

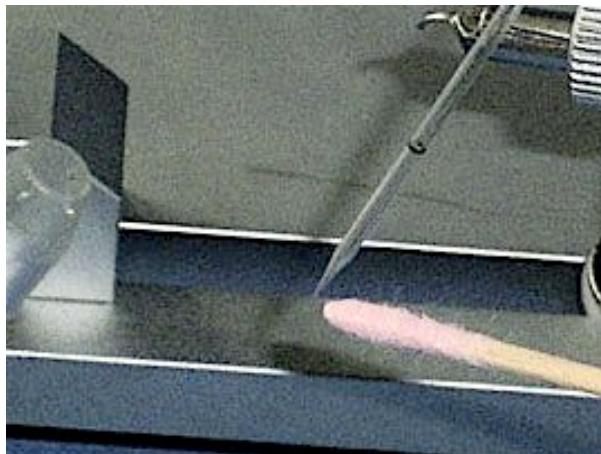
Cleaning and Disinfecting

Losses and infertility problems with inseminated queen bees are often the result of improper insemination techniques and infection due to contamination during the procedure. To achieve optimum results, an essential requirement is keeping the work area and the equipment as clean as possible.

Nature, by no means, functions under sterile conditions. However, it is important to avoid harmful pathogens. Therefore, all instrument parts, direct or indirect, which come into contact with the sperm and the reproductive organs of the queen bee must be kept sterile.

The potential problem is exposure to pathogens, which under certain circumstances, may become harmful to bees. The spore-forming bacteria *Bacillus cereus* was e.g. determined responsible for queens of entire productions dying off. The germ in and itself is not pathogenic. It can be found in the faeces of queens and drones. However, if the germ enters the reproductive tract of the queen, it will multiply rapidly. The excretion of poisonous metabolites leads to severe symptoms of poisoning. The aforementioned bacillus is also found in soil and dust and is quite similar to the anthrax bacillus. Also other pathogens, such as streptococcus bacteria will have a similar impact.

Special attention must be paid to the insemination glass tips. During semen collection, these must constantly be cleaned with cotton swabs rinsed with a diluent.



To prevent the glass tip opening clean, use sterile cotton swabs.

Following use, immediately place glass tips in soapy water to ensure no residues dry on the glass surface, as this will be difficult to remove.



Dishwashing detergent and Petri dish (Ø 100 x 15 mm)
for cleaning insemination glass tips.

Later, rinse glass tips thoroughly using a disposable syringe. Follow this with a rinse of water and alcohol rinse, and lastly with physiological saline solution to remove any remaining alcohol residue. If glass tips are to be stored dry, use acetone in as a final rinse. The acetone dries quickly and leaves no residue.

Alcohol

Alcohol disinfects and is universally used for this purpose. However, alcohol may not come in contact with sperm, and is also not suitable for cleaning the queen holding tubes. The Plexiglass tubes do not tolerate alcohol, which will become cloudy, brittle, and crack with prolonged exposure to alcohol.

Use technological isopropyl alcohol (70%), available in any pharmacy. This is also preferred to clean the hooks and the hand held probe. The HARBO syringe and its connecting tubing are also rinsed with alcohol and followed by rinsing with distilled water. The base of the insemination syringe's rubber bulb can also be wiped and cleaned with alcohol, or the entire spindle can be placed in the pressure cooker.

Pressure Cooker

A safe and easy method of sterilization is to use a pressure cooker. At a pressure of 1 bar, regulated by a pressure relief valve, the temperature will be about 120 degrees C. After an actual treatment period of 10 minutes (not including preheating), the parts will be sterile.



Sterilization in the pressure cooker

Place items to be sterilized above the water level in the vapour phase area. Use a strainer insert with a spacer in the pot. Sterilize the **Insemination glass tips (with the squeezing seal tubing attached), syringe cylinder, cotton swabs on wooden core, disposable syringes with rubber plunger, steel needle tips for the disposable syringes and any other syringe parts; such as sleeve and plunger spindle.** Loosely wrap the parts in aluminium foil.

Bacteria Filter

To sterilize the filtering distilled water and physiological saline solution, use disposable bacteria filters with pore size 0.2 μm and a diameter of 30 mm. The filtration process is intended as added safety to prevent infection. This is especially important if the physiological saline solution used in the insemination syringe has been used for several days. The filter is threaded to fit between the steel needle tip and the disposable syringe. The filtration process is quite simple and recommended.



Disposable filter 0.20 μm

Bacteria filtering as added safety. The air drawn is also filtered.

Summary

- 1) Clean the equipment with a damp cloth. During eversion of the drones for semen collection, turn away from the work area to avoid faeces spraying onto equipment. Wash hands frequently.
- 2) Alcohol is primarily used to clean the hooks and the handheld probe. Alcohol can also be used to flush the HARBO syringe. Do not allow the in feed tube and the holding tube to come in contact with alcohol. The opening of the insemination glass tip is cleaned with cotton swabs moistened with sterile saline solution.
- 3) Only place heat resistant items, which come in direct or indirect contact with semen, in the pressure cooker. This includes insemination glass tips with the attached squeezing seal, syringe cylinder, disposable syringes with rubber plunger used for filling the syringe, steel needle tips and cotton swabs.
- 4) If distilled water and physiological saline solution have been in use for several days, it is recommended to run these through disposable bacteria filters to ensure the solutions are sterile.

Above recommendations only represent guidelines and reference points for beginners. Experienced users may modify sterilization techniques as desired.

Peter Schley