How to Carry Out the FKB Test for Hygienic Behaviour

Hygienic behaviour is a natural form of disease resistance effective against American foulbrood, chalkbrood, deformed wing virus, and varroa. Beekeepers can breed hygienic bees by rearing queens (or males to mate or inseminate queens) from hygienic colonies. But how do you know which colonies are hygienic? This pamphlet gives background information on quantifying hygienic behaviour using the freeze-killed brood (FKB) test.

British Oxygen liquid nitrogen truck filling up Dewar flask near LASI apiary

Transporting Dewar flask of liquid nitrogen by vehicle but not in passenger compartment.

Sack cart being used to transport strapped in Dewar flask 1 mile on foot to apiary.

Handcart (optional) for moving Dewar flask. Flask can also be carried by handles.

Dewar flask, 25 litres, to hold a large volume of liquid nitrogen.

Wide top cryogenic flask, 4 litres, to hold liquid nitrogen during FKB test.

Metal cylinders to press into sealed brood to hold liquid nitrogen.

Funnel & ladle, 50 ml, for transferring liquid nitrogen into cylinders.

Hand & eye protection. Follow regulations, use care and common sense.

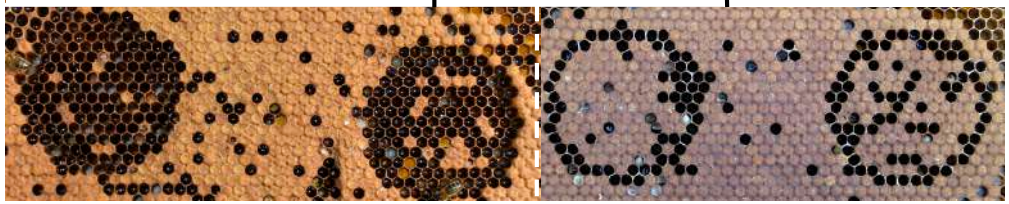
LASI has been using the FKB test since 2004 to test hundreds of colonies. The FKB test was developed at the University of Minnesota by Professor Marla Spivak and colleagues. Its main advantage is that it can be done in a single apiary visit, with a second visit 2 days later to see the results. An apparent disadvantage is that it uses liquid nitrogen, a material most beekeepers are unfamiliar with. A large Dewar flask to hold sufficient liquid nitrogen is also needed. But liquid nitrogen is cheap and a Dewar flask lasts indefinitely. Like many things, it is easy when you are set up and know the ropes.

The basic method is simple. A hive is opened and a frame with sealed brood removed. Two metal cylinders (LASI uses 6.5 cm diameter × 8 cm height) are pushed into suitable brood to the mid rib. Do not use brood within a few days of emerging. A small amount of liquid nitrogen is then poured into each cylinder using a ladle for transfer from a wide mouthed cryogenic thermos flask part filled from the Dewar flask. The nitrogen boils away rapidly. Over a few minutes gradually add c. 150 ml per cylinder to kill the brood. When all has boiled away, wait a few minutes and remove the cylinders from the brood. If this is done too soon the cylinders will be frozen tight and will pull out some of the brood. A photo is taken, the frame is put back, and the hive is closed. After 2 days the treated frame is removed and another photo is taken. The number of freeze-killed capped cells that have been fully cleaned out is determined by comparing the two photos. Do not count empty cells made by the cylinder as this damages a circle of brood, which are quickly removed. Only count the cells inside the circle. Colonies than have cleaned out >95% of the previously-sealed freeze-killed cells are considered highly hygienic. LASI makes 3 or 4 tests per colony at 1-2 week intervals. Colonies being tested should have the same queen throughout and should not receive brood from other colonies. If you are screening hives for the first time for breeding stock, try to test 25-30 or more to have a good chance of finding one or more that are highly hygienic.

Freezing sealed brood with liquid nitrogen, 150 ml per cylinder. Ladle transfers nitrogen from 4 litre flask, 3 scoops per cylinder, over a few minutes.

Brood from several colonies can be treated at once. Another person removes and replaces test frames.

Liquid nitrogen boils at -196°C so is soon all gone. The cylinders now have frost on them.



Brood frames two days after freeze-killing with liquid nitrogen. The left colony has a high level of hygienic behaviour, about 85%. Note that some cells have not been cleaned out. The colony on the right has a low level, about 10%. Very few freeze-killed cells have been cleaned out. Circles of cleaned out cells can clearly be seen. These are the cells that contained brood damaged by the metal cylinders. These are quickly cleaned out. Do not count these. Only count the cells inside the circles. To determine the proportion killed, divide the number of cells not cleaned out with the number that were capped at the time of freeze killing.

In the UK liquid nitrogen is sold by British Oxygen "Cryospeed" who will deliver to your door and may also rent out a Dewar flask. Liquid nitrogen is kept by universities and hospitals which may be able to supply small amounts by arrangement. Liquid nitrogen is potentially hazardous and any beekeeper planning to carry out FKB tests should carefully study the relevant safety information and follow any regulations. Key safety points are: 1) Do not transport a flask of liquid nitrogen inside a vehicle. Although nitrogen is non-toxic, it could asphyxiate if it filled an enclosed space such as a passenger compartment or room and displaced the oxygen; 2) Do not get liquid nitrogen on your skin or eyes as it can cause frostbite. The use of gloves and goggles is recommended unless you are highly experienced; 3) Flasks of liquid nitrogen should be stored, transported, and used in ways that minimize the chance of being knocked over.

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