

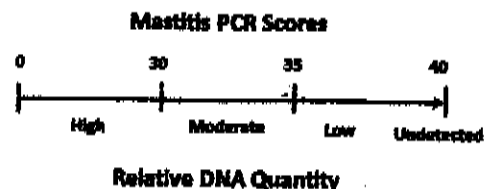
## Mastitis PCR

### Frequently Asked Questions

**What is PCR?** Polymerase chain reaction is an advanced laboratory technique that amplifies DNA so it can be detected even when present in very, very small quantities. It is the same technique used to investigate crime scenes.

**Why try to detect the DNA?** DNA provide a unique 'signature' for each mastitis pathogen. If a unique DNA signature is detected, we know that particular pathogen is in the milk sample. Unlike culture, which relies on pathogen growth, DNA can be detected whether the pathogen is alive or dead in the sample.

**How does PCR determine how much DNA is detected?** PCR replicates DNA in similar ways to nature, but at a drastically increased rate. When DNA is detected, the test returns a value or score that can be used to determine the relative abundance of particular pathogens. It is important to note that the scores are inversely related to the quantity, lower scores mean higher quantities of DNA. This is because more positive samples have more DNA and it takes less time to reach the positive threshold.



**Are test results valid if DNA was detected from dead pathogens?** The presence of the pathogen, whether dead or alive, along with evidence of a pre-existing udder infection (e.g., high SCC or clinical appearance) is strongly indicative that the pathogen is responsible for existing health conditions.

**What if DNA from more than one species of pathogen is detected in a milk sample?** The pathogen with the lower PCR score should be considered first, since the lower score indicates higher numbers of that pathogen. This is especially true when one of the detected pathogens has a much lower score relative to the rest (e.g., 26 vs 35 or +++ vs +).

**What do I do with positive results, when PCR scores for different organisms are similar?** In many cases, multiple samples are submitted for testing. If the scores in one sample are similar, look at pathogens that were detected in other samples. If one particular pathogen is detected with higher frequency in a group of samples, that pathogen should be considered first.

**What other factors should be considered when interpreting mastitis PCR test results?** Like the interpretation of any other diagnostic test result, testing history, clinical observations, environmental conditions, and management factors need to be considered.

**When can pooling be considered for mastitis PCR testing?** Pooling is recommended for general mastitis surveillance to determine the presence and relative quantity of pathogens on the dairy, or in groups of cows. Once the pathogen of concern has been identified, test individual samples to identify infected cows for treatment or management.

**Why use PCR when it costs so much more than culture?** Not only is PCR faster and more sensitive than culture, it can be used where culture can't; on milk samples from treated cows and from DHI test day. Without additional effort on the part of the producer the very same high SCC sample can be used to detect causative pathogens, rather than waiting days to obtain a hand-stripped sample for testing.

**Can high SCC samples be pooled?** While identifying high SCC samples for mastitis testing is a great way to target problem cows, pooling those samples to limit costs even further is NOT recommended. We expect to find more positive results in high SCC samples, increasing the likelihood of a positive result. In a positive pool, individual samples would need to be retested to identify infected cow(s), thereby *increasing* overall testing costs versus running the samples as individuals in the first place.

**If the test is that sensitive, isn't contamination a concern for PCR on DHI milk samples?** Yes. DHI Specialists should always be concerned with contamination and the importance of controlling and reducing potential carryover, regardless of the type of testing. However, even with minor contamination, quantitative PCR scores and experience are able to differentiate causative pathogens from contamination.

**How can fresh cows be tested by PCR when they are not in the milking herd yet?** Milk samples from fresh cows can be collected, frozen and shipped directly to the laboratory by farm staff. For convenience, the laboratory can arrange to recirculate a shipper as part of an ongoing surveillance program for the fresh cow pen. The speed of PCR can make test results available before cows are returned to the milking herd.

**What is the turnaround time for PCR?** Once samples reach the laboratory, the PCR assay only takes four hours and is run daily. Therefore most test results are available within 24-48 hours of when the sample arrives in the lab. Allow additional time for sample shipping.

**How do results from PCR compare to culture?** While both PCR and culture provide information on mastitis, they are two different tools detecting pathogens by very different methods. Therefore, it is more effective to compare or interpret results within test type rather than across test type. Comparing within PCR test results you can tell which milk samples contain pathogen DNA, which contain more and which have less. Attempting to compare across test types to determine *what* PCR score might be equivalent to *how many* culture colonies doesn't add information to help diagnose and manage mastitis.

**For the mastitis PCR promotion, what producers would make good candidates to receive the complementary testing coupons?** Consider herds that are expressing concerns over mastitis or high bulk tank SCC. For ongoing mastitis concerns, the complementary tests may be suitable for hospital cows (sick pen) or recently fresh cows (fresh pen). If a predominant pathogen is identified, veterinarians can advise herds on treatment and management protocols. For herds with high bulk tank SCC, testing the highest SCC DHI samples can identify responsible pathogen(s).

**Is there someone available to help interpret PCR results?** During the mastitis promotion program (Jan-Mar), AntelBio will follow up with the herds' DHI Specialist to get any available background information, and then will contact the herd to discuss results. Any information you can pass on about the situation on-farm may help with interpretation.