

Stallion: \_\_\_\_\_

Straws per AI Dose: \_\_\_\_\_

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## Instructions for Handling & Thawing Frozen Semen

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### Thawing semen packaged in 0.5ml straws

**Materials needed for semen thawing:**

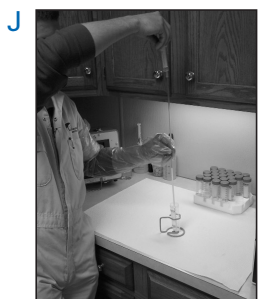
- 37°C constant temperature water bath  
 (straw dimensions: length = 13.2 cm (5 3/16"))

Note: A thick walled styrofoam box is okay to use. Use an accurate thermometer & maintain the bath at 37°C

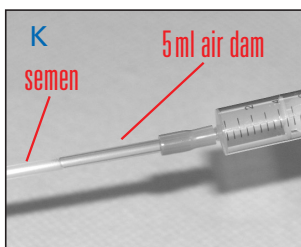
- long handled tweezers or hemostats
- sterile insemination supplies warmed to 37°C

1. Prepare the mare aseptically prior to opening the container.
2. Using a pre-cooled hemostat, or tweezers, (A) remove one straw from the nitrogen container & immediately place it into the 37°C water bath, being careful not to submerge the hemostats or tweezers into the water. It is important to make this transfer as quickly as possible, exposing the straw to room temperature for no more than 2 to 3 seconds (B, C, D).
3. Leave the straw in the water bath for at least 20 seconds to ensure proper thawing (E).
4. The individual straw may remain in the water bath while the remaining straws are removed from the tank and thawed (E). The number of straws that comprise a single insemination dose may vary according to the freezing procedure employed. The information for your stallion is at the top of this page.
5. Once all of the straws for the insemination dose have been thawed, remove them one by one and wipe the straws completely (F). Water leaking into the straw would be spermicidal.
6. Holding the straw vertically so that the air bubble is positioned on the top of the straw (the top is crimped or sealed with a sealing ball) cut off the crimped or sealed end (G).
7. Place the open end of the straw over a sterile, pre-warmed, 37°C container (a centrifuge tube or red top blood tube are both suitable) and cut off the plug on the other end of the straw (H, I). Allow the semen to empty into the sterile container. Tap the end of the straw to ensure that all of the semen is removed.

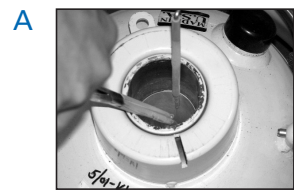
Repeat this procedure for all of the thawed straws, combining the contents into a single container.



8. We do not recommend adding extender to the semen.
9. Draw the thawed semen into a sterile pre-warmed insemination pipette using an all plastic syringe (J) and inseminate the mare just as you would with fresh semen. Use a 5 ml air dam behind the semen to ensure that all the semen is deposited in the mare's uterus (K).



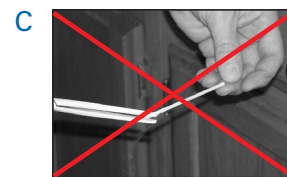
Each insemination dose contains 600-1000 x 10<sup>6</sup> total sperm concentrated into the breeding dose. A minimum of 30% progressive motility is expected upon thawing. It is important to deposit the entire insemination dose into the uterus for optimum results.



Pre-cool the tweezers or hemostats and do not lift the canister above the frost line.



Pulling the canister above the neck of the tank will cause damage to the semen



Do not touch the frozen straw with your hand when transferring.



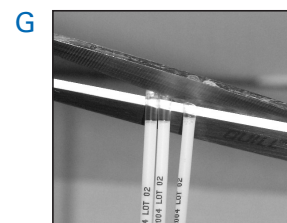
The transfer of the straws to the waterbath should be quick (2-3 sec).



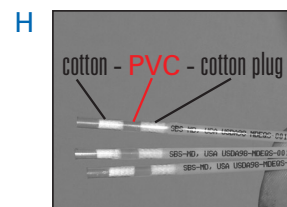
Transfer the straws one at a time. Leave each straw in the water bath for at least 20 seconds.



Dry each straw completely. Water is spermicidal and will damage the semen.



Hold the straw vertically so the sealing ball or crimped end is at the top. Then, cut off this end above the air bubble.



The bottom of the straw showing the cotton plug.



Invert the straw over a pre-warmed, sterile 37°C container & empty the semen by cutting off the plug on the end.