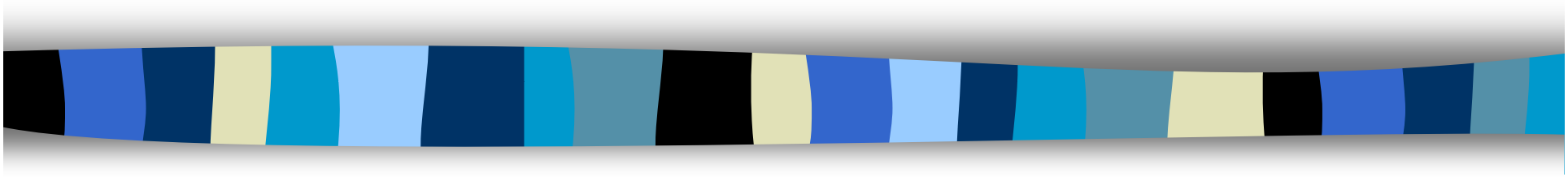
Session II. QC. Straw handling



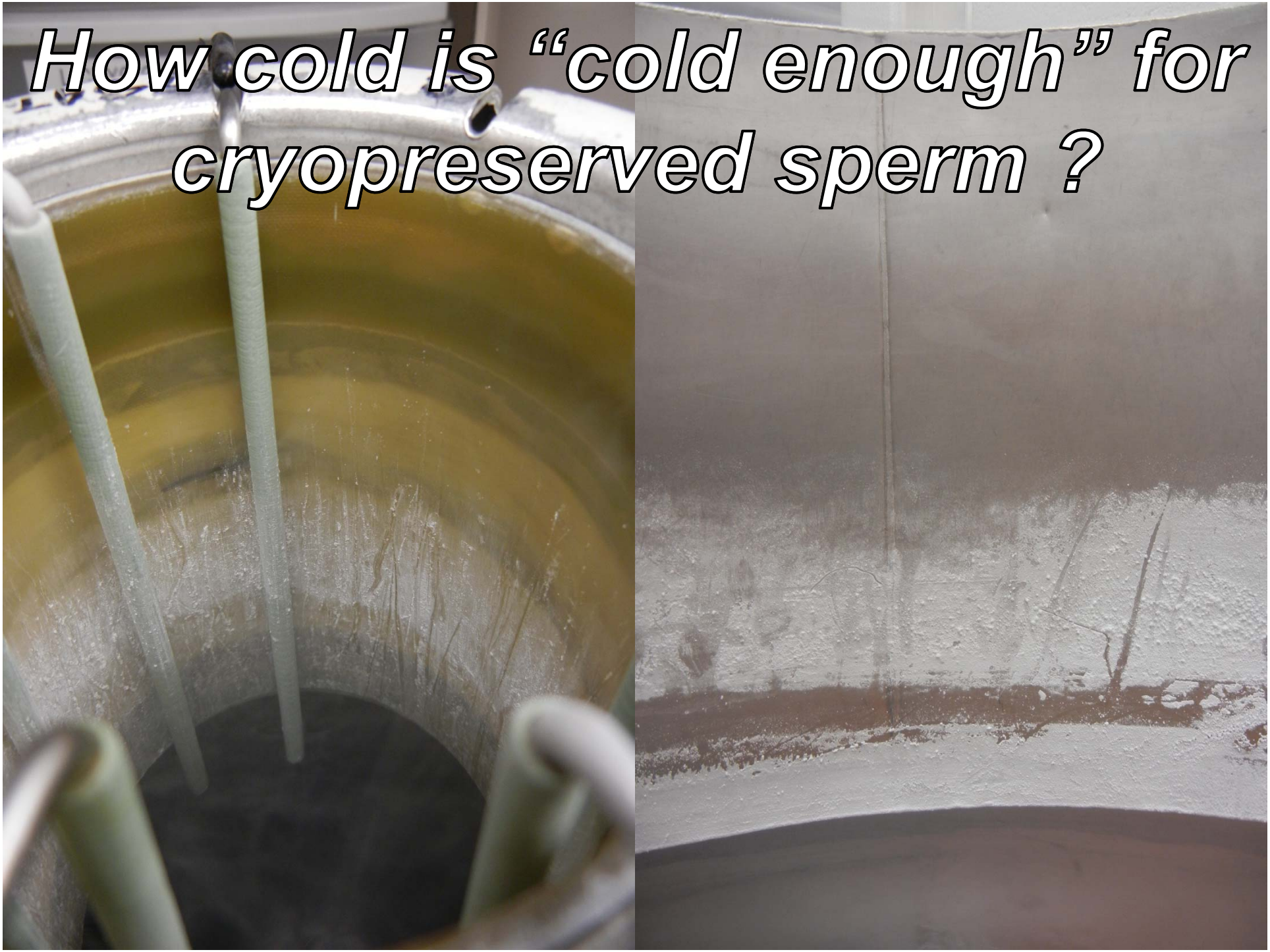
Maintaining cryopreserved semen quality

Prepared for staffers handling cryopreserved straws.

2014 Anand Workshop

Michael Kaproth, PhD
AVP Labs and Production Technology
and Genex Production staff

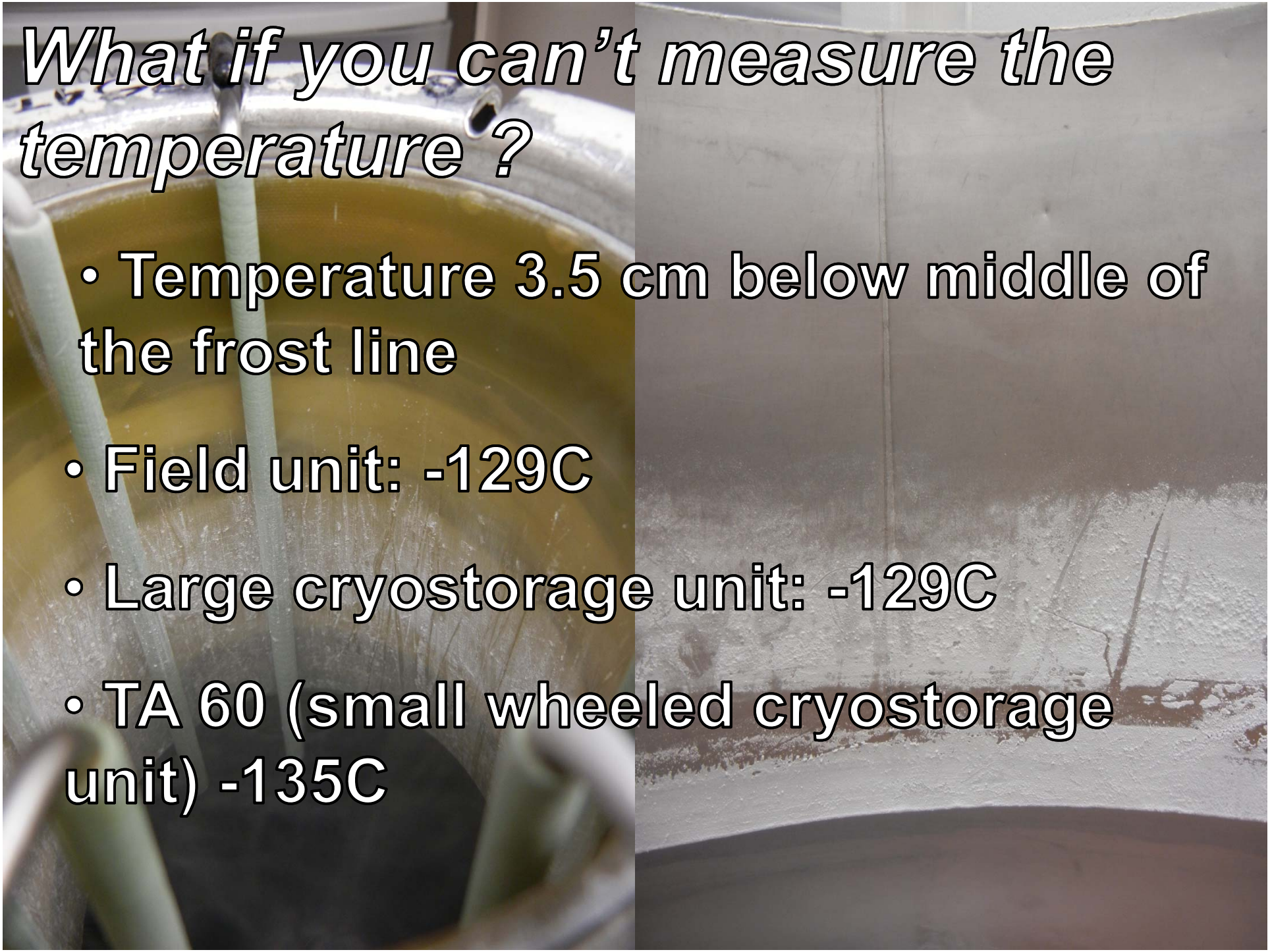
Genex/CRI


How cold is “cold enough” for cryopreserved sperm ?

“-130°C ?”

Best: Suggested by Berndtson and coworkers as the limit for safely holding frozen straws of semen.

Below -132°C, all water is in a glass or crystalline state. The sperm is inert.



What if you can't measure the temperature ?

- Temperature 3.5 cm below middle of the frost line
- Field unit: -129C
- Large cryostorage unit: -129C
- TA 60 (small wheeled cryostorage unit) -135C

Rule of thumb:

In lieu of temperature measurements, consider 3.5 cm below the middle of the frost line a safe starting point
(for a tank with reasonable liquid nitrogen content).

Do cryopreserved cells need to stay at a constant temperature?

When tissue culture samples endure ***cyclic changes*** from -196°C up to -150°C, -130°C or -100°C, or cycle between these elevated temperatures…

…***there is an additional cell die-off*** that is not seen for cells maintained at a single cryogenic temperature.

Do cryopreserved cells need to stay at a constant temperature? Examples from tissue culture science: …

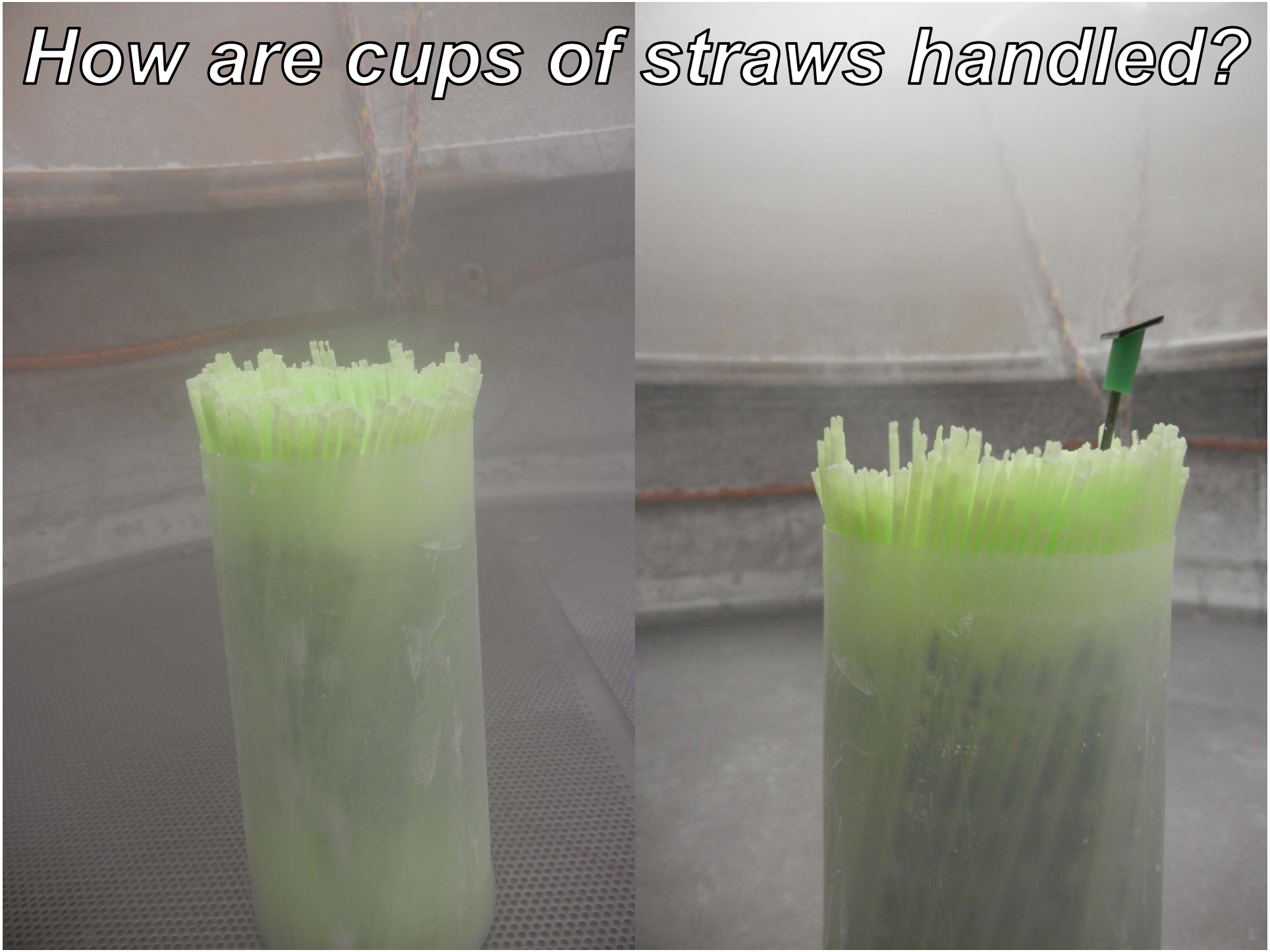
Samples, frozen and stored at -196 °C:

- Cycle 10x to -150 °C: 85% of standard recovery,
- Cycle 10x to -130 °C: 80%,
- Cycle 5x to -100 °C: 48%,
- Cycle 10x to -100 °C: 28%,

No Cycling, store at -196°C: 100%
No Cycling, store at -150°C: 100%
No Cycling, store at -130°C: 100%
No Cycling, store at -100°C: 100%

Rule of thumb:

Limit the number of temperature cycles that cryopreserved sperm must endure


How are cups of straws handled?

How are straws handled once they are frozen and submerged ?

- Do not expose straws to temperatures above -130°C.

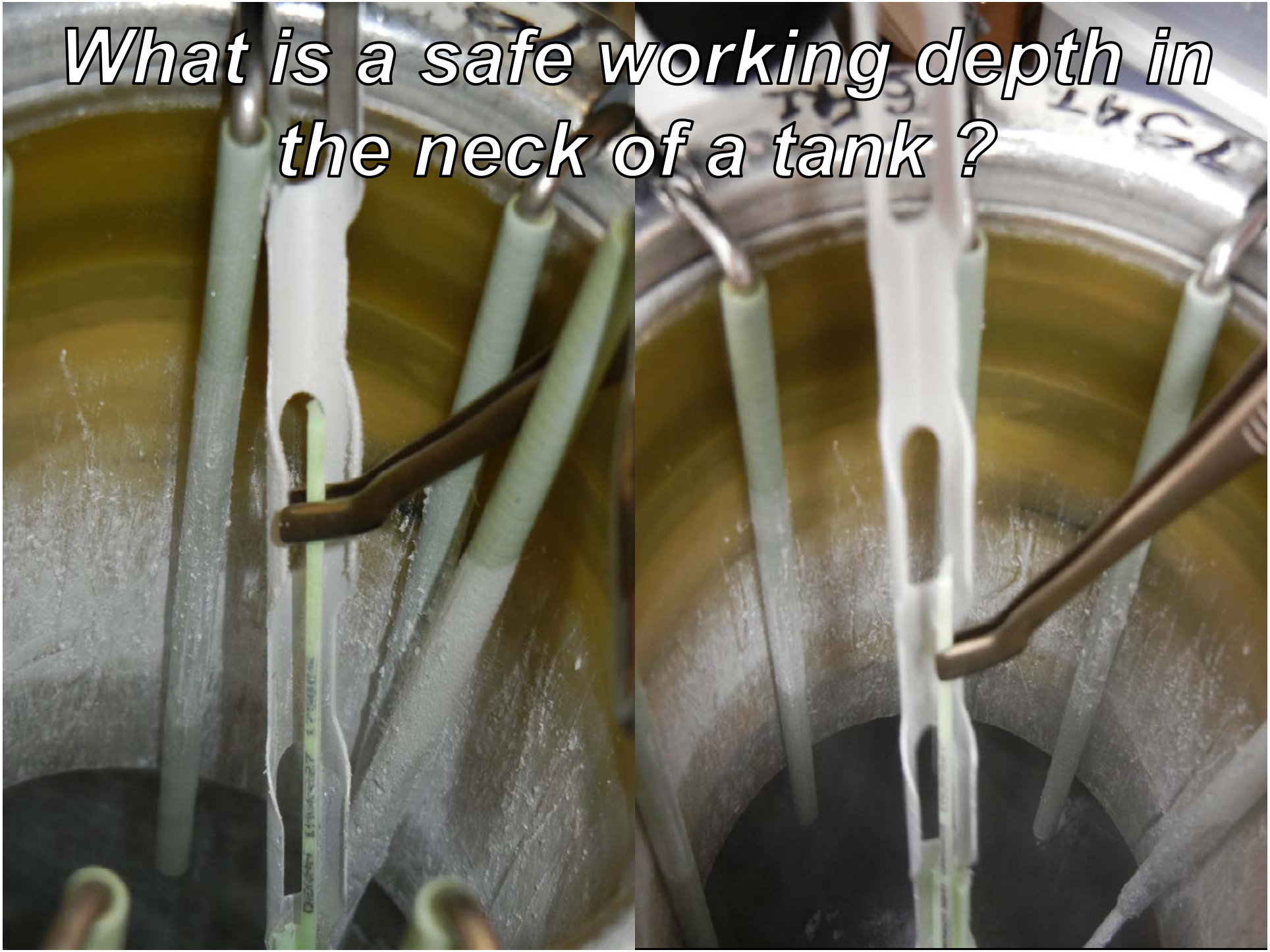
- Pre-chill anything coming into contact with the straws.

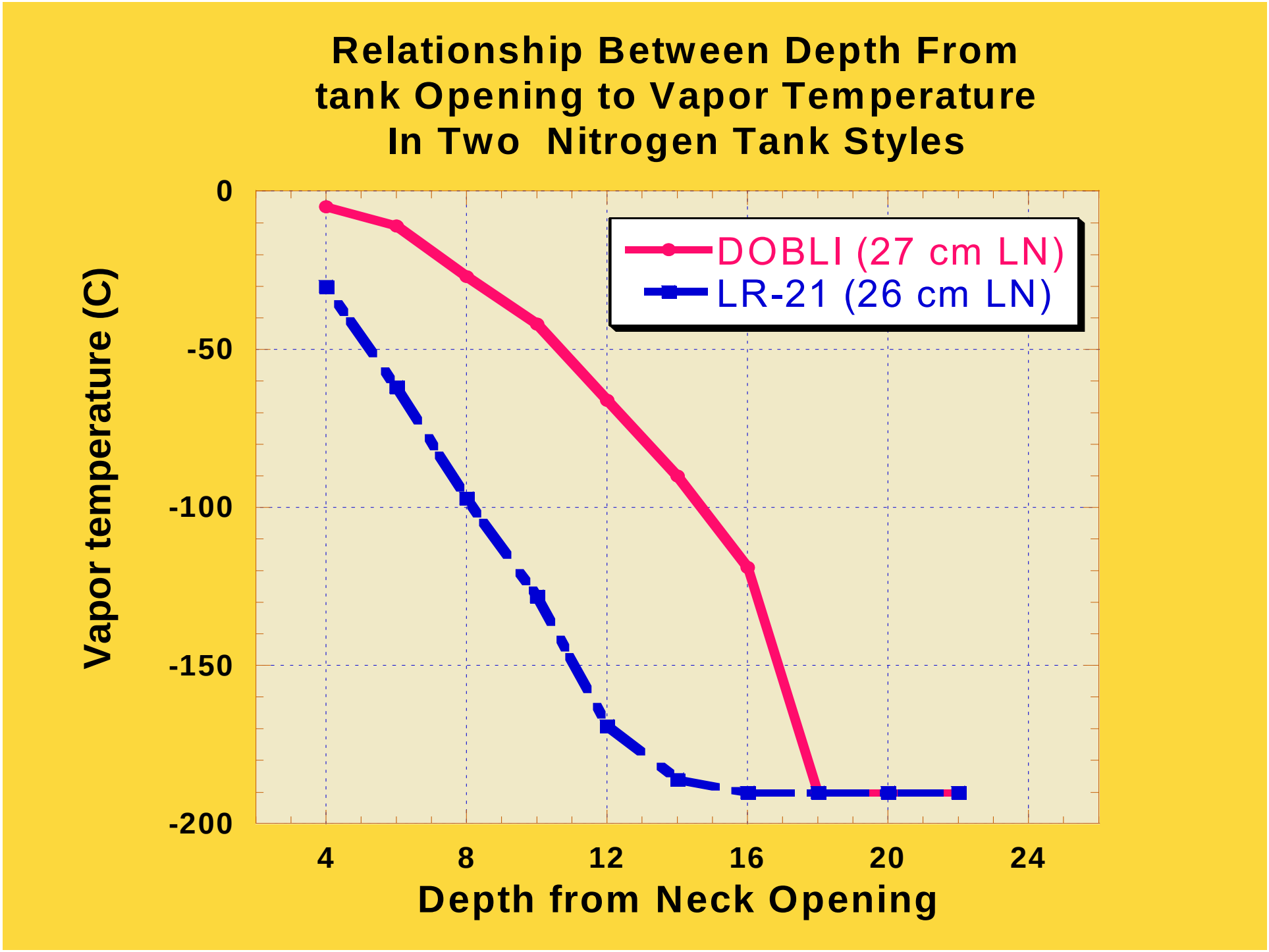
- Work with the straws in liquid or in the vapor as close to the liquid as possible and never above the lip of a tank.

- Limit the time out of the liquid to 3 sec. before re-submerging.

How are cups of straws handled?

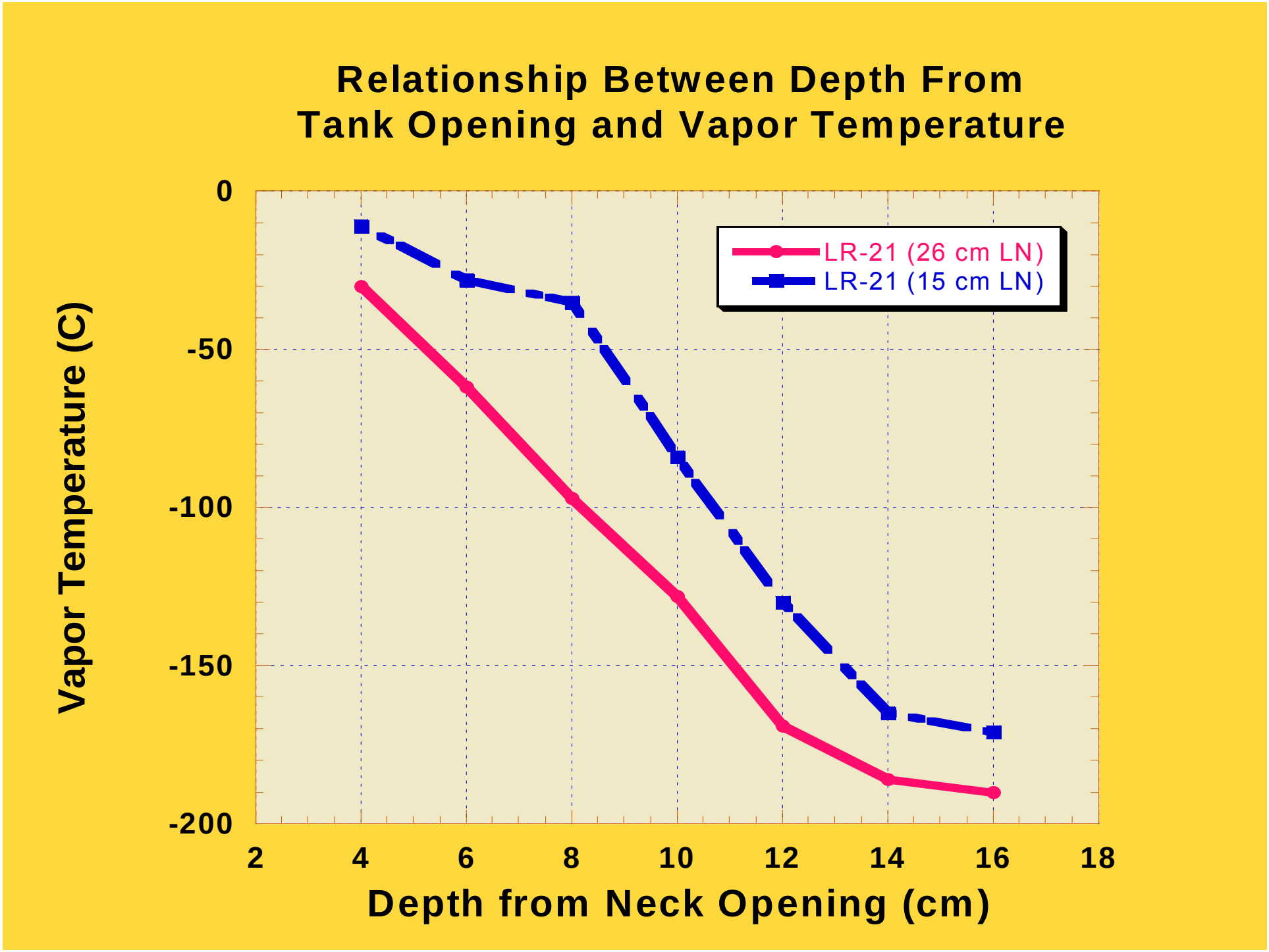
How are cups of straws handled?


What is a safe working depth in the neck of a tank ?


Relationship Between Depth From
tank Opening to Vapor Temperature
In Two Nitrogen Tank Styles
Vapor temperature (C)
0
-50
-100
-150
-200
4
8
12
16
20
24
Depth from Neck Opening
DOBLI (27 cm LN)
LR-21 (26 cm LN)

Rule of thumb:

Make sure you know your tank, because its design impacts neck temperatures which alters available “working time”

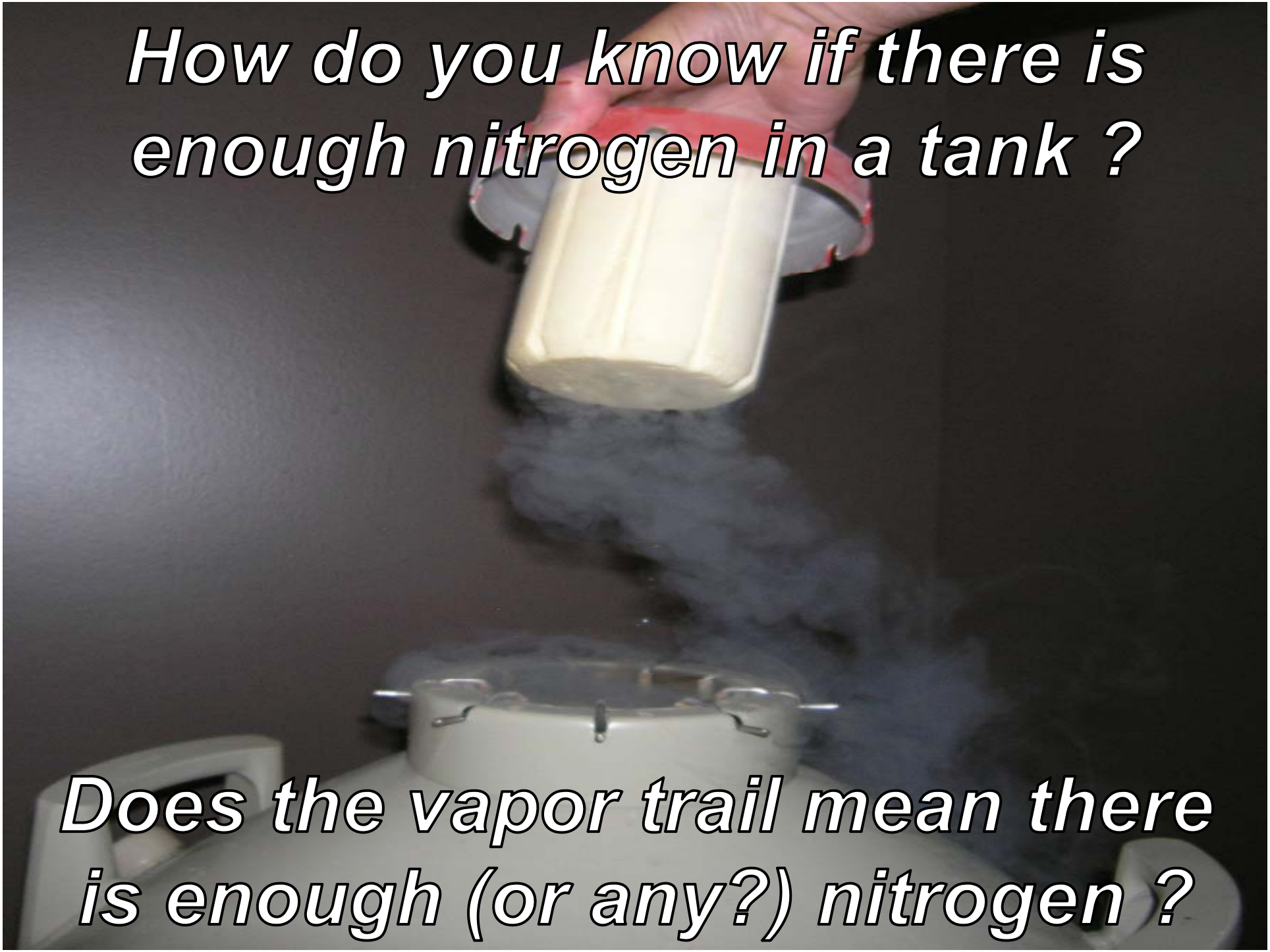

Relationship Between Depth From
Tank Opening and Vapor Temperature
Vapor Temperature (C)
0
-50
-100
-150
-200
LR-21 (26 cm LN)
LR-21 (15 cm LN)
2
4
6
8
10
12
14
16
18
Depth from Neck Opening (cm)

Rule of thumb:

Understand that the nitrogen vapor at the top of a field unit is warm and inefficient in "recooling" a cane of straws.

Rule of thumb:

Likely important that the "experts" put straws into the final goblets as would be used by the customer


How do you know if there is enough nitrogen in a tank ?
Does the vapor trail mean there is enough (or any?) nitrogen ?


The tank was empty: Only -50°

Rule of thumb:

A foggy vapor trail does not indicate a tank has sufficient liquid nitrogen


Does straw temperature
impact motility ?

There is a likely range of effects from exceeding critical temperatures:

- Lower PT motility scores (rarely complete).
- Cracks the coat surrounding the sperm.
- Residual water (as ice) in sperm slowly grow from small crystals to large crystals.
- Lower viability scores.
- Shortened longevity.
- Loss of important cell subpopulations.
- Lower fertility. May impact certain herds more, due AI timing relative to ovulation.

What is the effect of exposure time on straw temperature ?

- A straw taken from a goblet with liquid nitrogen in it will reach the critical temperature of -130°C in less than 10 seconds.

- A straw taken from an empty goblet in the lower neck area of a tank, perhaps -160°C, reaches critical temperature of -130°C in less than 3 seconds.

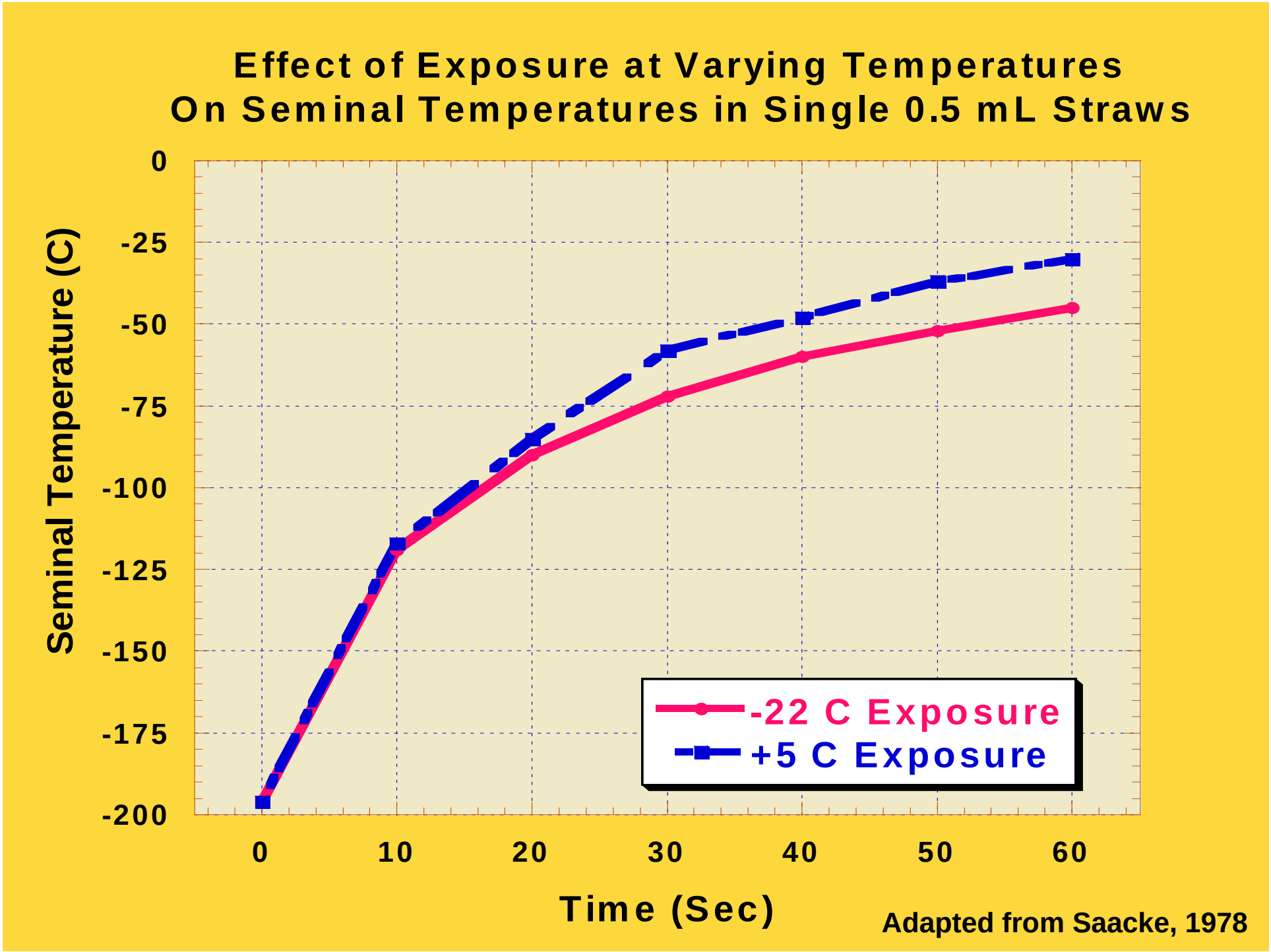
The speed of chemical processes slows down as temperatures decrease

•Calculated as relative energies of activation for an example reaction.

•Each second spent at -100°C is chemically the equivalent of:

•51 seconds at -128°C.

•2767 years at -196°C.


Effect of Exposure at Varying Temperatures
On Seminal Temperatures in Single 0.5 mL Straws
Seminal Temperature (C)
0
-25
-50
-75
-100
-125
-150
-175
-200
0
10
20
30
40
50
60
Time (Sec)
-22 C Exposure
+5 C Exposure
Adapted from Saacke, 1978

Good Lab Practices:

- Keep straws below -130°C .

- When moving canes, boxes, and canisters, complete the transfer in:
  - **two quick steps**,
  - **and under 3 seconds**.

- Use small wheeled tanks, otherwise.

Good Lab Practices:

•Avoid cycling through a range of temperatures.

• While handling individual straws, try to use a stream of nitrogen vapor to keep below -130^{o}C.

•Prechill forceps and gloves before touching straws.

Good Lab Practices:

•Keep the liquid level above the straws in any tank where a lab vacuum sucker is to used.

• In tanks where the liquid level drops due to activity, straw temperatures may reach the limit of temperature safety when the level has dropped 4-5 inches below straw tips.

Good Lab Practices:

•Lab suction devices can only be used if the tips of the straws are submerged.

• The atmosphere in a tank with an active suction device can be as warm as -10°C.

•Consider running a nitrogen vapor line into the tank: When streaming vapor down to the liquid level, you get a clear, cold, safe atmosphere.

Rule of thumb:

Straw temperature depends not only on exposure time but starting temperature, so you need to account for both

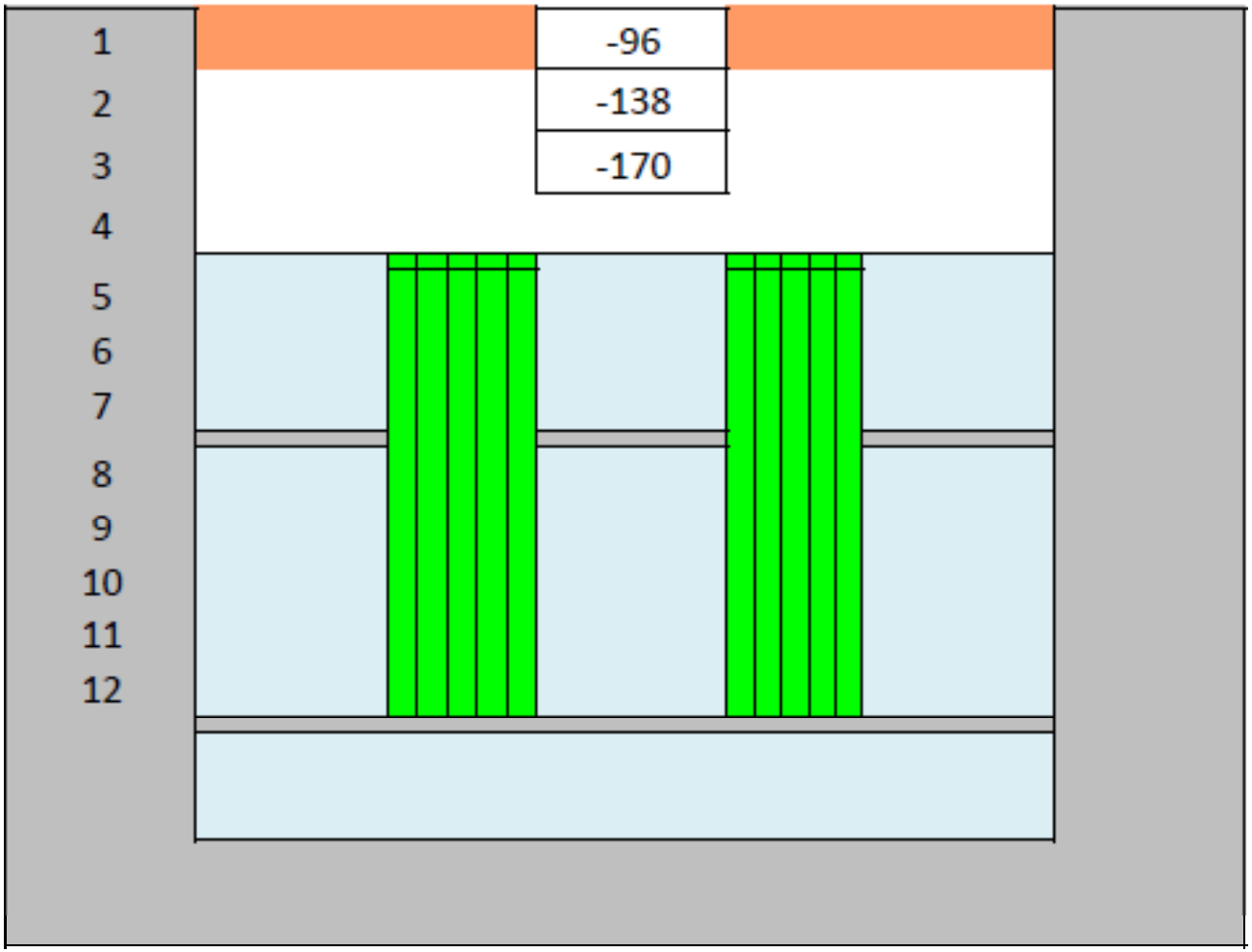
Observations on equipment.

Would like to see deeper, more spacious work areas for working with cryopreserved straws.

TA21

Processing lab

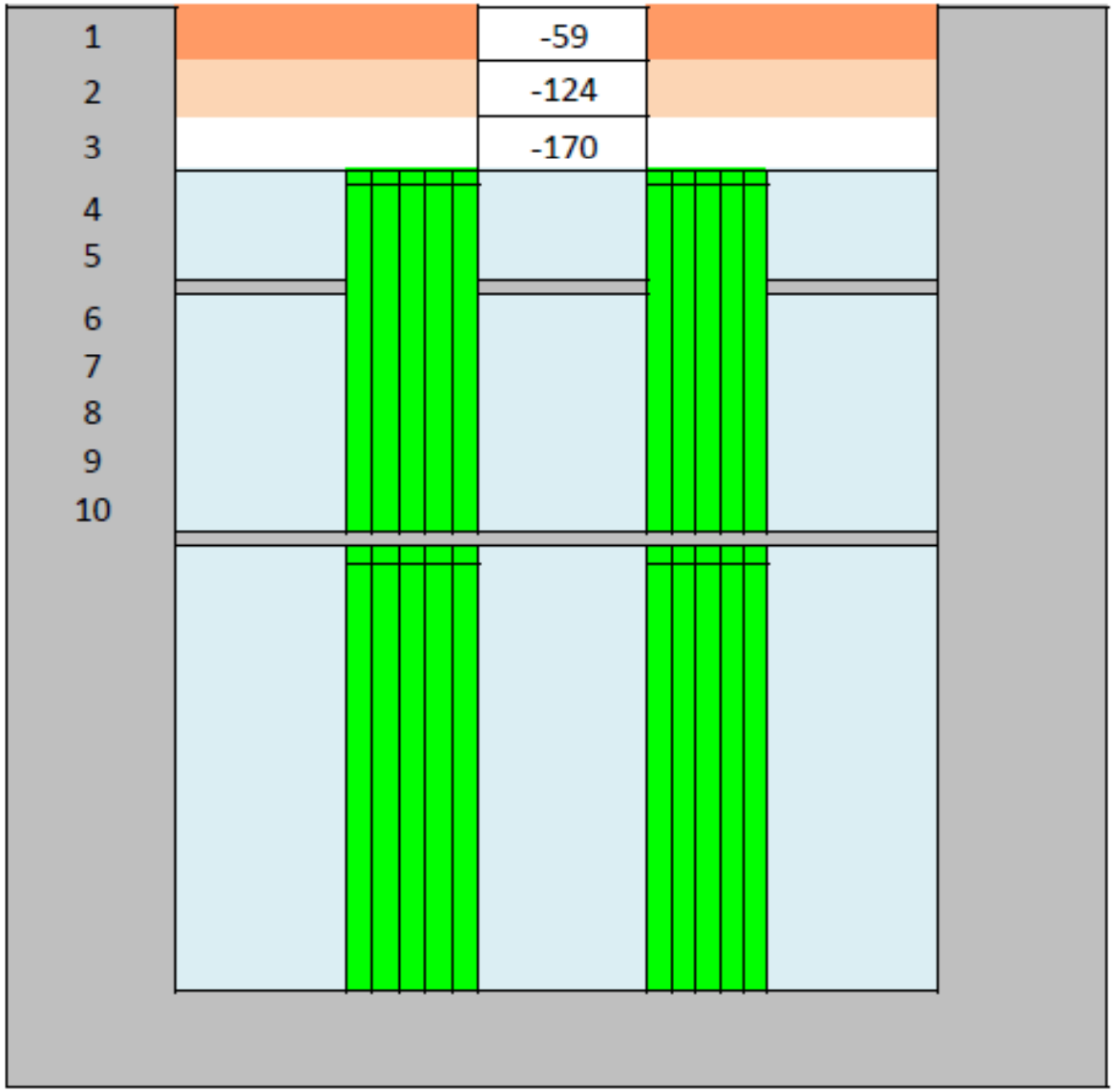
Three inches of working space



TA21

Processing lab

~1 1/2 inches of working space

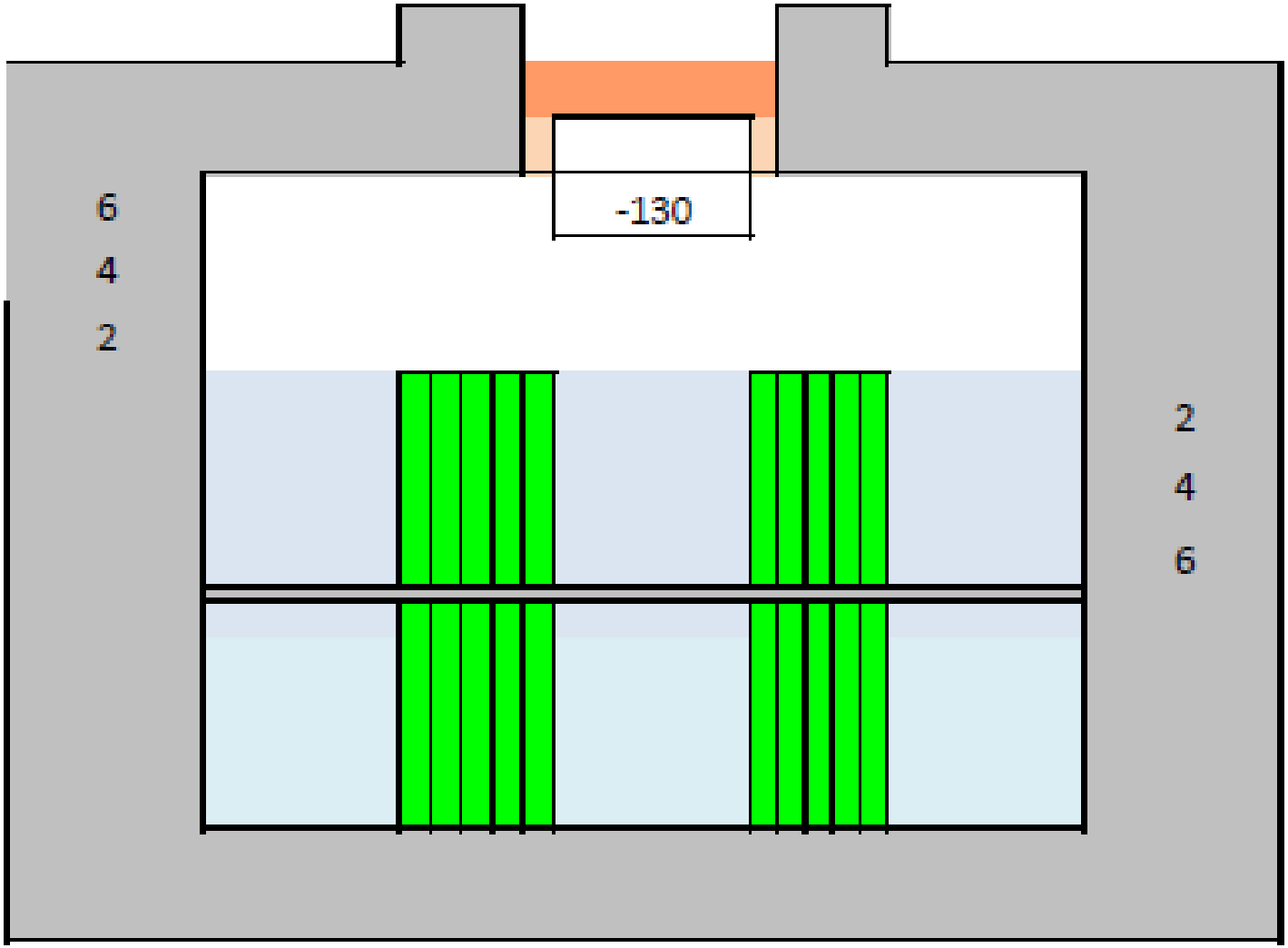


Liquid nitrogen level in large cryostorage tanks controls the atmosphere in the tanks.

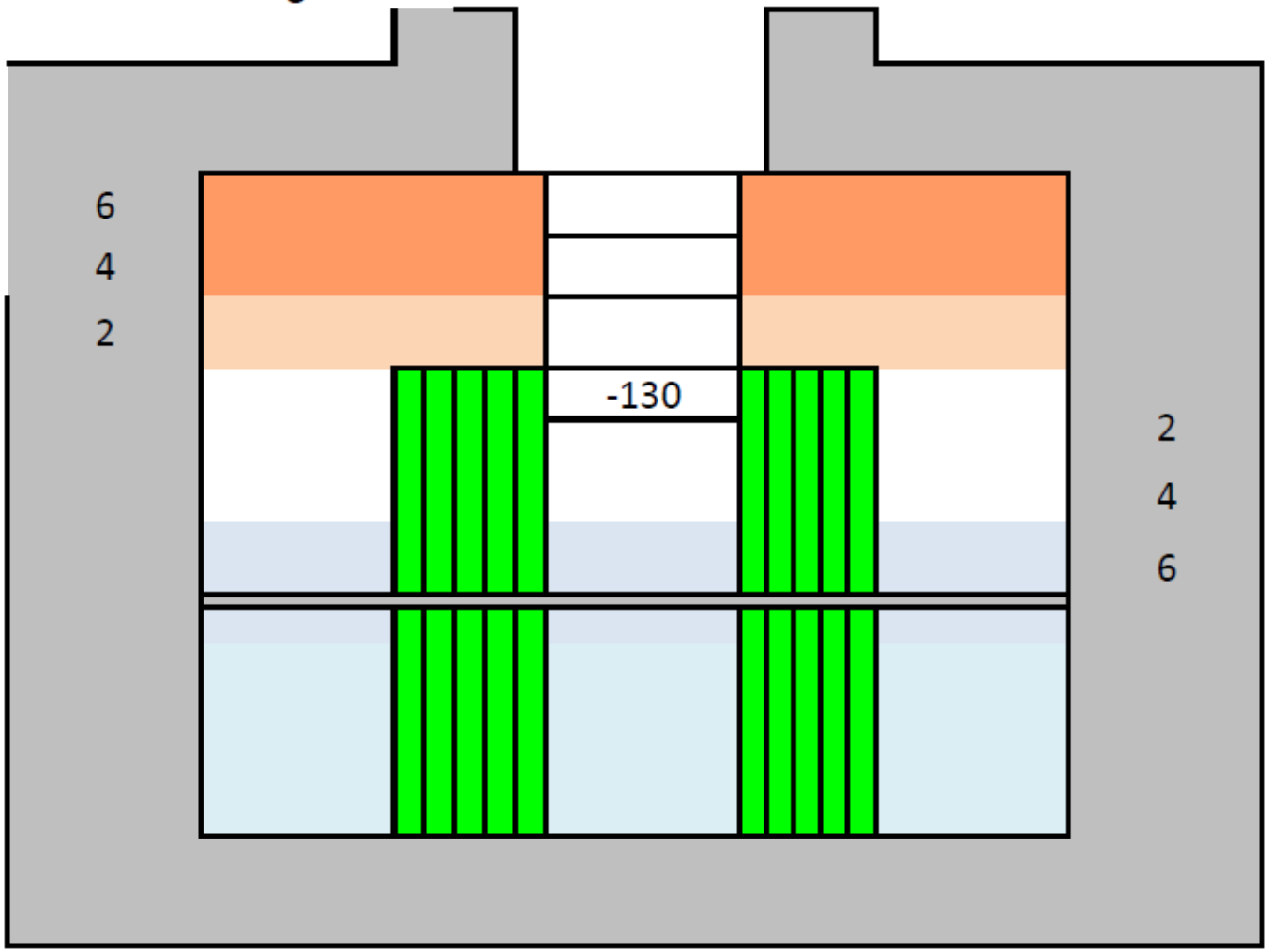
Any Large Storage tank

Distibution Lab

4 to 5 inches above the liquid level is save.



Any Large Storage tank
Distibution Lab
When liquid drops 4 to 5 inches below the straw tips, straws are at the limit of allowed warming.



Straws too high in worktank

Safe suction only when the liquid level is covering the straws

Liquid level too low: Don’t run the suction

What the distribution lab of a 10 million unit operation looks like

Create safe working tanks

Measure the working distance and drop the screen until we are safe for straws.

Consider gobletting by handling straws under the liquid level.

South America

Done every day across the world this way.

Montana