RESEARCH

HOW DO LABS CONDUCT THE MPI MĀNUKA TESTS?

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In April 2017, MPI announced a proposed new definition for mānuka honey, which includes some new requirements for laboratory testing. This article gives an overview of how the testing is carried out by laboratories, and some thoughts about how to understand the results. The testing uses well-established techniques, and prices are in the range of $180–$200 + GST per sample if all five tests are requested for a honey sample.

MPI's proposed mānuka definition was announced on 11 April 2017

A great deal of useful information is available on MPI's website (www.mpi.govt.nz) about the proposed definition of mānuka honey.

The proposed definition includes testing for a combination of five attributes (four chemicals and one DNA marker from mānuka pollen) to distinguish mānuka honey from other honey types, and to identify monofloral and multifloral mānuka honey. A summary of these tests and required results is given in Table 1.

All four chemical markers can be analysed in a single test carried out by the laboratory. The DNA pollen test is a separate test using entirely different equipment and testing procedures.

	Required levels for multifloral mānuka honey	Required levels for monofloral mānuka honey
Chemical markers		
3-phenyllactic acid	Greater than or equal to 20 mg/kg and less than 400 mg/kg	Greater than or equal to 400 mg/kg
2'-methoxyacetophenone	Greater than or equal to 1 mg/kg	
2-methoxybenzoic acid	Greater than or equal to 1 mg/kg	
4-hydroxyphenyllactic acid	Greater than or equal to 1 mg/kg	
DNA pollen	Cq value of less than 36*	

Table 1. Summary of tests and required results.

** The results of an MPI mānuka pollen DNA test are reported as a 'Cq' value, and for a sample to meet MPI's standard for mānuka honey, the Cq value needs to be less than 36.*

Take care if your test results are close to the required levels

If a honey sample is tested a number of times, you will not get exactly the same result each time. Because there is variability in both a sample and in the testing process, results will naturally vary across a range. Laboratories measure this when setting up a test, and refer to it as **Uncertainty of Measurement** (UoM). Many readers will have experienced this when asking for re-tests of samples in the past.

UoM is very important to take into account when comparing your test results against required levels such as those in the proposed MPI mānuka honey definition. *If a result for a sample is quite close to the threshold, there is a chance it will produce a result on the other side of the threshold if re-tested (by you or by someone else).* For example:

- Assume that a method has a UoM of plus or minus 10%, and you are aiming for a minimum of 400 mg/kg.
- You submit a sample, and get an actual result of 420 mg/kg, which is above the threshold.
- However, it falls within the UoM range of 360–440 mg/kg for the method compared with the threshold level of 400, and there is a reasonable chance that your sample could give a result below 400 mg/kg if re-tested.

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If you have test results close to an important threshold, we suggest that you talk with your laboratory about their UoM for that testing method. In general, most methods would have a UoM of at least plus or minus 10%, and it can easily be higher.

MPI MĀNUKA POLLEN DNA TEST

What is the background to the test?

MPI's **Mānuka Pollen DNA test** works on the principle that bees collecting nectar from mānuka plants will also collect some mānuka pollen at the same time, which will end up in the honey. As pollen grains contain DNA, we can use DNA testing techniques to test honey for mānuka pollen.

DNA stands for 'deoxyribonucleic acid', and is found in cells of every living organism (including plants like mānuka). The DNA of all plants is actually quite similar; however, different species or varieties of plants have their own unique sequences of DNA, and DNA tests are designed to look for these differences.

How is it done?

The pollen DNA test uses a technique called Real Time PCR, which is one of the common ways of doing DNA testing. Generally, the test requires about three days to complete from start to finish. The equipment needed is not too expensive (perhaps $100,000 in total). However, the testing process is quite expensive, with a number of manual steps and expensive consumables. Overall, labs are tending to charge $90–$100 + GST per sample.

There are five main steps in the testing process:

Isolate the pollen from the honey sample – some honey is weighed into a testing tube, and after being dissolved with water the sample is spun in a centrifuge. The pollen forms a pellet in the bottom of the tube as it spins, and the rest of the dissolved honey can then be poured away.

Break up the pollen and release the DNA from inside it – after washing the pollen to remove any honey residues, an extraction solution is added. Among other things, it contains very small and hard micro-beads. The sample is put onto a very aggressive shaker (called a bead beater) for a few minutes, and the micro-beads bash the pollen grains and break them open. This releases their DNA into the liquid in the tube.

Isolate the DNA – the liquid from the tube is poured through a special 'affinity' column which binds any DNA in the sample onto it, while letting the liquid and other things in the sample pass through. After a couple of washing steps, the purified DNA is washed off the column and is available for analysis.

Real Time PCR – the purified DNA has special reagents added to it, including small sequences of DNA (primers) that have been specifically designed to target DNA sequences that are unique to mānuka, and some DNA probes that have chemicals bound on to them that fluoresce. The sample is loaded onto a PCR instrument, which is pre-programmed to correctly run the test.

Results are reviewed and approved for reporting – the PCR instrument will produce a result for each of the samples that have been tested. A suitably qualified member of the lab team will review these results, including quality control samples, before approving results for reporting.

Quality control

Laboratories carry out a number of tests and checks each time that they analyse samples for quality control purposes. In the case of DNA pollen tests, these will include:

- Blank QC samples, which should have nothing in them, and therefore check for contamination in the lab.
- Control samples, which are honey samples the lab uses repeatedly to check that the method is working correctly. The lab will know what results to expect for these honeys, which are usually chosen to be at the bottom and top of the normal range seen in commercial samples.
- Duplicate samples, which may be the same sample tested twice in the same batch, or may be a sample from a previous batch that is re-tested. The purpose is again to confirm that the testing is producing consistent results using a 'live' sample submitted by a customer.
- An 'internal control'. Each sample is not only tested for mānuka DNA, but also for DNA found in all plants, to confirm that the testing process has worked for that sample. These general plant DNA tests should work well in all samples—if there is a poor result for the plant DNA markers, it will cause the lab to consider repeating the sample.

What results come from it, and how do I understand them?

The results of an MPI mānuka pollen DNA test are reported as a 'Cq' value, and for a sample to meet MPI's standard for mānuka honey, the Cq value needs to be less than 36.

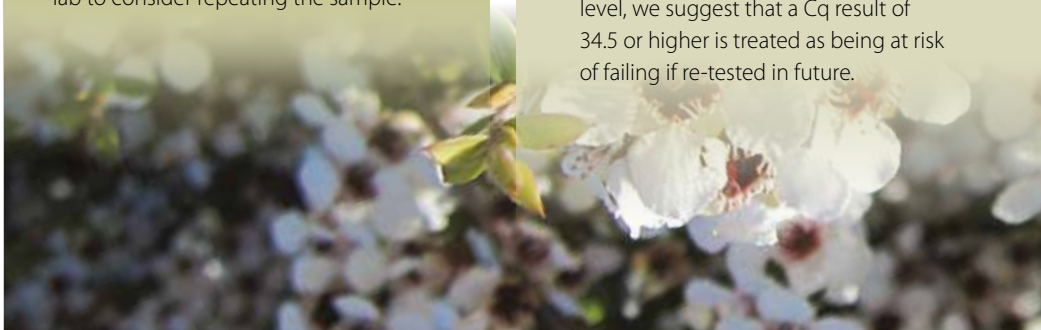
To understand this, a brief explanation is needed. Real Time PCR relies on things called 'primers' and 'probes' that are specific to mānuka DNA, and are added to the sample as part of the PCR reagents. When testing a sample, the PCR instrument goes through a series of cycles, and in each cycle:

- The primers and probes attach to any mānuka DNA which is there; and
- A copy of the targeted areas of DNA is made; so that
- At the end of the cycle there are two times as many copies of the mānuka DNA than there were at the start of the cycle.

For example, if there were 10 pieces of mānuka DNA in a sample at the start, there will be 20 at the end of the first cycle; 40 at the end of the second cycle, 80 at the end of the third cycle; and so on.

MPI has set a threshold level of mānuka DNA that must be in a sample being tested by PCR, and the Cq value is the number of PCR cycles needed to reach that threshold. The more mānuka DNA in the sample at the start, the fewer cycles will be needed to reach the threshold. If a sample takes 36 or more cycles to reach the threshold (a Cq value of 36), then the original honey sample does not have enough DNA in it to meet MPI's proposed mānuka definition.

A Cq result of 36 is very close to the method's reporting limit. Because of Uncertainty of Measurement at this level, we suggest that a Cq result of 34.5 or higher is treated as being at risk of failing if re-tested in future.



MPI MĀNUKA CHEMICAL MARKERS TEST

What is the background to the test?

MPI's **Mānuka Chemical Marker test** looks for naturally occurring chemical compounds in honey samples that are also known to be found in mānuka nectar. As honey is primarily made from nectar, these chemical markers confirm that the honey includes enough nectar from mānuka to be called a monofloral or multifloral mānuka honey.

How is it done?

The Chemical Marker test uses a technique called 'liquid chromatography–tandem mass spectrometry' (LC-MS/MS), which is used for other tests such as tutin. The test requires one to two days to complete from start to finish. The equipment needed is quite expensive (over $600,000), but the preparation process is relatively inexpensive in terms of both labour and consumables. Labs are tending to charge $90–$100 + GST per sample.

A summary of the testing process is:

Weigh and dilute the honey sample – after weighing some honey into a testing tube, it is dissolved and diluted with a pre-prepared extraction reagent. This prepares the sample for analysis, putting it in the right condition to be run on the mass spectrometer.

Analyse the sample on the mass spectrometer – the diluted honey is put into small vials and analysed on the mass spectrometer. Liquid chromatography is used to separate the various compounds found in the sample, before the concentration of those compounds is measured by the tandem mass spectrometer. All four of MPI's chemical markers are able to be analysed in the same run through the instrument if the lab wishes to do so.

Results are reviewed and approved for reporting – the mass spectrometer produces a result for each of the samples that have been tested, which is reviewed by a member of the lab team. After confirming they are satisfied with the results, and with quality control samples, they are approved for reporting.

Quality control

Laboratories carry out checks each time that they analyse samples for quality control purposes. In the case of chemical marker testing, these will include:

- Blank QC samples, which should have nothing in them, and therefore check for contamination in the lab.
- Control samples, which include honey samples the lab uses repeatedly, and samples which have had known amounts of the chemical markers added to them by the lab. The lab will know what results to expect for these honeys and will check this when reviewing results.
- Duplicate samples, which may be the same sample tested twice in the same batch, or may be a sample from a previous batch that is re-tested. The purpose is again to confirm that the testing is producing consistent results using a 'live' sample submitted by a customer.
- An internal control (or system monitoring compound), which confirms that the preparation and analysis process has gone well. A known amount of this internal control is added to each sample during preparation, and the lab expects to see this in the results for that sample after testing. If the amount of system monitoring compound is low (or high), it is an indication that the sample may need to be retested.

What results come from it, and how do I understand them?

Results are reported in milligrams per kilogram (mg/kg) in the honey. One mg/kg is sometimes called a 'part per million' (there are one thousand milligrams in a gram, and one million milligrams in a kilogram). These are the same units used to report other tests that the honey industry may be familiar with—tutin, DHA, MG, HMF, and leptosperin.

To interpret the results for a sample, simply compare them to the levels in MPI's proposed definition. However, if you have a result that is close to the minimum level required by MPI, there is a chance that a re-test of the honey may see that result change to fall below that minimum level due to the variability in honey samples and in testing methods. See comments earlier in this article.

This is particularly important for the three chemical markers that have a required level of 1 mg/kg (which is very close to the method's reporting limit). In this case, we suggest that a result of 1.5 mg/kg or lower is treated as being at risk of failing if re-tested in future.

Left to right: Analytica technicians Luca and Kris with the AB Sciex 6500 mass spectrometer, used for analysis of compounds like tutin and MPI chemical markers in honey. Photo courtesy of Analytica Laboratories.