

HSV Device Loading Protocol

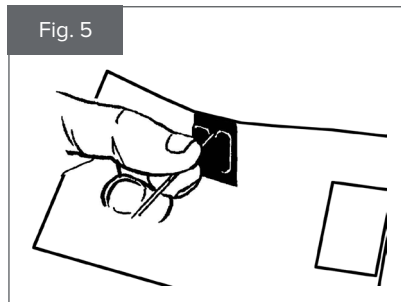
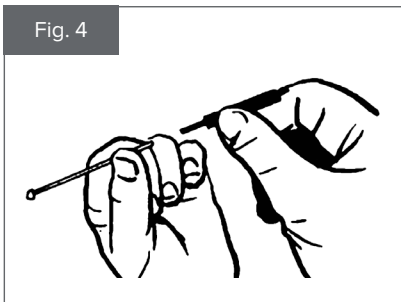
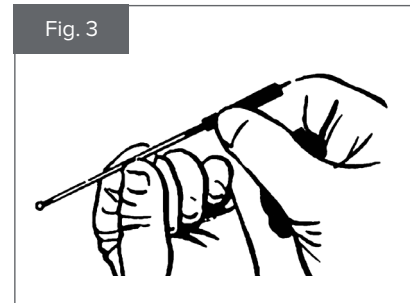
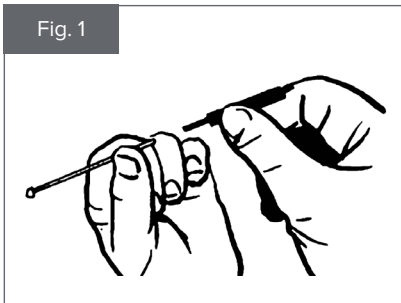
ALL PROCEDURES MUST BE PERFORMED AT ROOM TEMPERATURE (22–27°C)

Have all necessary materials, tools and equipment ready and easily accessible before starting procedure.

LOAD, SEAL, AND VITRIFY WITHIN 80 SECONDS

1. Prepare the identification label (liquid nitrogen resistant) for the HSV straw and apply it approximately 20 mm (0.8 in) from the flared end of the straw.
2. Connect the thin end of the blue plastic insertion device to the colored end of the handling rod (Fig. 1).
3. Prepare the specimen(s) for vitrification according to laboratory protocol.
4. Using a micropipette, carefully deposit the specimen(s) into the curved spatula (or gutter) at least 1 mm from the end. The drop holding the specimen(s) must not be greater than 0.5 μ l in order to avoid the risk of contact with outer straw. Deposit a maximum of 2 droplets, holding 1 specimen each. The two droplets must be at least 1 mm apart (Fig. 2).
5. Immediately place the curved spatula with handling rod in the flared end of the straw and push linearly with the insertion device to avoid any contact between the sample and the inner wall of the HSV straw. Push until the rectangular portion of the insertion device comes into contact with the flared end of the straw (Fig. 3).
6. Slightly pinch the straw between your thumb and finger in the area covering the colored handling rod and remove the insertion device (Fig. 4).
7. While still holding the straw in place, seal the open end using a SYMS sealer (Fig. 5).
8. Hold the straw using tweezers in the area of the handling rod.
9. Quickly plunge the entire straw into LN₂ vertically. Gently stir the straw in LN₂ for a few seconds so as to avoid formation of an isolating air bubble layer around the straw (Fig. 6).

 See reverse side for tips.



HSV Device Loading Protocol

Tips

- Have all necessary materials, tools, and equipment ready and easily accessible before starting procedure (e.g., LN₂ filled holding reservoir, cryocane and goblets, SYMS sealer, tongs or forceps, micropipette).
- HSV Device should be pre-labeled with patient information and the thin end of the blue plastic insertion device should be connected to the colored end of the handling rod.
- The recommended HSV Device capacity is a MAXIMUM of 2 specimens.
- The timing for exposure to VS is CRITICAL:
 - Load, seal, and plunge the HSV Device into LN₂ within 80 seconds after exposure to VS (or VS4 drop for multidrop protocol).
- The specimen(s) should be carefully deposited into the gutter at 1 mm from the end with minimal volume of VS (less than 0.5 µl).
- Use only SYMS sealers to seal the open end of HSV straw.
- HSV Device must remain submerged in LN₂ until ready to thaw. When transferring HSV Device from LN₂ filled holding reservoir, or between LN₂ storage tanks, HSV Device should remain submerged in a LN₂ filled goblet to prevent uncontrolled/premature thawing in air.

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