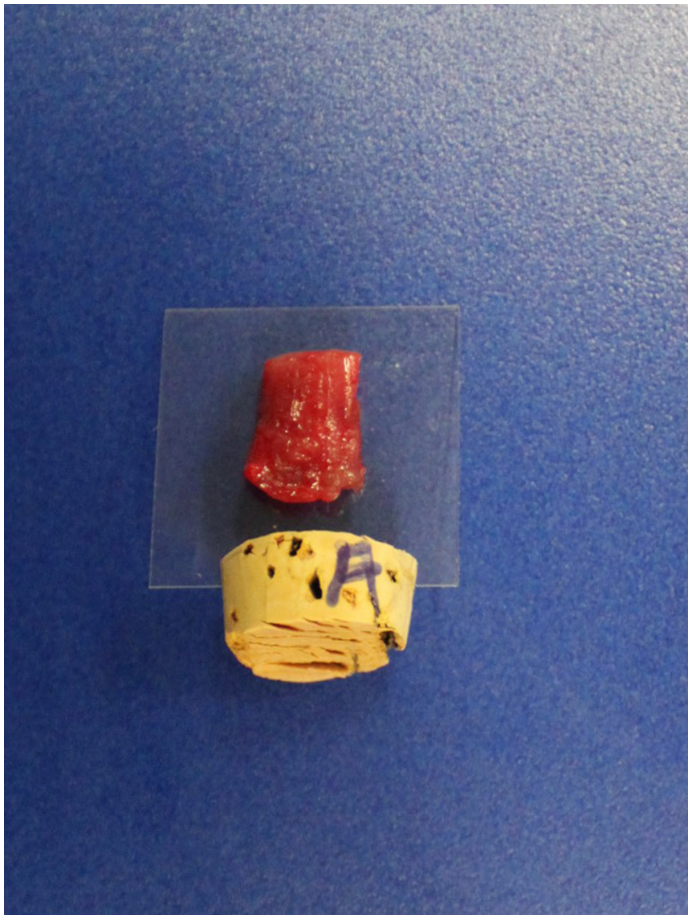
5) Fix a portion in formalin (clamped or unclamped)
6) Freeze a portion in liquid N_2 for biochemical or molecular studies



https://medicine.uiowa.edu/uidl/faculty-services/muscular-dystrophymuscle-biopsy/muscle-biopsy-general-evaluation
UIDL website

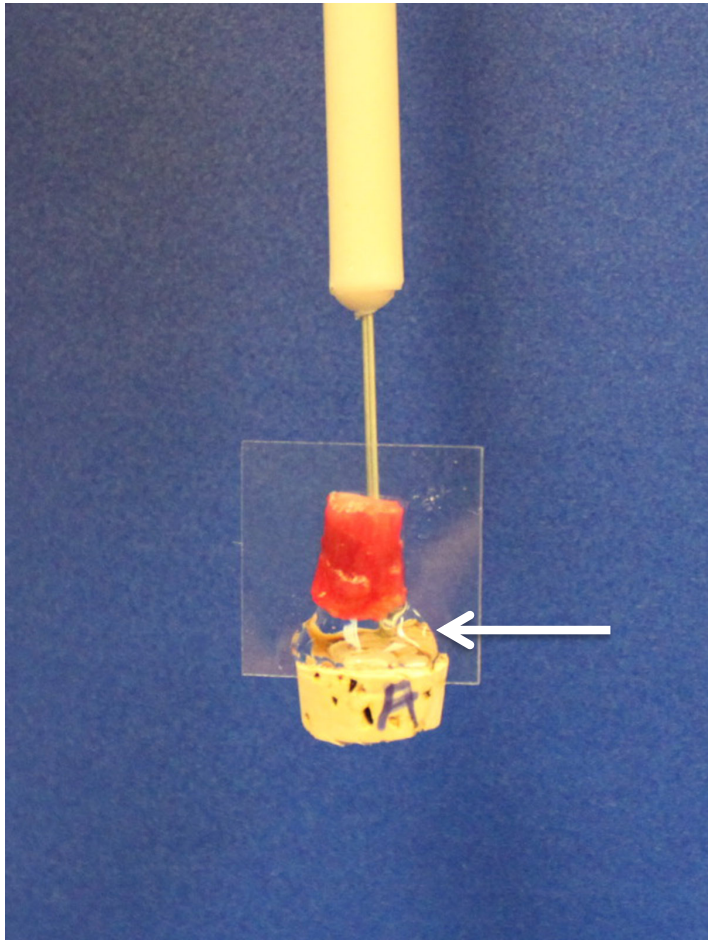
Cut the biopsy from between the forks of the clamp and position on a pre-assembled cork/coverslip.

Trim one or more 0.1 cm diameter strips the entire length of the biopsy to fix in glutaraldehyde.



The tissue to be frozen is positioned on a pre-assembled cork/coverslip.

A dissecting needle placed into the cork behind the coverslip serves as a handle. Fill the gap with OCT.




Product of
United States
Class IA
4 L
O3551-4
2-Methylbutane
Certified
Maximum Limits of Impurities
ASSAY
COLOR
IDENTIFICATION
C5H12
CAS 78-78-4
For laboratory and manufacturing use only, not for drug, food or household use
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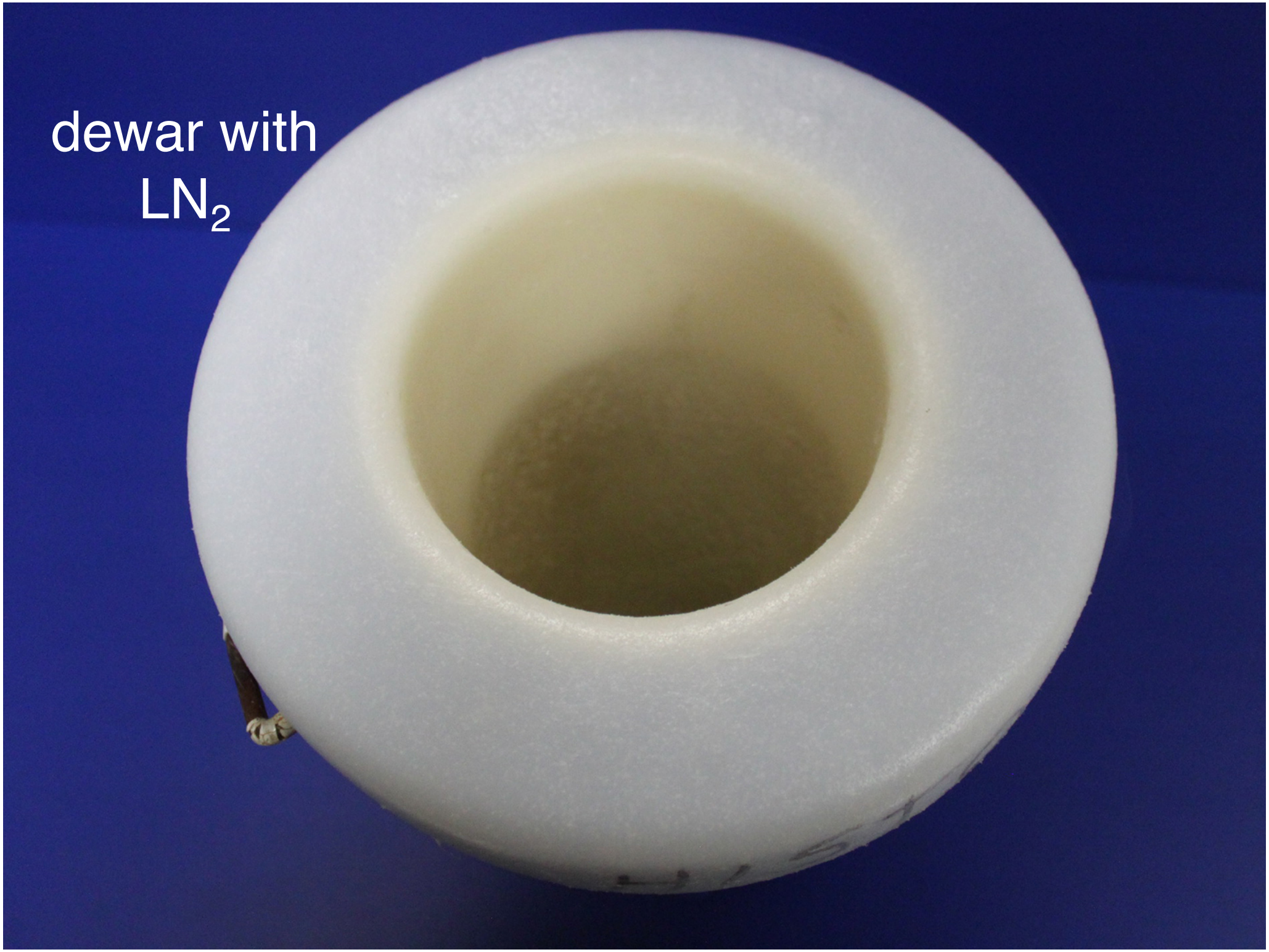
isopentane


Place isopentane in a metal cup with a flexible wire to suspend in LN_2.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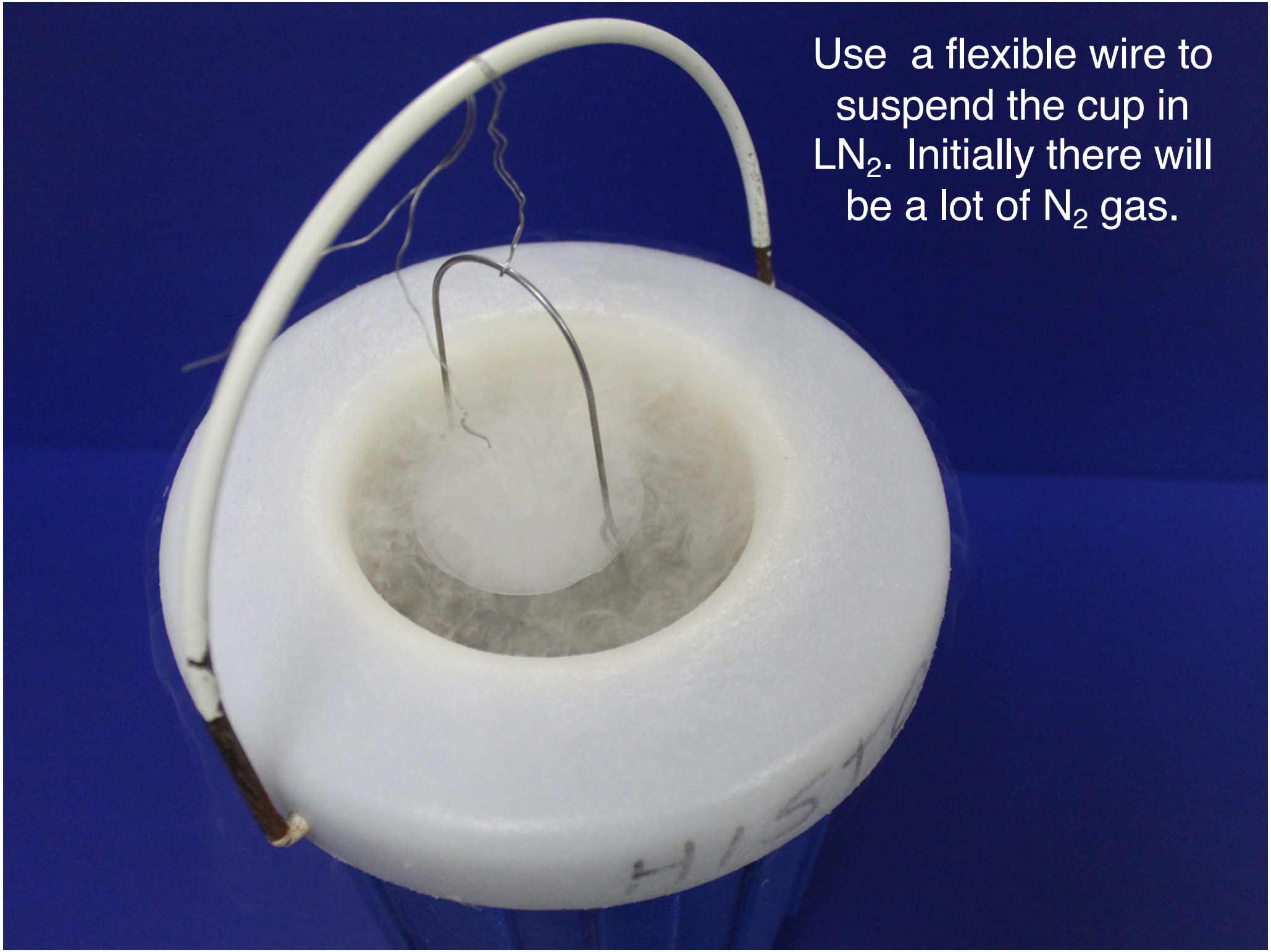
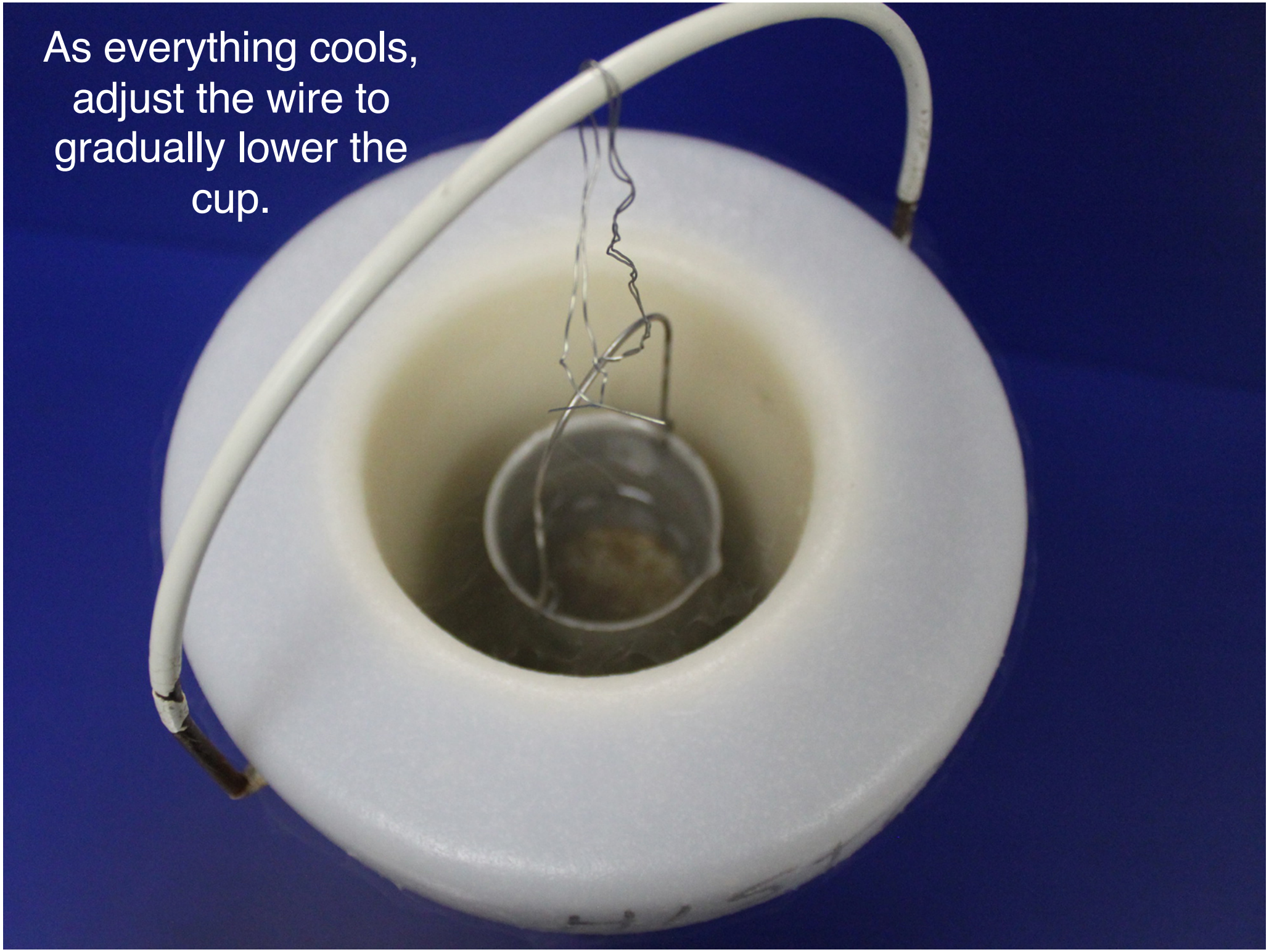
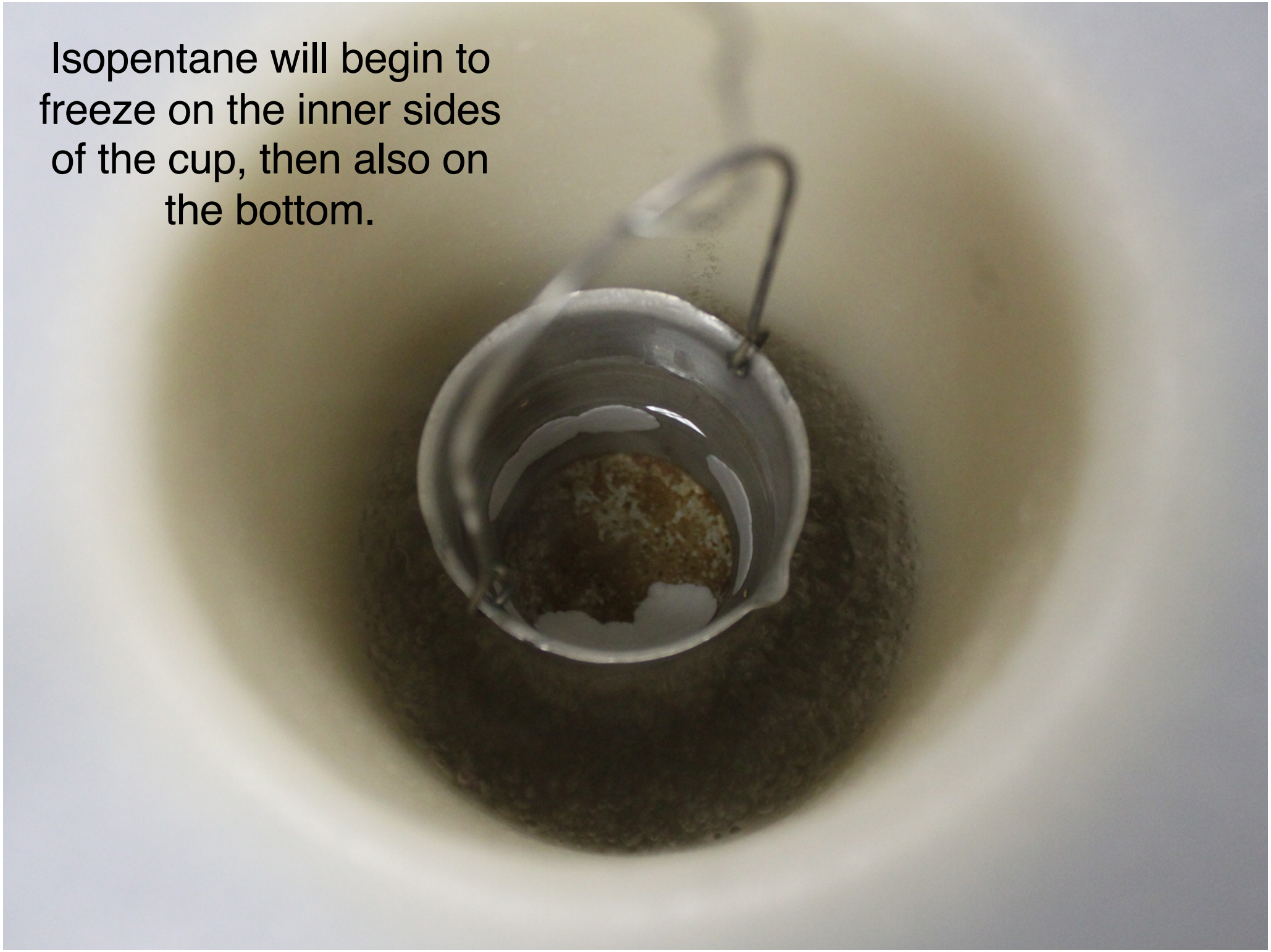
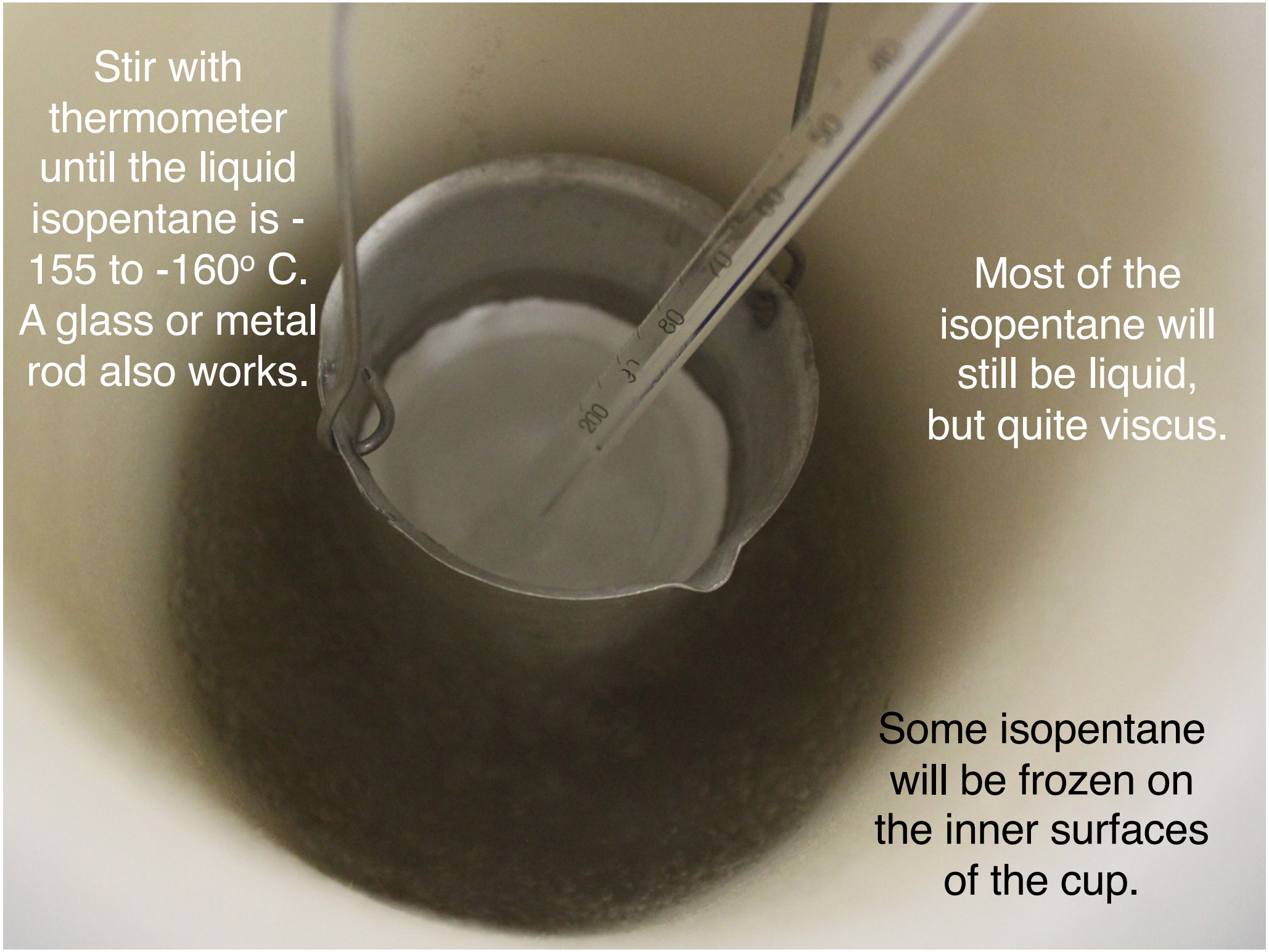
LN_2


Use a flexible wire to suspend the cup in LN_2. Initially there will be a lot of N_2 gas.


As everything cools, adjust the wire to gradually lower the cup.

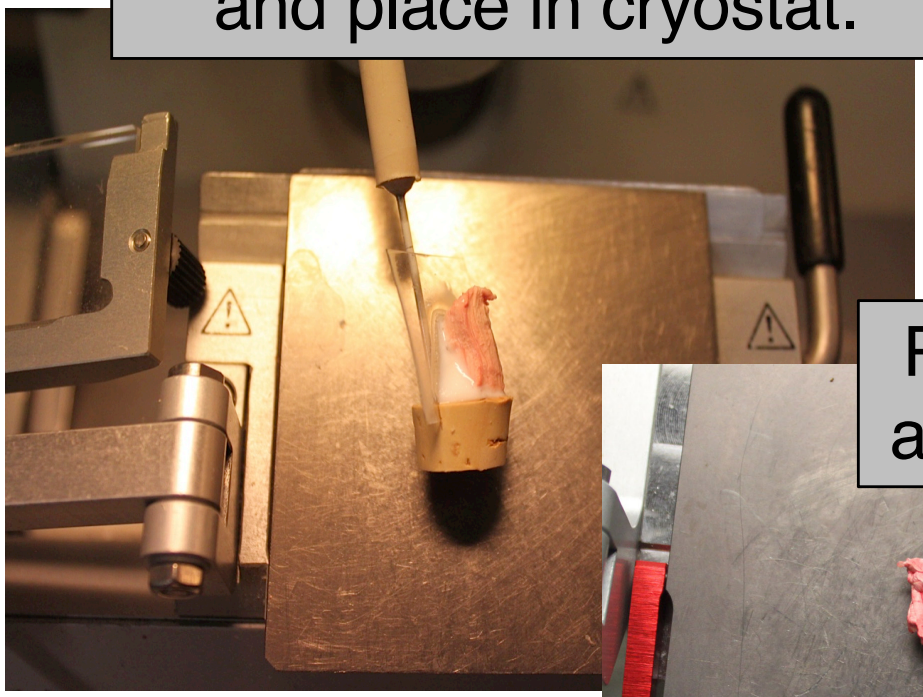

Isopentane will begin to freeze on the inner sides of the cup, then also on the bottom.


Stir with thermometer until the liquid isopentane is -155 to -160° C. A glass or metal rod also works.
Most of the isopentane will still be liquid, but quite viscus.
Some isopentane will be frozen on the inner surfaces of the cup.

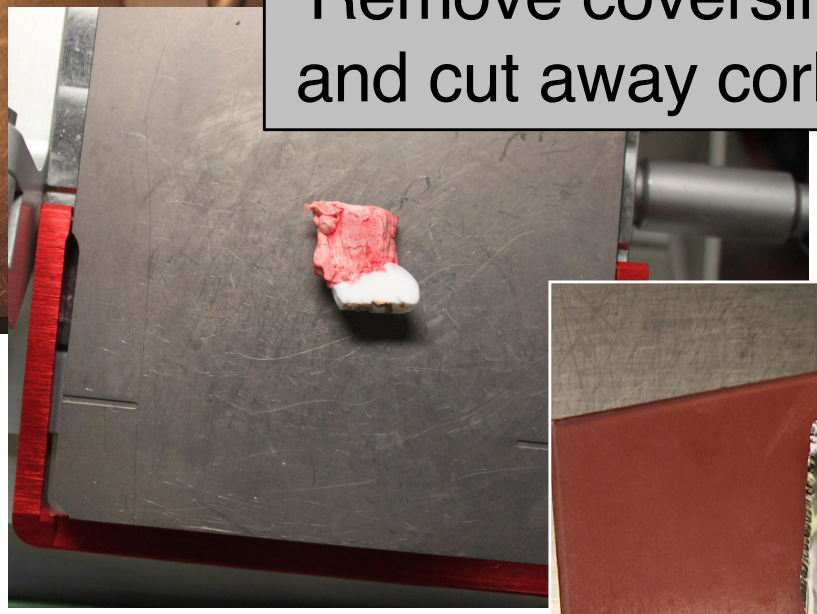
Plunge muscle biopsy into isopentane.

Packing isopentane container in dry ice is an alternative (temperature approximately -80^{o} C).

Remove from isopentane and place in cryostat.

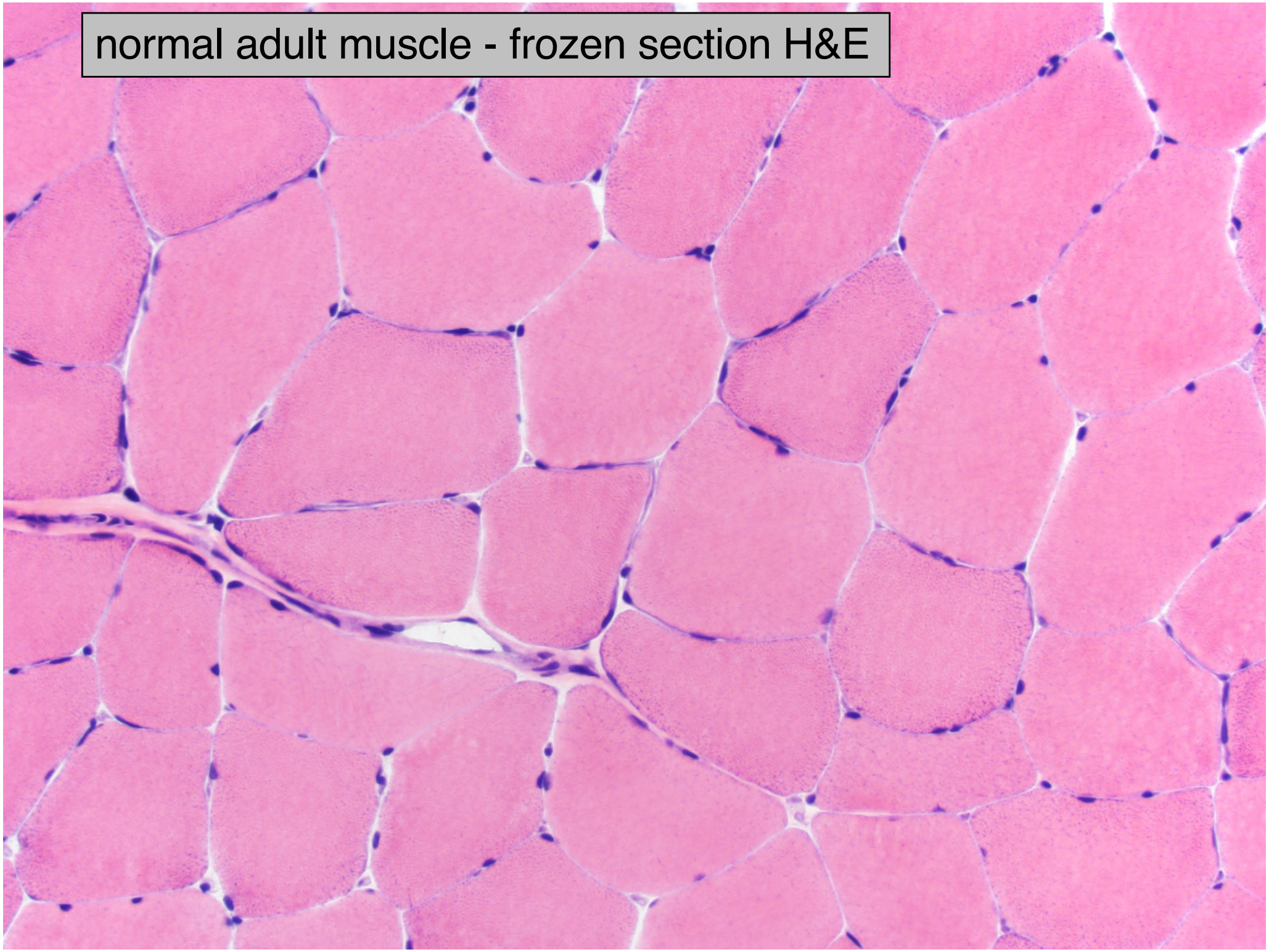


Remove coverslip and cut away cork.



Wrap in precooled foil.


store in prelabeled polycon


normal adult muscle - frozen section H&E