TECHNICAL SUMMARY

AFB and mānuka DNA testing in the lab

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For many years, Analytica Laboratories has been performing Polymerase Chain Reaction (PCR) testing to detect the levels of American foulbrood (AFB) spores and mānuka pollen DNA in honey. The basis of this test is rather simple; we extract, amplify, and quantify a section of DNA code in the honey sample that is specific to the analyte we are looking for. In this case it is the DNA of the AFB bacteria or the DNA of the mānuka flower's pollen.

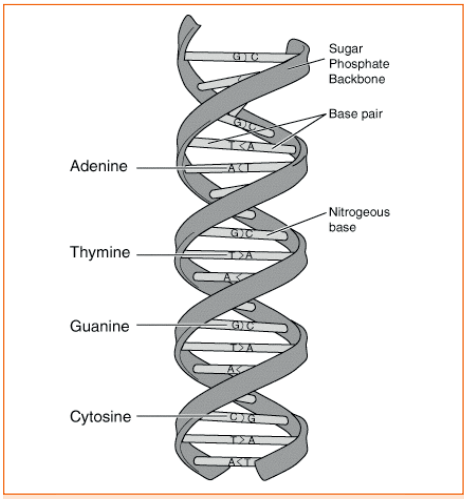

Figure 1: Diagram of a section of DNA (Wikipedia).

WHAT IS DNA?

Deoxyribonucleic acid (DNA) can be thought of as the blueprint for life. DNA is a string of genetic code that holds the information used for the development and functioning of an organism. Using science, we have been able to visualise DNA sequences which allows us to distinguish sections of code that are unique to that organism. Above is a section of DNA made up of Adenine, Thymine, Guanine and Cytosine: four bases that form the genetic code for all living things. The arrangement of these four bases will provide the blueprint for creating life like AFB and mānuka trees.

The process of testing for AFB and mānuka pollen DNA is a two-step process.

THE EXTRACTION PROCESS

The first step of these tests is to extract the DNA from its protective 'case' using an extractant solution and process. We need to perform this step because the protective case would prevent any solutions we add from reaching the DNA and therefore inhibit the PCR process from working effectively.

THE POLYMERASE CHAIN REACTION (PCR) PROCESS

Once the DNA has been extracted from the protective casing, we then add reagents to the sample which contain 'DNA probe' with specific target sequences called 'primers'. For ease, I'll refer to the primers and probes just as the 'DNA probe'. These DNA probes are specifically designed and only target exact regions of the genetic sequence.

Depending on what test we are running we will add either the mānuka pollen DNA probe or the AFB bacteria DNA probe.

Once the probe has been added, we then put the sample on the PCR instrument. This instrument works by repeating a number of 'cycles'. Each cycle has three steps as described below.

1. **Denaturation:** The extracted DNA is heated, which splits the double helix into two single strands.
2. **Annealing:** The sample is then cooled, allowing the DNA probes we added earlier to find and recombine to the specific section of DNA.
3. **Elongation:** An enzyme called 'polymerase' will then copy any DNA that the probe has attached to using nucleotides (spare bases) found in the cell, thus amplifying the DNA region we are targeting. For example, if our probes found five strands of DNA during cycle one, the polymerase would replicate these, and we would start cycle two with 10 strands of DNA. This would repeat until we had amplified enough DNA to reach the detection threshold.

This process is then repeated until we have completed 40–45 cycles (Cq) and we can determine a concentration or level of either AFB or mānuka DNA.

UNDERSTANDING THE MEASUREMENT UNCERTAINTY OF PCR TESTING

At Analytica we get many questions about how we can test the same sample for AFB twice and get a 'detect' for one test and a 'non-detect' for the other. All scientific testing has a level of measurement uncertainty, and for PCR testing this sits in the range of +/- 1.5 Cq. This means that the result we provide is within +/- 1.5 Cq values of the 'true' result. With PCR testing, we know that the closer the result is to the detection limit, the higher the level of uncertainty becomes.

DO YOU HAVE QUESTIONS ABOUT YOUR RESULTS?

Analytica prides itself on being a helpful and knowledgeable resource that can help you understand your results (within the limitations of our accreditation). If you ever need help, do not hesitate to contact us!

Figure 2: PCR reaction process (https://www.onlinebiologynotes.com/polymerase-chain-reaction-pcr-principle-procedure-steps-types-application/)