**Ian Marsh is working at the Elizabeth Macarthur Agricultural Institute (EMAI, New South Wales Department of Primary Industries). This is the NSW Centre for Animal and Plant Biosecurity that undertakes plant and animal research and diagnostics**

**Why did Ian choose the Mic?**

1. The ability to generate accurate and precise data,
2. Software to analyse that data simply and rapidly.
3. Software that facilitated high end research analysis.
4. Software that made day to day diagnostic work easy and understandable.

**Other important factors included,**

- **Price and low maintenance cost.**

The Mic offered 48 samples (with no unnecessary use of consumables for runs of less than 48 samples), no calibration, no maintenance, is extremely affordable and maintenance free.

- **Speed.**

With the Mic we are developing assays that run between 30 and 60 minutes that are highly accurate, precise and increasingly affordable.

- **Size.**

Mic has a very small space requirement.

**What is the Mic being used for?**

- Real-Time PCR assay to confirm the presence of *Erysipelothrix rhusiopathiae.*
- Mic and pyrosequencing to differentiate the genomically similar species of Chlamydia within hours of DNA extraction.
- Field-based research using the MIC for bovine Campylobacteriosis.
- Improving soils for greater productivity with nitrogen fixating Rhizobia.

## Efficiencies in PCR culture validation

**6 ul reaction volume and project functionality of Mic software**

Ian developed a new Real-Time PCR assay to confirm the presence of *Erysipelothrix rhusiopathiae. E. rhusiopathiae* is an animal pathogen, causing a disease known as erysipelas in animals, Turkeys and pigs are most commonly affected, the disease is also known as "diamond skin disease".

He also evaluated the use of 6 µL Real-Time PCR reactions in the Mic to minimise reagent costs. To determine how effective this was he used the project function of the Mic software to analyse the results. This function allows up to 10 runs to be imported into a single file and be analysed simultaneously.

Three Real-Time PCR runs were undertaken on a total of 138 samples

**Conclusion:**

Using the Project function he could immediately see there was uniformity across runs. The majority of samples could be analysed by Linear Regression with an average reaction efficiency of 88.6% and R2 value of 0.9997. This suggests that the smaller volume is well suited to this purpose.

**Mic's unique features highlighted:**

## Pyrosequencing to confirm Real-Time PCR results and/or strain type

**Get results in 2 hours instead of 2 days or weeks**

Especially when index cases from a previously unaffected source are detected or for samples that produce very high Cq values. The World Organisation for Animal Health (OIE) advices to do  a subsequent sequencing step to confirm Real-Time PCR positive results, We incorporate Real-Time PCR on the Mic with pyrosequencing to confirm Real-Time PCR results that exceed the cut off parameters set for assays that target notifiable diseases.

Occasionally results are obtained from a sample were the Cq value is beyond the diagnostic cut off. This is most likely the result of inhibition that may have prolonged the initiation of amplification.

**Conclusion:**

Using Real-Time PCR assays designed and optimised on the Mic in conjunction with in house pyrosequencing, we can now achieve results in hours that previously took days to weeks when outsourcing the sequencing component. We typically find the combination of a 16 µL Real-Time PCR reaction (to save on cost and reagents) in 30-60 minute runs to be highly efficient whilst not compromising on sensitivity.

## Mic's  unique features highlighted:

## Mic and pyrosequencing to differentiate the genomically similar species of Chlamydia within hours of DNA extraction

Classified bacteria within the Chlamydiaceae family include:
*C. trachomatis, C. muridarum, C. suis, C. psittaci, C. abortus, C. caviae, C. felis, C.pneumoniae* and *C. pecorum*.

These cause a variety of diseases in both human and animal hosts and whilst host specificity was thought to be well understood, recent insights in this field have been gained to question this.

**Conclusion**

Compared to conventional PCR and restriction enzyme digest analysis which takes up to 2 days to complete you can now differentiate within hours of DNA extraction between the genomically similar species of Chlamydia by using a combination of Mic-based Real-Time PCR and pyrosequencing.

## Mic's unique features highlighted:

## Field-based research using the MIC for bovine Campylobacteriosis

Real-Time PCR testing using the Mic was combined with culture to evaluate preputial samples for bovine Campylobacteriosis.

Bovine Campylobacteriosis is a venereal disease characterised by infertility, early embryonic death, and occasionally abortion in cattle. Bacterial culture is used to examine samples, however, culture may lack sensitivity and specificity.

Bovine Campylobacteriosis remains difficult to diagnose and continued problem in cattle production, the Real-Time PCR results were integrated with the bacterial culture results to develop a better understanding of the potential of this technology to better diagnose difficult bovine diseases.

**Conclusion:**

Relocating the Mic to the farm presented no problems.
The results we achieved with the Mic are very encouraging with regard to the use of this technology in more field-based research

## Mic's unique features highlighted:

## Improving soils for greater productivity with nitrogen fixating Rhizobia

**Using the Mic as a conventional PCR instrument for a 1200 bp amplicon resulting in consistent and more reliable sequence data.**

Rhizobia are specialized bacteria that associate with legumes to 'fix' atmospheric nitrogen into soil. It is estimated that the legume-rhizobia symbiosis fixes 2.7 million tonnes of nitrogen into Australian agricultural systems each year, boosting productivity by $4 billion annually

The commercial rhizobial collection curated by the The Australian Inoculants Research Group(AIRG) is issued to industry to manufacture commercial quantities of legume inoculant products. Each year the AIRG authenticate and check the performance of up to 40 legume inoculant strains that are used on over 90 host legumes before issue.

The commercial product made with these inoculant strains is then assessed by the AIRG to ensure that each batch contains:

1. The correct rhizobial strain for the intended host plant
2. There are sufficient numbers of that strain
3. The Rhizobia strains are still functional.
   (able to associate with the host plant and fix nitrogen).

We use the Mic to generate a PCR product in real time which is then sequenced to confirm the genetic identity of each strain. To achieve this, we generate PCR amplicons greater than 1200 base pairs long and require the entire length to be sequenced to facilitate speciation.

**Conclusion:**

Whilst achievable on other platforms, the results were variable and the resulting sequence data was unreliable. The reliability of this process has been vastly improved with the Mic which has resulted in a high degree of success.

The Mic software is very simple to use and is highly intuitive allowing us to validate this process and shortening turnaround times significantly compared to sequencing from conventional PCR methods.

## Mic's unique features highlighted: