

## PEST AND DISEASE CONTROL

# AFB testing: commonly asked questions

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Laboratories are regularly asked questions about AFB testing, and this has increased over the last 12 months as a result of changes to the requirements for honey being exported to China. This article sets out to answer some common questions.

## BACKGROUND

American foulbrood (AFB) is a disease caused by bacteria (*Paenibacillus larvae larvae*) that infect developing brood, and in doing so weaken and kill the hive. AFB is a notifiable disease that has been governed by a National Pest Management Plan (NPMP) since 1998. AFB has been found in New Zealand hives for over a century, and the first legislation to control it was passed in 1906.

The current Pest Management Strategy for AFB became law in 1998, and all beekeepers in New Zealand should be familiar with its requirements to inspect hives regularly, destroy any infected hives, and report the results of inspections to the AFB Management Agency. The Management Agency's website ([www.afb.org.nz](http://www.afb.org.nz)) provides a great deal of useful information.

Requirements for AFB testing changed significantly in late 2020, when the Ministry for Primary Industries (MPI) informed the industry that honey exported to China may be tested for the presence of AFB bacteria or spores by Chinese laboratories. As a result, any honey being exported to China must now be tested for AFB by an MPI Recognised Laboratory Programme (RLP) Approved Laboratory, and there must be no AFB detected in the sample before it is approved to be exported to China. While Chinese laboratories may still test honey arriving in China for AFB, this does ensure that no honey with detectable AFB in it is exported there.

These requirements have created an entirely new level of need for AFB testing in New Zealand. Not only are shipments of honey sent to China being tested, but also bulk honey offered for sale in New Zealand is also being tested to check whether it is suitable to use in manufacturing honey for export to China.

## HOW LABORATORIES TEST FOR AFB

AFB is tested using a very sensitive DNA testing technique called polymerase chain reaction (PCR), which specifically targets AFB while ignoring any other bacteria in the honey. This is similar to the test understood to be used in Chinese laboratories, but is different to the one traditionally used by the AFB Management Agency when investigating hives with visual symptoms of AFB.

In simple terms, the sample is prepared so that DNA is released from any spores or cells in it. Then it is analysed using the PCR technique, which targets DNA that is specific to the bacteria which causes AFB (in a similar way to the MPI Mānuka DNA test, which targets DNA that is specific to the mānuka plant).

PCR is fast and very sensitive—much more so than traditional microbiological plating tests that have been used for AFB testing in the past. What this does mean is that it can easily detect AFB in honey from hives that are not showing any visual signs of the disease. It can also detect DNA from bacteria or spores that are dead, as well as those which are still alive.

Most laboratories' test reports will include an estimated number of bacteria and/or spores per gram of honey in the sample that was supplied to them.

## HOW TO UNDERSTAND RESULTS

### Cq values and AFB spores per gram

PCR testing expresses results using a measure called a *Cq Value* (which stands for 'cycles quantified'). This measures the number of PCR cycles it took to detect AFB DNA in a sample at a threshold level. If there is more AFB DNA in the sample, it will take fewer cycles (a lower Cq value) to reach the threshold. Or, put another way, a higher Cq value means there is less AFB DNA in the sample.

Analytica's AFB test can detect and quantify AFB bacteria or spores in a sample up to 35.00 cycles—this is known as the limit of quantification (LoQ).

The following table provides a guide to how Cq values relate to numbers of AFB bacteria or spores in a gram of honey in the test method used by Analytica. At one extreme, a Cq value of 16.9 equates to over 7 million spores/bacteria per gram of honey. At the other, a Cq value of 35 equates to 92 spores/bacteria per gram of honey.

Average Cq	AFB spores (and/or cells) per g honey
16.90	7,142,857
20.63	714,286
24.24	71,429
27.82	7,143
31.61	714
33.83	179
35.00	92

### A result of Cq 35 or less is Positive for AFB

A Cq value of 35 or less means there are definitely some AFB bacteria or spores in the honey, and the honey will not comply with requirements for export to China.

It's not as clear how to interpret these results when it comes to managing AFB in hives. If there is a positive AFB test result, then at least one of the hives that contributed to that batch must have had some AFB bacteria in it. However, the hive(s) may not have been showing any visual or clinical signs of AFB so could be impossible to find. The AFB Management Agency is best equipped to provide you with advice on how to proceed if your honey sample returns a positive result for AFB.

### **A Positive result in a honey sample will not increase over time**

AFB bacteria only grow inside a bee larva, and don't grow in honey. So the test result from that same sample will not increase over time if re-tested (other than normal variability arising from testing). Please be aware, though, that AFB is able to survive for a long period in honey, and if bees have access to honey containing AFB bacteria or spores, this can result in spread of the disease.

### **A result of Cq 40 or more means that AFB was Not Detected**

A result of Cq 40 means that no AFB DNA was detected in the sample by the test and will usually be described as Not Detected on a test report. This result does not necessarily mean there are no AFB spores/bacteria in the sample; but if there are some, they are too few to be detected by the test.

A test result only relates to the time that sample was taken. Hives may develop AFB after the honey has been taken off, and so a Negative result for AFB should not be used as a reason to stop doing normal visual AFB checks on hives at other times of the year.

### **A result of between Cq 35 and 40 is a weak detection of AFB in the sample**

Some samples return a Cq result between 35 and 40, and these are more difficult to interpret. They do mean that a small amount of AFB DNA was detected in the sample. However, if that sample was to be re-tested,

or if a new sample from the batch was to be taken and tested, it's quite possible it could return a Positive or a Negative result. Once again, for help interpreting a weak detection in a test, contact the AFB Management Agency.

### **Beware of False Positives from cross-contamination**

PCR is a very sensitive testing technique, and even small amounts of cross contamination can cause a 'false positive' result which leads you to wrong conclusions about that sample—especially if one of your batches of honey has a lot of AFB spores/bacteria in it. You can avoid cross-contamination by carefully cleaning and sterilising sampling equipment with methylated spirits between samples.

### **HOW CAN I GET VALUE FROM MY AFB TEST RESULTS?**

1. A Negative result for AFB means that the honey is suitable for export to China. This is not only useful for those packing honey to send to China—it's also a way of adding value to your bulk honey if you are wanting to attract interest in it from potential buyers.
2. A Positive result for AFB is telling you that there was a hive or some hives with an AFB infection at the time when the honey was being produced by the bees. If you can identify the apiary sites linked to that batch, you can take a careful look

at the hives at those sites to see if any are showing clinical signs.

3. You can use the test to check out samples from hives that you are concerned may have AFB. Call your laboratory to check what kind of samples they are able to accept, but if you have a hive you are concerned about, then a sample of honey or brood from the hive can be tested to see if any AFB is present.

### **REFERENCES**

Analytica Laboratories. American Foulbrood (AFB) Testing. Retrieved September 6, 2021, from <https://www.analytica.co.nz/News-Resources/american-foulbrood-afb-testing>

Corbett, G. (2021, April/May). Testing for AFB. *The New Zealand BeeKeeper*, 29(3), 35.

The Management Agency, National American Foulbrood Pest Management Plan. Interpreting results. Retrieved September 6, 2021, from <https://afb.org.nz/interpreting-results/>

The Management Agency, National American Foulbrood Pest Management Plan. What is AFB? Retrieved September 6, 2021, from <https://afb.org.nz/what-is-afb/>



*Frame of bees heavily infected with AFB. Photo: Dwayne Hill.*