



## I.C.E. CUBES # 3

### Tiny bits of information to help improve your cryopreservation practice

Hello, "ICE Cubes" is the official e-newsletter of Innovative Cryo Enterprises I.C.E. I am publishing this to provide an ongoing connection to current users and others interested in cryopreservation. This e-letter will be published randomly, possibly on a bi-yearly basis, or as important news comes up. This e-letter will provide information and advice on all aspects of cryopreservation in the reproductive field. You can also check out our website for even more information and past newsletters at: [www.icevitrification.com](http://www.icevitrification.com). Thank you for your support!

## Minor Change!

Nobody likes changes, nor do we. However, we are going to be using a slightly larger cryovial for vitrification media. We have changed from a 4ml vial to a 5ml vial. Please note that the vials are made by the same manufacturer, and are identical to the ones we have always used. The major differences are 1) the vial is a bit larger; 2) the cap color is red not orange; and 3) the labels on the vials read "Sigma" in white and not "Corning" in black. All the tubes are made by Corning and are identical in composition to the old ones. This change is due to the fact that the vials cost us nearly 50% less, and that they are essentially the same vials we have always used. Please contact us if you have any questions or concerns.

## Meetings!

I.C.E. will be at the upcoming meetings this year, and will be presenting either posters or oral talks.

PCRS: March 19-22, 2014; AAB: May 15-17, 2014; and ASRM October 18-22, 2014

James will be available to meet and talk to you during these conferences. Please let me know that you will be there and we can set something up.

## Tips and Tricks!!!

### Heat-Sealing: Updated tips to obtain perfect seals every time.

This is an expanded explanation of heat-sealing, mandated by the fact that some groups are or have had issues with getting perfect heat-seals. We hope that this will put an end to these problems. Please



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refer to the full description on the website: <http://icevitrification.com/HeatSealing.html>. The website also has more information about straw types and where to obtain straws and heat-sealers.

Proper heat-sealing is essential to ensure perfect seals and no explosions of straws upon warming. Unfortunately many labs have had one or more poor thaws due to a faulty heat-seal. This seemingly simple act of heat-sealing has led to many problems for some and it is time we perfected it, hopefully once and for all.

There are 2 basic faults when heat-sealing. The first is not using a hot enough setting on the sealer. This can be easily corrected. The second is damaging the straw during heat-sealing, sometimes by using too much heat.

The ICE vitrification system was designed to be used with the standard 0.25cc cryo-straw. Embryologists have used these straws for years. Unfortunately poor seals or damaged straws can lead to rupturing and explosions upon warming. Because of the rapid cooling upon direct contact with liquid nitrogen, the straws contract and can pull apart. If the plastic straw is not totally melted together, the straw may form an opening for liquid nitrogen to leak in. Liquid nitrogen seeps inside the straw through a faulty seal or crack and then expands quickly upon warming the straw. If the crack is large enough the liquid nitrogen will simply shoot out the opening. The end result is that the cells inside the straw are usually lost or dead.

## Proper Heat-Sealing

1) To heat-seal, set the control knob to the desired setting (5-7) in most cases; for standard 0.25cc cryo-straws.

You can perform a test on a blank straw by heat-sealing at different settings and check your seals under a microscope to see which setting works best for the straws you are using (See images below).

2) Pre-flattening the ends of the straw is recommended in order to help prevent damage to the edges of the straw while heat-sealing. (see last 2 images below)

3) Insert straw between sealer jaws so that the end is sticking out past the heating element. This will ensure a complete, 5mm wide seal.

4) Press and hold the sealer top jaw down firmly. When the red light goes off and the sealer beeps, wait 1-2 sec more before opening the sealer to remove the straw.

5) The straw may stick to the teflon. Do not pull it off as this may disrupt the columns of media and your embryos inside. Gently pry the straw off the teflon using your fingernail. Touching the straw with your fingertip will cool the straw so it can be removed easily. Although the straw will be very warm it should not burn you when you touch it.

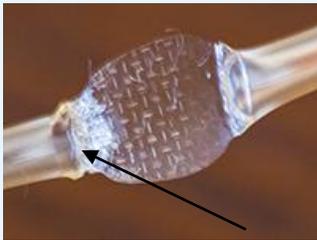
6) IMPORTANT! Check your heat seal under a microscope to ensure proper sealing! See images below.



7) Recalibrate your sealer every 4-6 months to ensure it is working properly.

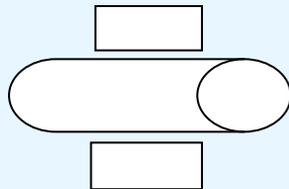


Settings #6 & 7 on a AIE-205 sealer: Sealed, along length of seal, note how the straw flattens to form a large oval, with marks from teflon.

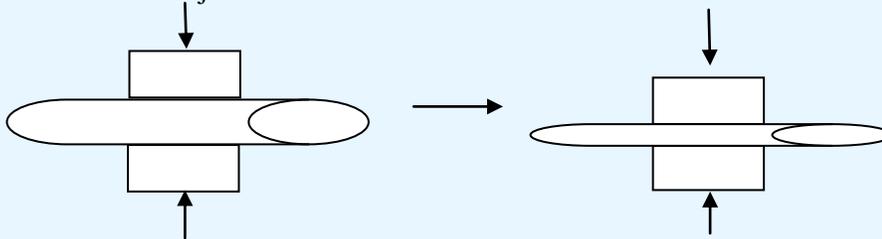


Setting #8: Sealed, but the straw is a bit damaged on the left edge of the seal due to too high a setting.

### Possible damage during heat sealing of a 0.25cc or 0.50cc straw



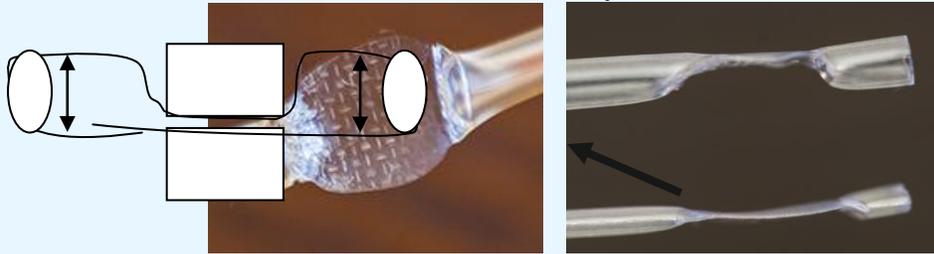
Straw between heat-sealer jaws. Head-on view.



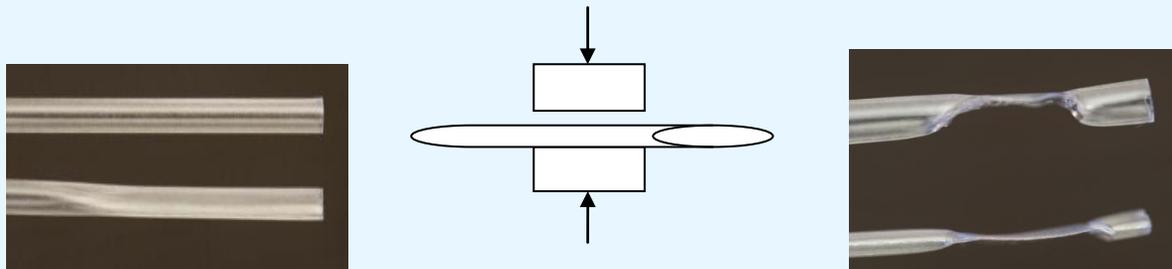
Jaws compress the straw during heat-sealing.

potential weak spots, where tears can occur





As the straw is compressed and heated, the sides of the straw that are outside the jaws will want to regain their original shape. When this happens the edge of the straw near the heat seal will pull away to regain original form, thus stretching the plastic making it thinner or, if the heat is too high, creating a weak spot and/or hole in the straw. See the arrows in the pictures above for places of potential damage.



By pre-flattening the straw with a pen (see picture on left of a regular and pre-flattened straw), the straw will resist opening up to original shape during heat-sealing, and thus prevent damage at the edges of the heat-seal. See picture on right of a regular vs. pre flattened heat sealed straw. Keep in mind that pre-flattening does not work for CBS type straws, due to the different plastic composition.

### Keep In Mind:

- A proper heat seal will melt the straw together.
- No inner lumen should be showing and the straw should flatten out in a large oval shape.
- Check the sides of the straw near the heat seal to ensure there is no damage to the straw at the inner edge of the seal. Notice in the last image that the jaws of the heat-sealer press down to make the seal.
- During sealing, as the straw melts, it will try to regain its shape, which can weaken the straw at the edges of the heat seal. The straw will thin out and may tear at the edge of the seal as it regains its shape.
- Pre-flattening the straw prior to heat sealing will eliminate damage at the edges of the seal.
- Clean the jaws periodically with alcohol. Replace teflon if it is damaged. Avoid always sealing in one place along the jaws of the sealer, as this will cause wear or damage in that spot.
- A proper heat seal ensures no liquid nitrogen will enter the straw during storage and prevent the straw from exploding upon warming.

Please refer to the website for a complete description of heat-sealing and where to obtain straws and sealers. <http://icevitrification.com/HeatSealing.html>



Innovative Cryo Enterprises L.L.C.

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## Useful Item!



I have recently discovered a very nice 6-well dish to use for vitrification and thawing. The AgTech Inc D18, 6-well dish. The dish is about the same size as a Nunc 4-well dish. The wells hold about 1ml of media and are rounded with a flat bottom. This means embryos will not get stuck or hidden around the edges, but collect at the bottom, in the center of the well. This makes for easy manipulation without the risk of embryo loss. The 3x2 design means you can do 2 rounds of vitrification with 1 dish, or 1 round of thawing (T1-T5) with one extra well for expelling the embryo out of the straw.

They come in a 10 pack, each individually wrapped and sterilized, so you only have to open 1 at a time.

The cost is only \$1.60 per dish and the dishes are embryo tested as well. If you decide to try these please let me know your thoughts.

### [6 well Dish, Agtech Solution\(tm\)](#)

SKU: D18



Our price: \$15.95

AgTech Inc. 1-800-367-4016

I thank you for your continued support. We are always trying to improve this already successful system in hopes of increasing survival and pregnancy rates so that *all* clinics using the system will achieve results similar to their fresh/nonfrozen embryo transfer rates. Many of you have already achieved this lofty goal and we are so glad that we could help. If you have any questions or comments about this newsletter or how to improve our system please contact me.

Best,

James J. Stachecki Ph.D.

Innovative Cryo Enterprises LLC

973-632-8635