

# Improvements to the UMF assay

With the increasing price of manuka honey there are increasing calls for a more precise measurement of UMF activity. Two years ago I gave to the AMHA executive a written report on some work they had commissioned me to do investigating variation in the results in the assay. In this report I made some recommendations for improvements, but these were never adopted. Instead their approach has been to seek an alternative method, firstly assay by MIC, and then assay by MGO content, despite my explaining why these methods would not be more accurate. So the honey industry is now two years further down the track and nothing has changed, other than some moves to give small improvements in accuracy that could be made by the testing laboratory without the approval of AMHA to change the testing methodology. (These were to introduce regular checking of the incubator temperature and the pH of the phenol standard solutions.)

There are also increasing calls for something to be done about the problem of partial inhibition that is seen with some samples of manuka honey. This problem is increasing as more samples of manuka honey are tested early in the season, before the UMF activity has had a chance to increase, and as manuka sites are more heavily stocked with hives so that the honey produced has a higher proportion of non-manuka nectar in it and thus has a lower UMF activity. Again, in my report two years ago I recommended how this problem could be dealt with, but this has not been conveyed to beekeepers.

Consequently I am repeating these recommendations in this article so that beekeepers are made aware of them. (The numbering here has been changed from that in my report.) After each recommendation I have added a brief explanation.

*Recommendation 1: Where honeys are found to give partial inhibition, re-assay them blended 50/50 with a UMF 20 honey that has its activity known with certainty. That will give clear activity that is the average of UMF 20 and the true UMF activity of the honey that showed partial activity, thus true activity can be determined. (For example, if blending a "partial" honey with a UMF 20 honey gave a result of UMF 15, then the true UMF value of the "partial" honey would be 10, since the average of 10 & 20 is 15.)*

Our research had shown that partial inhibition results from the honey having a different type of antibacterial activity, such that at a quite wide range of lower concentrations it only slows the growth of bacteria, not stops it. (This would not be effective in clearing an infection.) At a higher level of activity it is possible to measure the area on the plate where the growth is completely stopped, and thus get a rating of the true therapeutically useful level of antibacterial activity. Knowing the level of honeys giving partial inhibition would allow blending with honey of higher activity to obtain a product with certifiable UMF activity.

The safest way of obtaining a blended honey with the correct activity would be to mix together in measured proportions truly representative samples of a partially inhibiting honey

and a honey with a high UMF activity, and get the activity of the blend measured to find what the activity will be when the bulk quantities of these honeys are blended.

*Recommendation 2: Use manuka honeys of known activity as the day-to-day standards for the agar diffusion assay, instead of using phenol. Thus any variation in temperature, bacterial culture sensitivity, or culture inoculum will affect equally the samples being assayed and the standards, and thus will not influence the results. (The activity of the standard honeys will have to be carefully and repeatedly checked against phenol to ensure that the activity level they are said to have is correct.)*

It is standard practice in any type of assay to use a standard for comparison. This ensures that if the assay is giving high results one day or low results another day, the results for the standard will go up or down also. But when results for the samples being assayed are calculated relative to the standard they will be correct, because the results for the sample and the standard go up or down together. The problem with the UMF assay is that the phenol used as a standard is not the same substance as that which gives manuka honey its antibacterial activity. Thus the bacteria may increase in sensitivity to phenol on one day and decrease on another day, but their sensitivity to the antibacterial components of manuka honey may not change. Or vice versa. But if, for example, two different honeys (standard and sample) were both UMF 15, then no matter how much the bacteria and conditions varied and the size of the inhibition zone varied as a consequence of this, the zones of inhibition of these two honeys would always be the same size as each other.

There would be extra costs involved because of the assays needing to be done to establish and confirm the UMF rating of the standard honeys to be used. But these would be offset by fewer assays having to be done overall, since there would be no more instances of assays having to be thrown out and repeated when the results for the control honey of known activity are found to vary too much. This would also increase the throughput of assays at busy times.

*Recommendation 3: Until such time as Recommendation 2 is adopted, have the phenol standards on the agar plates positioned in a row with a space between that row and the honey samples.*

Our research found that the nature of the honey sample next to the phenol standard affected the size of the zone of inhibition from the phenol standards. Adopting this recommendation would decrease the number of samples that can be assayed on a plate, and thus would increase costs and decrease the number of samples that can be assayed at busy times. But if Recommendation 2 were adopted, this recommended procedure would need to be used only to find the UMF rating of the honeys to be used as standards on the plate so the activity of these standards could be known more accurately.

Recommendation 4: For honeys that are above UMF 20, require that for a certified result they be re-tested with a 12.5% honey solution instead of the standard 25% honey solution so that the diffusion of the activity into the agar is of an extent that allows the activity to be measured accurately. (This will require numerous samples in the range UMF 17 to UMF 20 to be measured as 25% and 12.5% solutions to get the correct multiplication factor, as was done when testing as 50% solutions for honey below UMF 8 was first started.)

The method currently used, which has honey as a 25% when put into the wells on the assay plate, was optimised for honey activities in the range UMF 10 to UMF 20, since activity above UMF 20 never used to be found. The reason for the lower accuracy at high UMF levels is that it is the area of the clear zone of inhibition on the plates that is proportional to the activity but it is the diameter of the zones that is measured. The area of a circle is proportional to the square of the diameter. So at around UMF 10 a difference of 1 mm in the diameter corresponds to a difference in activity of 0.85 UMF units, but at UMF 30 a difference of 1 mm in the diameter corresponds to a difference in activity of 3.6 UMF units.

The extra cost for finding the correct multiplication factor is a one-off cost of doing the number of repeated assays necessary to get a reliable figure. If the improvement to the method in Recommendation 2 is adopted then there should not be many repeated assays necessary.

With recommendations 2 and 4 adopted, any remaining inaccuracy would be due to errors in measuring precisely the diameter of the clear zones of inhibition, the edge of these zones not being a "black and white" difference but actually a narrow "grey" area of partial inhibition. Some preliminary work we have done has confirmed that the area of the zone can be measured by digital imaging, which should give much more accurate results and will avoid subjective judgement by technicians of where the edge of the zone actually is when measuring the diameter. This, however, would need further work that would have to be funded.

I can only advise on the assay of UMF activity. It is up to members of AMHA, through their executive, to implement improvements.

- Professor Peter Molan  
University of Waikato, Honey Research Unit



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## Comment: what beekeepers should be doing to get accurate activity results

In talking to Professor Molan while arranging for him to attend this year's conference, we discussed the varying results in manuka activity testing. Most beekeepers were blaming the lab for the differences in results to double blind tests; however, beekeepers should also be doing their bit to improve the accuracy of results so packers can be confident that the results they get back from core sample testing of a drum are accurate.

Most beekeepers would not be aware that the activity stratifies out in the drum vertically and horizontally. This means that a sample taken from one side of the drum could be different from the other.

Also, how the drums are stored compared to the samples beekeepers take could also mean varying results are received. That is, if the samples are stored inside and the drums outside, then the activity of the sample could, over 12 months, be slightly higher than the drum contents. Also, the amount of air in the sample could affect results, so these should be the same.

How do beekeepers producing manuka honey achieve an even result? By stirring honey for 24 hours at brood temperature with the right type of stirrer—one that folds the honey down on the outside and up in the middle. This type of stirrer folds the honey in on itself without adding air to the honey.

If you are having your honey contract extracted, the cost is often higher for manuka. Along with this extra cost comes an obligation/responsibility by/for contract extractors to give due care to what is essentially a beekeeper's hard-earned income. If your contract extractor is simply running your honey into a drum, then you will not realise the true value of your crop.

Stirring also will bring the UMF activity up slightly, so the honey should be rested for two days before samples are sent off for testing.

- Frank Lindsay  
Lindsay's Apiaries



Gordana Aleksic, Laboratory Manager, NZ Labs.