The use of PCR testing in Australian Milk Quality Investigations

What is a PCR test?
Polymerase Chain Reaction (PCR) testing is a laboratory testing technique which has potential to assist Australian dairy farmers and their advisers when investigating milk quality problems. PCR tests detect fragments of mastitis pathogen DNA (genetic material) in the milk sample.

What are the advantages of milk PCR testing?
Milk PCR tests were originally designed for use on individual cow milk samples after the teat had been prepared hygienically for sampling. In Australia, PCRs are mostly been used in pooled milk samples (bulk tank or pooled hospital milk) to test for bovine mastitis bacteria milk. PCR testing is highly sensitive and can identify bacteria from a single infected cow within a group (e.g. in a bulk milk vat sample of the milking herd), making this test cost-effective on bulk milk samples.

What are the disadvantages of PCR testing?
Whilst PCR tests detect DNA of target organisms in the milk sample, it cannot determine if the organisms are alive or dead or how many organisms are present.

Therefore, the test can only be used to determine the ‘presence’ or ‘absence’ of bacterial DNA in the sample. Additionally, it cannot determine if they were causing mastitis or merely contaminants of the sample. In Australia, as the test is predominantly used on bulk samples from teats that have not been sterilised, there is a greater chance of many types of bacteria being present in the milk sample, and some of these could originate from the environment rather than from cows’ udders.

What are the different tests available?
One commercially available test is the ‘PCR-4’ which identifies four bacteria (i.e. their DNA) in one test; Streptococcus agalactiae, Mycoplasma bovis, Staphylococcus aureus and Streptococcus uberis. A ‘PCR-16’ test is also available which tests for several more bacteria, a yeast and a specific bacterial gene.

This more expansive test might be useful in specific situations but given current knowledge and the way the milk PCR test is used in Australia, the PCR-4 is all that is required in most circumstances.

In Australian dairy herds, the main value of PCR testing is for detecting Streptococcus agalactiae and Mycoplasma bovis in bulk milk samples. It is a quick, simple and cheap test since hundreds of cows can be screened with one sample.

Both these bacteria can only originate from infected udders, so the presence of their DNA generally means that there is at least one infected cow in the sample.

Note that Mycoplasma bovis can be shed intermittently from infected cows, so a ‘negative test’ result for this pathogen is not always predictive of an uninfected herd, even if the right cows are providing milk into the tested sample.
When to use milk PCR tests

The Milk PCR test is only one part of a mastitis investigation process (see Technote 13 and the Mastitis Investigation Pack). As the PCR only detects the presence or absence of certain mastitis pathogens it cannot be used to determine the source of infection or the likely spread within a herd. Whilst PCR testing has a very high level of accuracy, ‘false results’ need to be considered. These are more likely to occur when testing samples of milk that have very little likelihood of containing the pathogen of interest.

The probability of a result being truly accurate is more likely if the test is used on samples taken from groups of cows that are likely to be infected with the pathogens being tested for. The flow diagram below shows how to identify situations where the PCR is likely to be the most useful and yield the most accurate results.

Figure 1 How to use a milk PCR Test

**Step 1**

**Suspicious of Mycoplasma bovis?**
- High rates of clinical mastitis, poorly responsive to usual treatment*
- Clinical mastitis in multiple quarters of the same cow
- Swollen joints in cows and/or calves
- High rates of pneumonia in calves
- >50% ‘no growths’ from standard milk cultures

**Suspicious of Streptococcus agalactiae?**
- BMCC > 300,000 cells/ml
- High clinical mastitis case rates*
- Recently purchased cows
- Previous cultures positive for Streptococcus agalactiae

**Step 2**

- Sample bulk tank & pooled hospital milk
- Use PCR-4 test

**Step 3**

- Discuss with Countdown adviser
- Follow up milk cultures & other areas for investigation

*(more than 25 clinical mastitis cases per 100 cows over the past 12 months)

Interpretation of the PCR test results

*It is important to consider which cows contributed milk into the sample. For example, if infected cows are not being milked into the vat (eg. under treatment or dry), their milk will not be included in the sample tested and hence a “not detected” result is misleading.*

*Specifically collecting milk from cows likely to have mastitis pathogens in their milk, such as the ‘hospital’ or ‘treatment herd’, is a method to increase the chance of an accurate test result. (Note: the PCR test is not affected by antibiotic treatments.)*
In this example, the sample was taken from the bulk milk tank. This test result indicates that cows that contributed to the sample did not shed any *Mycoplasma bovis* or *Streptococcus agalactiae* on that day. We can make no inference about the ‘low DNA level’ recorded against *Staphylococcus aureus* or *Streptococcus uberis* as these bacteria may have come from the outside surface of the teats, meaning they are not causing the mastitis challenge.

If there was still suspicion that *Mycoplasma bovis* or *Streptococcus agalactiae* was causing mastitis, it would be appropriate to take individual milk samples from clinical and/or sub-clinical mastitis cows and send to a diagnostic lab that is able to perform *Mycoplasma bovis* cultures.

**Should PCR milk tests be used to screen herds?**

The use of PCR testing to screen herds with very few or no clinical signs (as exemplified by the signs discussed in figure1) increases the chance of returning test results which are difficult to interpret. This includes the situation where PCR testing might be used to screen herds prior to purchasing.

To obtain the most value from PCR tests in such situations (biosecurity screening; pre-purchase screening/testing) it is advisable to discuss testing with an experienced milk quality advisor before the testing is performed, not after results from a PCR test have been received.

Using very sensitive tests in situations of very low risk (chance/probability) increases the chance of producing results which are difficult to explain and this may have implications for the herd being tested.

**Milk PCR tests are just one part of an investigation process**

The PCR test result forms part of the evidence gathered within the investigation and needs to be combined with other findings (e.g. other presenting signs listed in the boxes above) to build a fuller picture of the mastitis problem within the herd.

The only way to confirm the presence of the pathogens indicated by the PCR test result is to use milk cultures as described in Fact Sheet A.