

The Management Agency's guidelines on the use of quantitative PCR (qPCR)/AFB spore testing

qPCR is a widely used diagnostic tool for the early detection of AFB in many countries.

qPCR testing for AFB is used by The Management Agency for:

- confirming whether reusing AFB contaminated gear (in storage) is the reason behind the high incidence of AFB identified in the field,
- confirming any presence or absence of AFB contamination on neglected apiaries where the state of decay or absence of the brood prevents the completion of a reliable visual AFB inspection,
- confirming whether there is AFB contamination in processed honey and,
- verifying that used beekeeping gear is left open and accessible to bees is not contaminated with AFB spores.

Note: Spore testing does not replace the requirement to carry out visual inspections.

How does the Management Agency determine if spore tests are necessary?

1. AP2 visual inspections completed following an AFB report.
2. AP2 visual inspections of an apiary find incidence of clinical AFB *higher* than the national incidence of AFB at that point in time (greater than 0.5% on average).
3. Further AP2 inspections of other apiaries belonging to the same beekeeper, find additional incidence of AFB at a higher rate than the national AFB incidence level.
4. AP2 inspections confirm that the beekeeper is unable to identify clinical symptoms or is not proactively inspecting hives for AFB.
5. Operations Managers (AP1s) assess the likely pathway of AFB spread across the beekeeper's operation. This is often linked to beekeeping equipment that has been previously used on AFB-positive hives and re-used across different hives and apiaries.

Note: Clause 28 of the AFB NPMP places an obligation on all beekeepers to destroy AFB-infected hives and associated gear within 7 days.

6. Operations Manager suspect that used beekeeping equipment in storage (that does not contain brood) may be contaminated with high levels of AFB spores. This cannot be verified by physical inspection due to the absence of brood.
7. Operations Managers/AP1s issue written permission to AP2s directing composite swab samples be collected.
8. The beekeeper's suspected AFB-contaminated beekeeping equipment is swabbed to confirm the presence or absence of AFB spores. The results will help determine

the likelihood of new cases of AFB arising, if this equipment was used again on healthy colonies.

How are swab samples taken?

1. AP2s can only swab used beekeeping equipment under written instruction from an AP1. **New beekeeping equipment not required to be swabbed.**
2. AP2s have received training from diagnostic labs on how best to collect swab samples.
3. AP2s make accurate notes related to the location and items swabbed, number and type of equipment associated with each swab.
4. The swab samples are sent to the lab with a unique number. I.e. the lab does not know where or who the samples have been collected from. All other details related to the samples are retained by The Management Agency against the beekeeper's records.

How the Management Agency reads the spore results

- Conducted research determines the minimum threshold of spores on used beekeeping equipment that would indicate equipment has previously been associated with clinical cases of AFB.
- Based on technical advice and statistical analysis, The Management Agency and diagnostic laboratories have been using the AFB spore count value from the CART analysis method (also used by MPI for the Manuka Honey definition).
- This form of analysis provides a threshold where a spore count of >18,000 spores has come from a hive (bottom board) showing visual signs of AFB or one where it was pre-clinical and showed visual signs of AFB infection within weeks of testing.
- The level of AFB contamination in a bottom board is expected to be the highest compared to other pieces of equipment (except brood frames), as it sits directly under the brood box and all AFB material falls onto the bottom board by gravity.

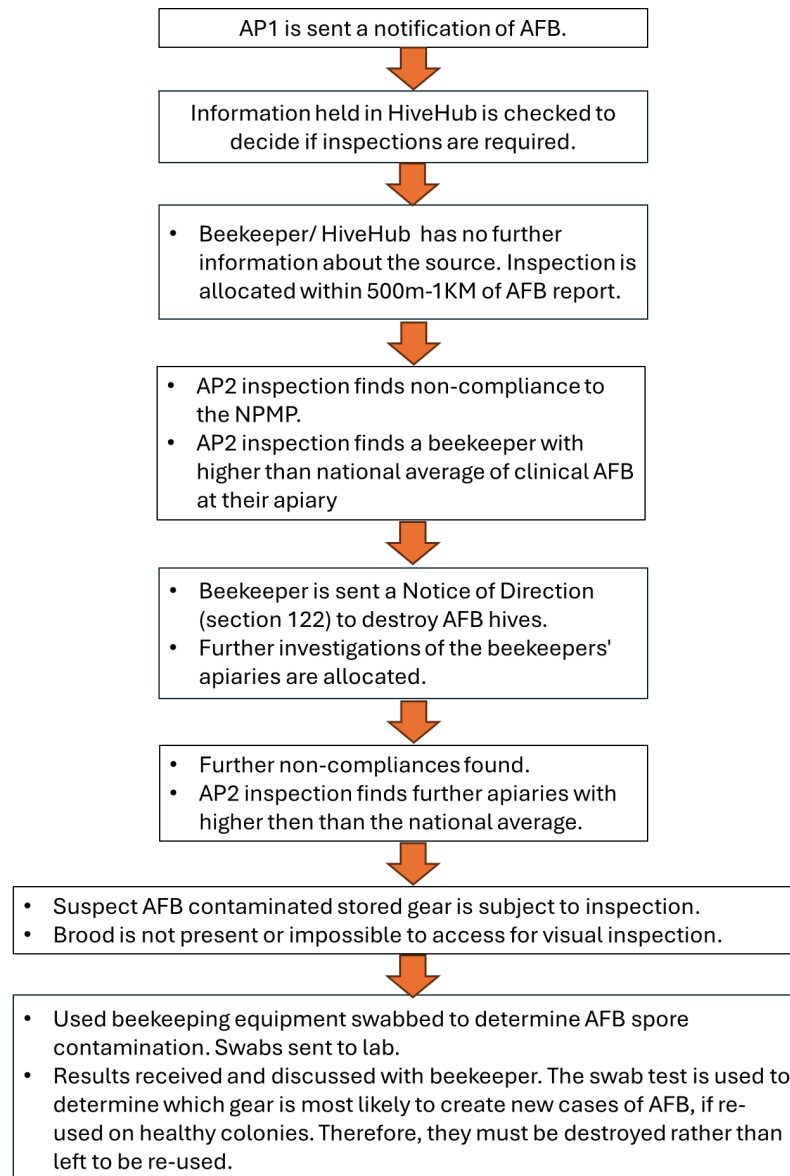
It is reasonable to expect that the level of spore contamination present on other beekeeping gear (e.g. honey supers) that are located above the brood boxes, especially those previously associated with clinical cases of AFB, will be lower than the 18,000-spore threshold.

Next steps

- Before deciding on next steps, The Management Agency's Operations Managers review the beekeeper's compliance history, record-keeping, general beekeeping and AFB management practices, adherence to all AFB NPMP rules, and the degree of cooperation the beekeeping operation has shown to The Management Agency's surveillance inspections.
- Once the swab results are received from the diagnostic lab, it is shared with the affected beekeeper.

- Where swab results show a contamination level of 18,000 spores or above, the affected beekeeper is directed to destroy the beekeeping equipment (i.e. a Notice to Destroy).
 - Where swab results contain spore numbers *less than 18,000*, the Operations Managers and the beekeeper will discuss the best ways to mitigate the further spread of AFB. Options could include keeping contaminated beekeeping equipment separate from other equipment and trace its usage.
 - Beekeepers are also encouraged to destroy potentially contaminated equipment to minimise future AFB risk.
 - Where swab results show 0 spores, no further action is required.
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- **Any directions given by an AP1 must be followed, however, we are open to discussion.**
 - **Please communicate with The Management Agency early and await a response, prior to actioning a Notice to Destroy. If your query has been sent to The Management Agency after business hours, the Operations Manager will contact you on the next business day.**

Steps taken by AP1s, prior to swab tests



Number of spores	Action taken by TMA
18000 +	Directed to destroy this equipment
Under 18000	Conversations with Operations Managers about next steps