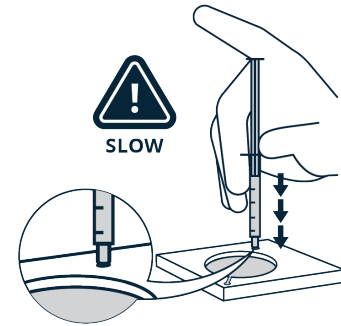
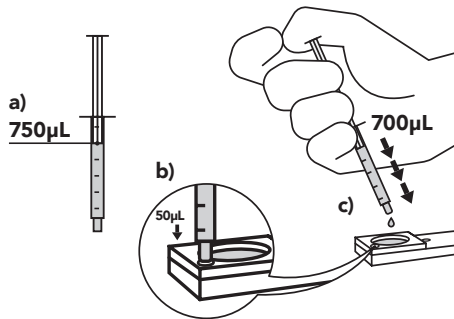


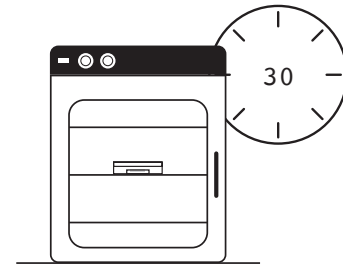
- 1**
- a) Thaw frozen semen (0.1-1 x 0.5mL straws) at 37°C for 30 seconds.
  - b) Extend thawed semen 1:1 (v:v) extender: semen.
  - c) Use 1mL syringe to draw 850 $\mu$ L aliquot.



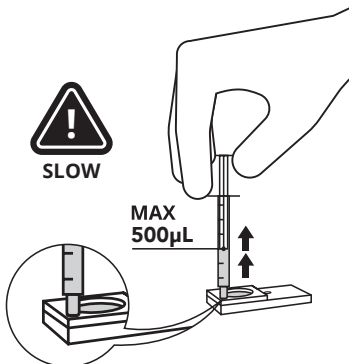
- 2**
- Achieve seal in the inlet port and apply slow and steady pressure to inject 850 $\mu$ L sample into bottom chamber via inlet port.



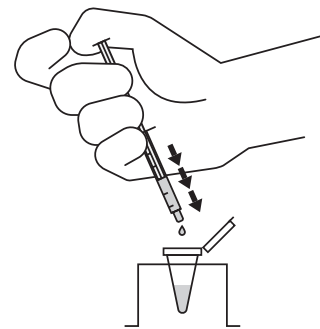
- 3**
- a) Draw 750 $\mu$ L of media.
  - b) Prime outlet channel with 50 $\mu$ L media.
  - c) Cover upper membrane with 700 $\mu$ L media.



- 4**
- Place device into a Petri dish and cover. Incubate at room temperature for 30 minutes.



- 5**
- Slowly aspirate 500 $\mu$ L of the sperm containing fluid from the top chamber.



- 6**
- Optional: Centrifuge at 300g for 5 minutes and then remove supernatant. Calculate the concentration of sperm and resuspend pellet as required.

**VetMotl Multi (850µL) Sperm Separation Device**  
**VME0850 Equine**  
**Equine ART Procedures: ICSI, IVF**

**FROZEN-THAWED SEMEN**

1. Thaw frozen semen (0.1-1 x 0.5mL straws) at 37°C for 30 seconds.
  - a) Extend thawed semen 1:1 (v:v) extender:semen. We recommend an extender compatible with your ICSI/IVF system.
  - b) Ensure there is a minimum of 850µl of extended semen to fill the lower chamber of the device to avoid any air gaps between the lower chamber and the membrane.
  - c) Use 1mL semen safe syringe to draw 850µL aliquot of extended semen.
2. Achieve seal in the inlet port and apply slow and steady pressure to inject 850µL sample into bottom chamber via inlet port. Achieve seal in the inlet port and apply slow and steady pressure to inject 3mL sample into bottom chamber via inlet port.
3. Use fresh 1mL semen safe syringe to draw 750µL media (e.g., ICSI/IVF media).
  - a) Prime outlet port with 50µL media.
  - b) Dispense the balance of the media onto the surface of the upper membrane by dropping from approximately 2cm above the membrane. Completely cover the upper membrane with media. Do not tilt the device to spread the media.
  - c) Gently tease out any air bubbles in the media and avoid penetrating the membrane.
4. Place device into a Petri dish and cover. Incubate at 30-37°C for 30 minutes in the conditions suitable for the ICSI/IVF media (e.g., 5% CO<sub>2</sub> or benchtop).
5. Insert a fresh 1mL syringe into the outlet port, achieving a firm connection. Very slowly aspirate 500µL of the sperm-containing fluid from the top chamber. Avoid aspirating too quickly, as this can pull unwanted material from the lower chamber through the membrane into the clean sample.
6. Optional: Centrifuge at 300g for 5 minutes and then remove supernatant. Calculate the concentration of sperm and resuspend pellet as required.