AFB PCR Testing

Hill Labs offers rapid, next day results, for the detection and quantification of American Foulbrood Bacteria (AFB) in honey.

What is AFB?

The bacterium called *Paenibacillus larvae* is the causative agent of American Foulbrood disease (AFB). *P. larvae* can exists in two forms, namely spores and vegetative cells. Spores can survive in the environment for a long time and are extremely difficult to eliminate due to their ability to withstand high temperatures and resistance to disinfectants. AFB is a fatal bacterial disease of honeybee brood. The disease is not able to be cured, resulting in the destruction of infected colonies and hives or irradiation of infection material being the only way to control AFB.

AFB is a notifiable disease in New Zealand and is managed by the AFB National Pest Management Agency. For more information please refer to: https://afb.org.nz/

Testing for the presence of AFB is an overseas market access requirement (OMAR) for China and a few other selected counties.

AFB Testing Requirements

- Ensure any sample submitted for testing is a good representation of the batch of honey it has been sourced from. For more information on collecting a representative honey sample please see this technical note
- Typically, a minimum of 5g is required, but if additional testing is required, further sample may be needed. Please contact our client service team to discuss your needs.

For more information, freephone 0508 HILL LAB 44 555 22 mail@hill-labs.co.nz hill-labs.co.nz

What Happens in the Lab?

In the lab, DNA from both AFB cells and spores is extracted from the honey sample. The DNA is then tested using a molecular technique called polymerase chain reaction (PCR). PCR reactions cycle through a series of temperatures, typically 40-45 times, during which any AFB DNA present is amplified to high levels. This amplification is detected in 'real-time' by the production of fluorescence, as the AFB DNA is being multiplied during each cycle of the PCR reaction. The point at which the level of AFB DNA in any test sample is detected is defined as the Cq value. Cq stands for quantification cycle and the Cq value is the point at which the fluorescence in a PCR reaction cross a pre-determined threshold **(Figure 1a**). The lower the Cq value the higher the starting concentration of DNA present in the original sample. The Cq value is converted into the corresponding number of genomic copies of *P. larvae* and reported as equivalent AFB cells and/or spores per gram of honey.

Figure 1a) Overview of PCR reactions

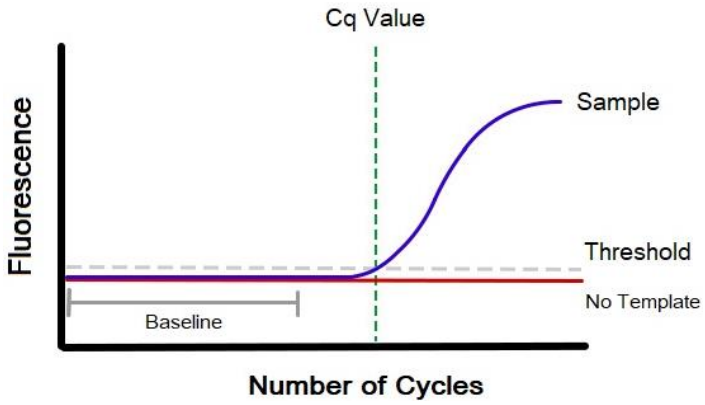
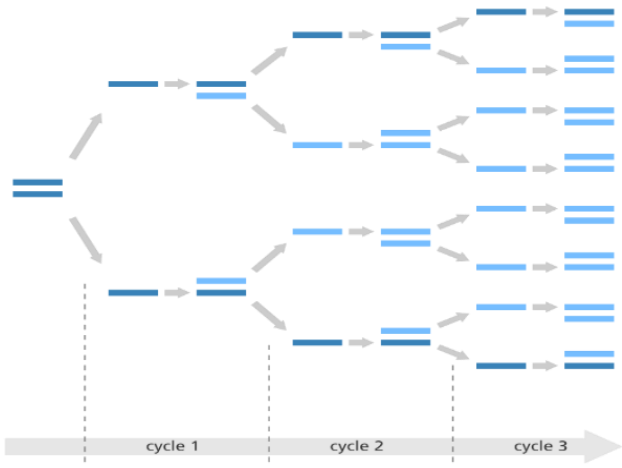


Figure 1b) Exponential amplification of DNA during PCR reactions



Theoretically after each cycle of a PCR reaction, with 100% efficiency, the amount of DNA present is doubled. For example, starting with 1 bacterial cell/genomic copy of AFB, after the 1st cycle of PCR this is doubled to 2 copies, after the 2nd cycle this increases to 4 copies and so on (**Figure 1b**). Therefore, as PCR can amplify very small starting amounts of target DNA, it is very important to be careful not to cross contaminate between samples during collection.

As with any analytical testing method there is uncertainty around the true value of the result. For PCR the uncertainty of measurement (UoM) typically can be in the range of ±1.5 Cq.

Contact

For further information, contact one of our friendly Food and Bioanalytical Client Service Managers on: fnb.csm@hill-labs.co.nz or 0508 HILL LAB (0508 44 555 22).