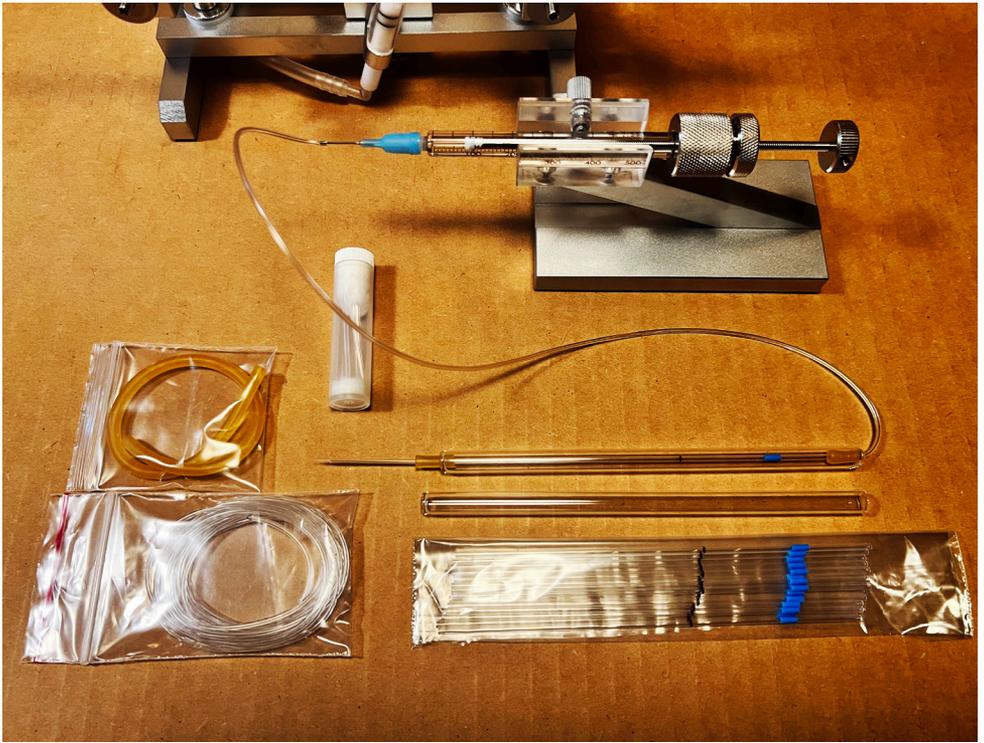


Susan W. Cobey

Harbo Syringe User Manual

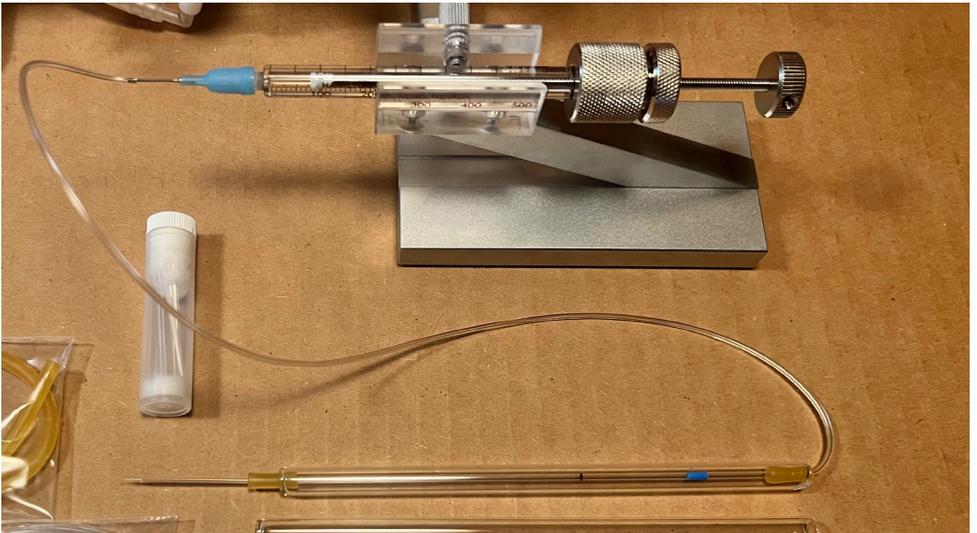


Washington State University
Honey Bee Program



Harbo Syringe Instruction Manual

The Harbo syringe is an innovative large capacity syringe designed to simplify the collection, handling, storage and shipment of honey bee semen. Semen is collected and can be stored in easily detachable capillary tubes, providing an unlimited capacity. The micrometer accurately measures semen dosage in both small and large quantities. The syringe increases efficiency for large scale production as well as specific requirements for specialized research purposes. Housed in a separate stand, the syringe is compatible with most insemination instruments on the market.



Features

Precise Measurement Of Semen Volume

The 500 μ L Hamilton micrometer enables the delivery of very accurate measurements. The calibration is 5.29 μ L per revolution. Two turns deliver a standard semen dosage per queen. The set screw marking on the plunger dial is a calibration indicator mark.

Unlimited Capacity

Semen is collected and stored in easily detachable capillary tubes, enabling efficient collection of an unlimited quantity of semen. The handling, storage and shipping of semen is simplified.

Detachable Glass Tips

Glass tips are easily changeable, connected to the capillary tube with a small piece of latex connector tubing. This also provides the advantage in giving the tip flexibility, and helps prevent breakage.

Protective Glass Barrel

A protective glass barrel houses the semen storage capillary tube. The barrel fits into the instrument syringe holder. It is sized to fit various instrument models.

Flexibility Of Connection Points Provides Convenience

The glass tips and semen capillary storage tubes are attached with a piece of flexible latex tubing providing easy and quick exchange of tips and storage tubes. The ability to remove the tip, for example to clear a mucus plug, without loss of semen is an advantage. Also, the various connections offer the ability to adjust the quantity of saline in the syringe, if necessary during semen collection and semen delivery, without loss of collected semen. For example, the tygon tubing connected to the needle head can be briefly removed for saline volume adjustments. Be sure to avoid air bubbles in the line during reconnection.

A Separate Syringe Stand

The syringe micrometer is held in its own separate stand. This helps reduce the chance of unwanted movement of the syringe tip once it is properly positioned. For production work, the separate stand allows the easy transfer of semen loads between instruments and operators.



CALIBRATION

The Hamilton micrometer syringe is highly precise, delivering 5.29 μL per revolution. Two turns deliver a standard semen dosage of 10.5 μL per queen. Use the set screw marking on the plunger dial as a calibration indicator mark.

CARE OF THE HAMILTON MICROMETER SYRINGE

To ensure years of use, cleanliness is important. After use, clean and empty the syringe for storage. Avoid withdraw of the plunger from the syringe. If the plunger is accidentally withdrawn completely from the syringe, clean the plunger with a lint – free cloth and wet with deionized or distilled water before re-inserting into the syringe barrel. Take care of the plunger tip, avoid scratches and physical abrasions, and avoid touching. Oil from your fingers may cause leakage when reassembled. Detergent based cleansers should not be used. Clean with alcohol and rinse with high grade water.

TO CLEAN

To clean the micrometer after use, expel the saline and fill with distilled water collecting from the needle. Repeat with alcohol and rinse again with distilled water. A plastic syringe can be used to pump fluid into the micrometer. Flush the alcohol and fill with deionized or distilled water, expel to rinse clean. Store the micrometer empty. The outside of the syringe can be wiped with alcohol and air dry. Note – Be sure the needle head is clean, a plugged needle can cause back pressure on the plunger.

STERILIZATION

Sterilize syringe parts before usage, as described..

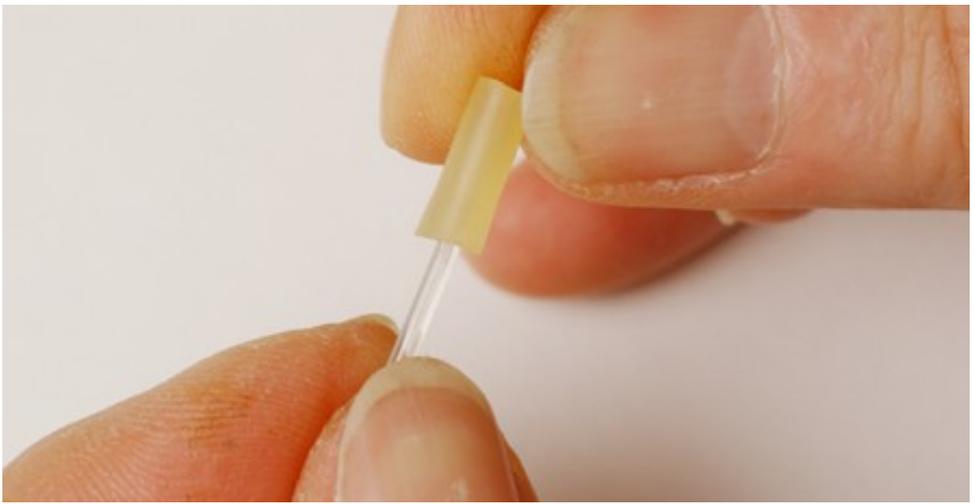
Flush the tygon tubing, capillary tubes and flexible tubing connectors with alcohol, followed by a thorough rinsing with distilled water. A plastic syringe, squeeze bottle or pipette can be used.

ASSEMBLY

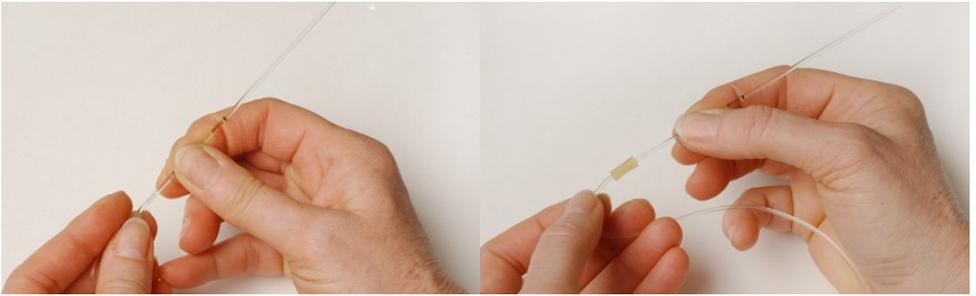
1. Wash hands and wipe the work area with alcohol, before assembly. Sterilize all syringe parts and accessories. During assembly, avoid touching parts that are in direct contact with saline diluent and semen.
2. Fill the micrometer barrel with saline solution, collecting fluid from the needle end. To remove air bubbles, hold the micrometer upright, tap gently and expel air bubbles from the tip. Collect additional saline in the micrometer, as needed to run saline through the system of tubing and



the attached capillary tube. Fit the tygon tubing securely over the needle head. At this stage the system is pre-assembled and filled with saline from the micrometer. To assemble the system before filling, following these steps.



3. Attach a piece of the flexible connector tubing to the tygon tubing.



4. Attach the capillary tube to the tygon, with a piece of connector tubing.



5. Pull the assembly of the capillary tube and tygon tubing through the protective glass barrel.
6. Push the capillary tube a short distance past the smaller opening of the protective glass barrel.
7. Fit the glass tip into another piece of connector tubing. Attach the tip with connector tubing to the exposed capillary tube.

8. Brace our fingers on the glass barrel and support the capillary tube to avoid breakage.
9. To secure the tip, twist the connector tubing with the attached glass tip to fit snugly into the smaller end of the protective glass barrel opening.
10. Fill the system with saline from the micrometer. Check there are no air bubbles in the system. At this



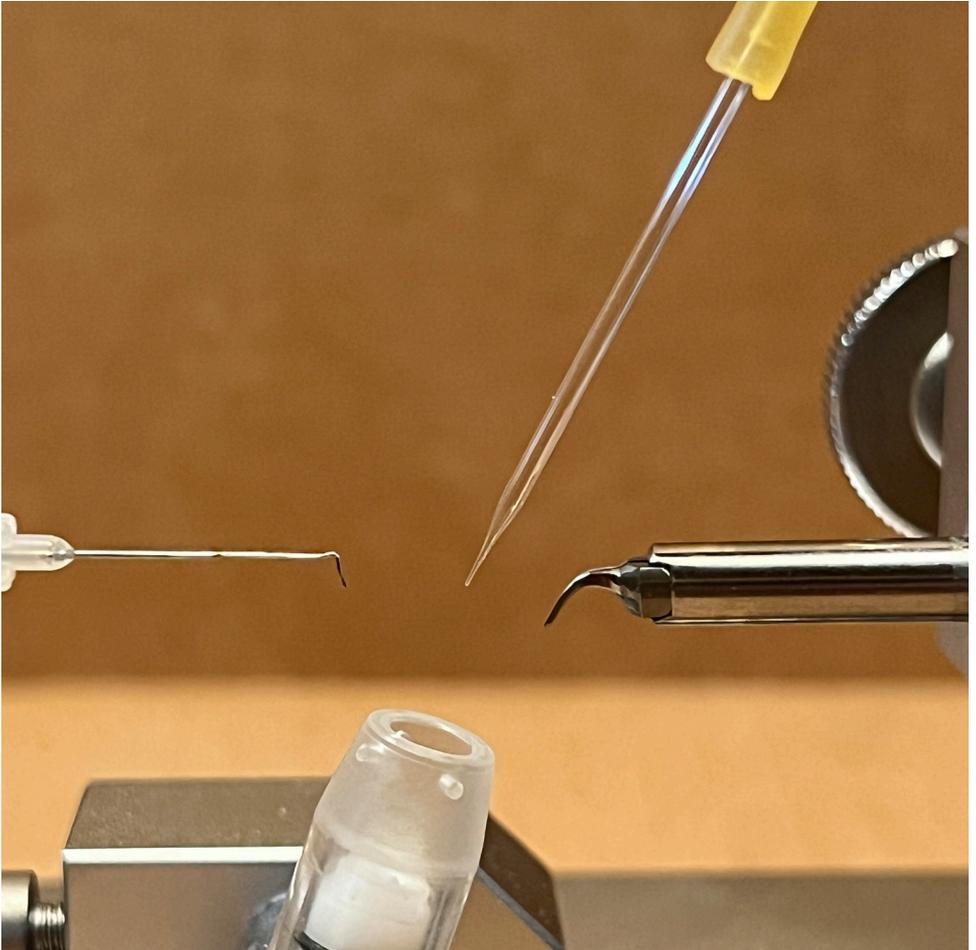
stage, more saline can be collected as needed, though allow enough space for semen collection.

11. The 100 μL capillary storage tube can hold about 130 μL (100 μL to the black line). The tip holds another 10 to 15 μL .
12. Before semen collection, be sure to create an air space between the saline solution and the semen to separate the semen column.



Glass Tips

Tips are cut, finely ground and fire polished. The tip finish is sized to the subspecies of honey bee inseminated, so please specify when ordering. Also the tip finish is straight or angled, based upon personal preference. The angled finish provides a larger surface area for semen collection and a leading edge for insemination. Therefore, the tips I make are angled unless requested with a straight finish. Tips vary in shape, size and length. The Harbo syringe uses a tip length of 3.5 cm. with the capillary storage tube. The European style syringe uses a tip length of 6 cm., as the tip and storage tube are one piece.



Semen Collection Points To Consider

Skim the semen from the endophallus into the syringe, avoiding the underlying viscous mucus layer. The semen column should be uniform in density. During the semen collection process, avoid adding air bubbles. Avoid adding excessive saline which will dilute the semen. During collection between drones keep the tip moist, collect a small drop of saline, as needed, to prevent drying.



SEMEN VOLUME MEASUREMENT

The Hamilton micrometer has a capacity of 500 μL . To calibrate semen delivery, one revolution is 5.29 μL . Two turns deliver a standard semen dosage per queen. For routine inseminations each queen requires a semen dosage of 8 to 12 μL . Each drone produces about 1 μL of semen. The set screw marking on the plunger dial serves as an indicator mark.

If the system is not highly responsive, check the line for air bubbles and leaks, as this can cause a “spongy” response.

SHORT TERM SEMEN STORAGE



To store semen, the filled capillary tube is sealed with a plug. A silicone cap, glass bead or petrolatum (Vaseline) seal is placed in each end of the semen filled capillary tube. Before sealing the tube, collect a small air space followed by several microliters of saline. Semen held at room temperature maintains good viability for about two weeks. For best results store at 13° C (55°F).

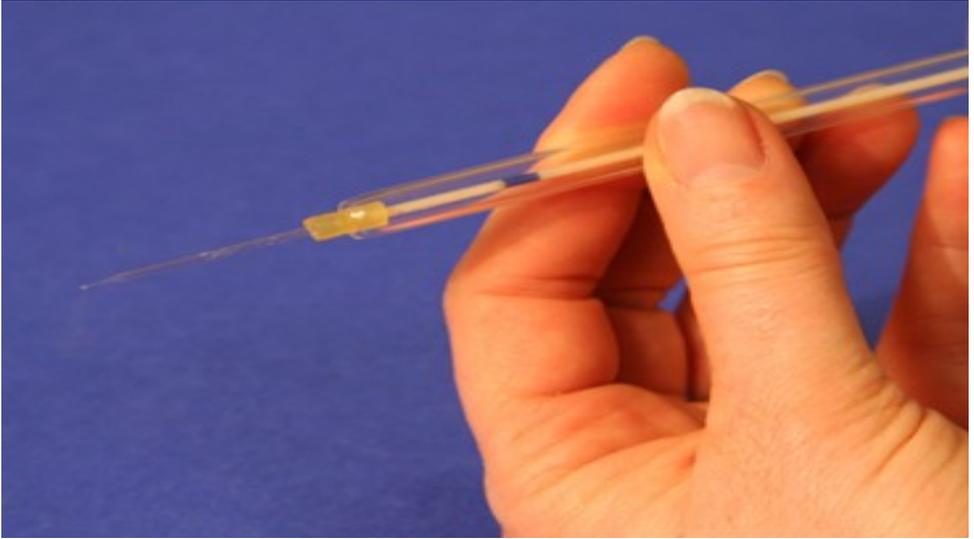
Do Not Refrigerate. Avoid Sunlight and Temperature Extremes.

Sterilize all syringe parts before use and maintain good sanitation throughout the collection procedure. This is especially important when storing semen.

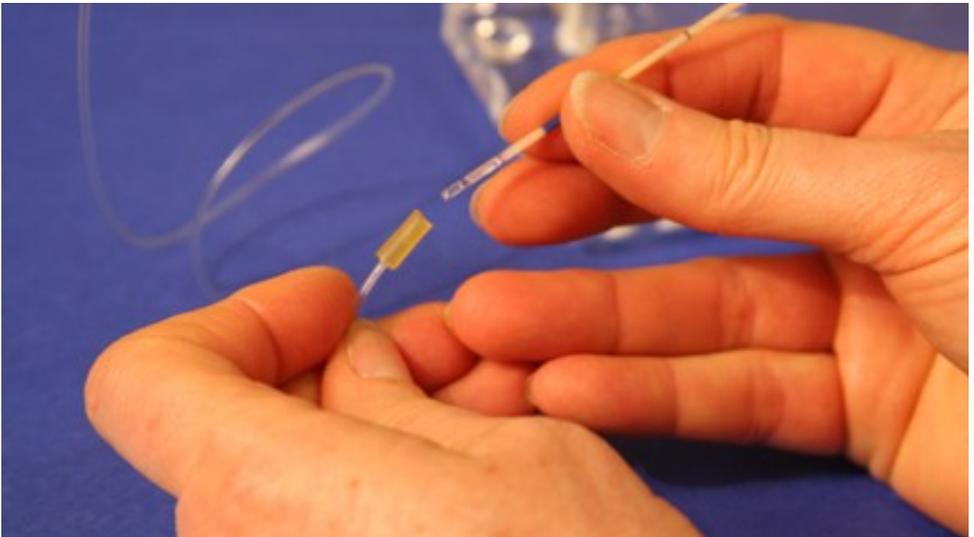


TO SEAL SEMEN STORAGE TUBE

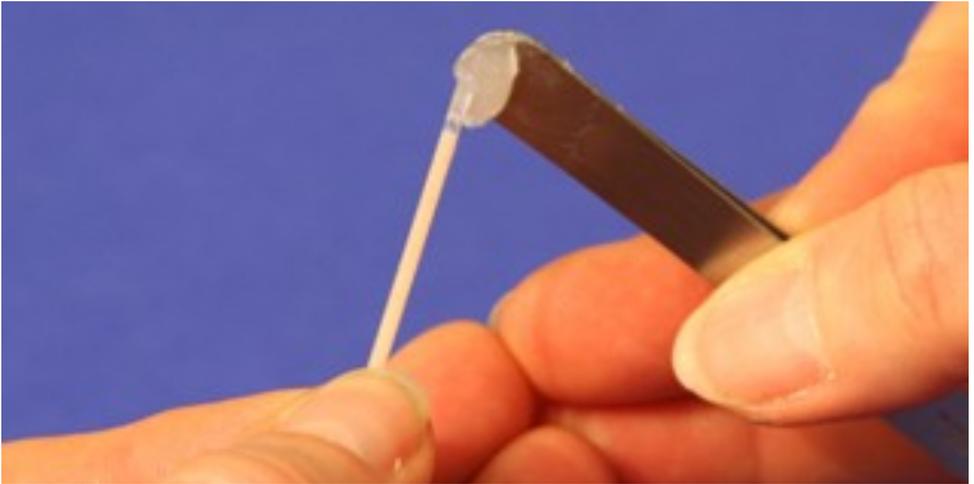
1. After semen collection, draw all the semen into the capillary tube. Collect an air space, followed by a few microliters of saline into the capillary tube.



2. Remove the glass tip and latex connector. Remove the capillary tube from the protective glass barrel, and detach from the micrometer. Place the plug or seal in the end of the capillary tube.

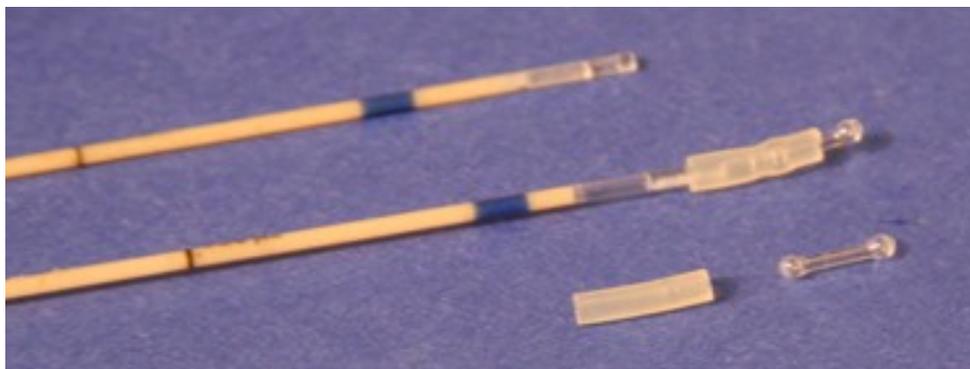


3. Repeat the process on the other end of the tube. If more space is needed to collect an air and saline spacers before sealing the opposite end, reconnect to the micrometer to push the column of semen. Allow enough space to seal both ends properly.
4. If the tube is not completely filled with semen, the capillary tube may be cut leaving enough space for a seal at both ends.
5. Place the sealed tubes of semen in protected packaging for storage & shipping.





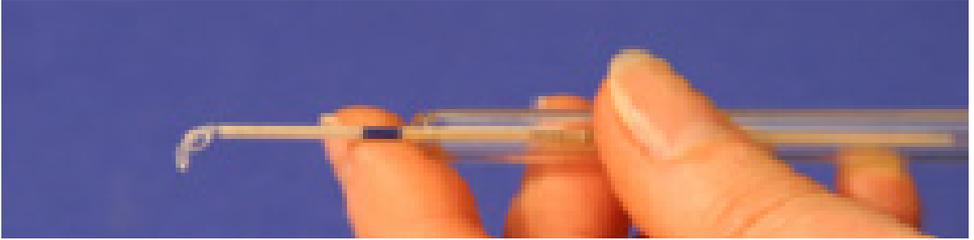
To seal the capillary tube for storage, a silicone cap or glass beads connected with pieces of silicone tubing can be used.



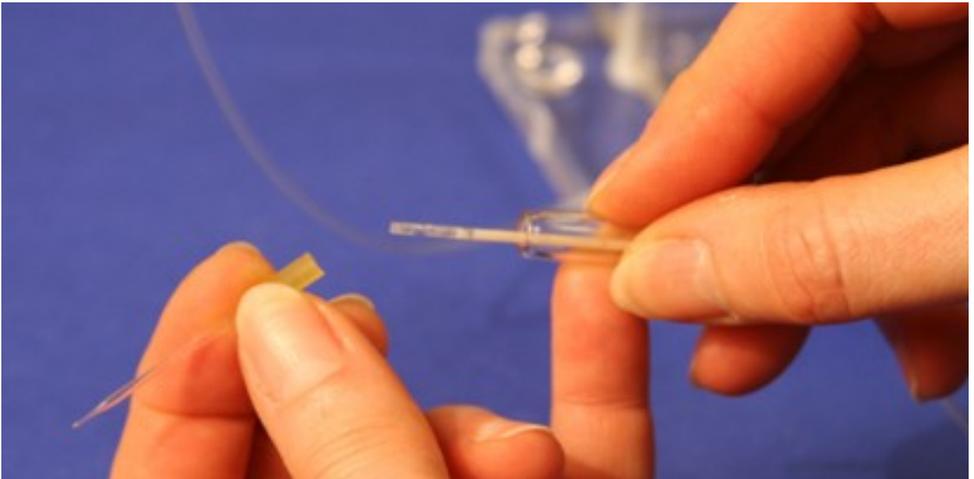
TO REATTACH THE SEMEN TUBE

1. Sterilize and fill the micrometer with saline. Attach tygon tubing and fill with saline. Collect a small air space before attaching the semen filled capillary tube to separate the saline and semen column. If using a petrolatum seal, this can remain or be removed by cutting this section of the tube. The plug at the other end must be removed before the tip is in place, use the micrometer to push out the petrolatum seal on the tip end of the capillary tube. The seal at the other end of the tube can be kept in place during insemination and will move with the column.

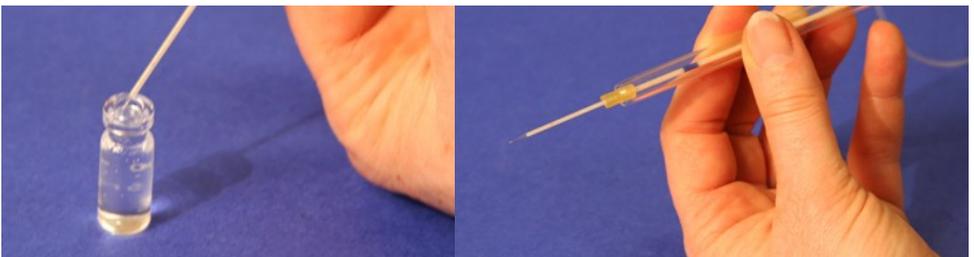
2. Take up a small air space and several microliters of saline in the capillary tube. Attach the glass tip and expel the saline to rinse the tip.
3. Collect a small drop (0.5 μ L) of saline in the tip to precede the first insemination.



4. After the inseminations are performed, take up saline to move the petrolatum seal out of the tip and into the capillary tube for disposal. Be sure not to get petrolatum in the narrow part of the glass tip as this will be very difficult to remove.



5. Dispose of the used capillary tube. Clean the syringe and glass tip before storage.



PROBLEM SOLVING

Unresponsive Syringe

If the system is not highly responsive, check the line for air bubbles and leaks, as this can cause a “spongy” response in collection and delivery of semen. The syringe system must be filled completely with saline to function accurately. Problems with air bubbles in the system are due to several factors:

Air bubbles in the micrometer glass barrel, needle head or in the tubing is problematic. A dirty micrometer plunger will also cause leakage. Check all connections. Check the needle head is clear of air bubbles, has not loosened or the base cracked, leaking in air. A dirty micrometer plunger will also cause leakage.

Important:

- Never grease the plunger of the micrometer.
- Avoid withdraw of the plunger from the syringe.
- Avoid detergent based cleansers
- Avoid touching the plunger. Oil from your fingers may cause leakage.

Care and Cleaning of the Micrometer

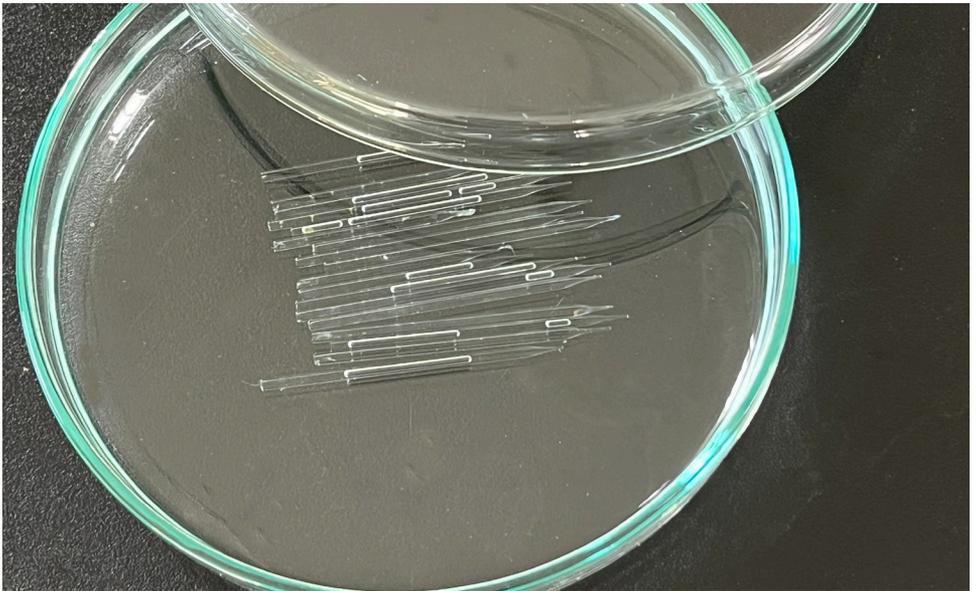
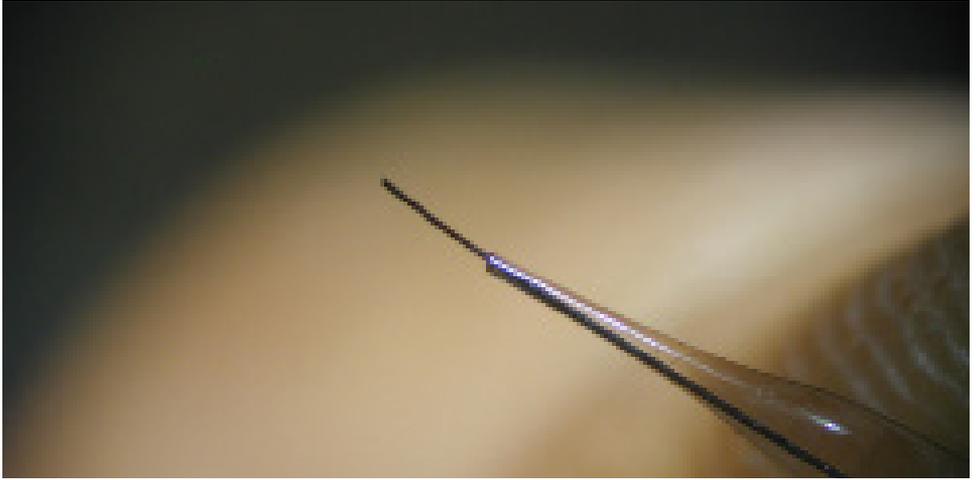
To clean, empty the syringe of saline and flush the micrometer with distilled water, followed by an alcohol rinse.

The outside of the micrometer can be washed with mild soap and water. A fine brush can be used to clean the ridged dial. Detergent based cleansers should not be used. Clean with alcohol and rinse with high grade water.

GLASS TIP CLEAN UP

After usage, remove and flush glass tips with distilled water. To remove semen residue, soak tips in a 10% hypochlorite (bleach) solution. This will loosen residue. Before re-use, flush well with distilled water.

To remove persistent residue from glass tips, use a cleaning wire. Insert the wire from the large end to avoid breakage of the fine tip. Do this under a microscope.



NOTES



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