

Mastitis 4 PCR Assay

Applied to DHI Preserved, Composite, Metered Samples

Frequently Asked Questions and Answers

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1 -Why did my milk culture results and PCR results differ?

Research from five Ontario dairy farms with a history of endemic *Staphylococcus aureus* (SA) mastitis has shown that PCR results from DHI samples and bacteriological culture results from hand-stripped, composite samples agreed in most cases for the major contagious pathogens, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis* and *Prototheca*. Where they disagreed the differences were usually readily explained.

A PCR result from a test on a DHI milk sample can differ from a routine bacteriological culture result for several reasons. They differ because:

- These PCR and culture are two different kinds of tests and test for different aspects of bacteria.
- The tests are done on different samples, and
- The two tests have a different spectrum of possible results.

Table 1 gives important and specific details about how the samples and tests differ.

2 - Why did a cow with a low SCC have a positive *Staphylococcus aureus* (SA) PCR result?

A positive PCR result indicates that DNA from SA was present in the DHI metered milk sample that was tested. Positive PCR test results from cows with elevated SCCs don't surprise us as SA is a frequent cause of subclinical mastitis. We may be more surprised to get a positive PCR result from a cow with a low SCC, but it still makes sense. When cows with high SCCs are tested and the SA PCR is positive, it is highly likely that an active, established infection with SA is present in the cow tested. After all, if we pick high SCC cows to test, this is a population of cows we already know by their SCCs are highly likely to have some kind of infection present. If, on the other hand, cows are selected for testing by some other criteria, i.e. all cows are tested on first DHI test, or all purchased cows are tested or the whole herd is tested at one time, then within these groups of cows we are now testing cows that have both high and low SCCs. In other words there are now cows being tested that have "mastitis" and cows that have no prior evidence of mastitis. When cows with low SCCs are tested it's not surprising that some are detected with SA DNA. What we are revealing is the population of cows that are exposed to SA but in whom it may be an early infection (not yet established, hence no SCC response), where infection is only transitory (it doesn't "take" for whatever reason) or where it is only a teat reservoir or streak canal inhabitant (not an infection of glandular tissue). In all these situations SA DNA is present but no reaction in the cow (elevated SCCs) is present at the same time.

We need to be aware that in herds with poor milking technique many cows in the herd are repeatedly challenged at the teat end with SA that is circulated at milking time from the chronically infected SA cows that are being milked in the herd. Poor milking hygiene, lack of a milking order, poor teat dipping etc allows this to happen. Many times when cows are exposed to SA at milking time infection does not occur, even though small numbers of organism may be present in milk from the quarter(s) at a given time. Herds with a high prevalence of cows infected with SA and who

have less than ideal milking procedures are more likely to have some cows with low SCCs that test positive with the Mastitis 4 test. This suggests that SA is likely circulating in an unrestricted manner among the udders of the cows milked in the herd.

Decisions about treatment or culling should not be based solely on the outcome of any diagnostic test. The Mastitis 4 result is one piece of information that can be added to the cow's mastitis history, her current and historical SCCs, CMT results and other health information to decide what action, if any, should follow.

Before setting up a sampling and testing protocol, make sure you know the question you want to answer with your test results. Ensure that the test results you receive provide the information you need.

3 - Why did a cow with clinical mastitis have a positive SA PCR result?

While SA is most commonly associated with subclinical infection, it is also an important cause of clinical mastitis. SA was the bacterial pathogen most frequently cultured from clinical mastitis cases in herds where all clinical cases were cultured as part of the CBMRN project (Olde Riekerink et al, 2008).

The Mastitis 4 PCR Assay tests for the 4 major contagious mastitis pathogens in herds: SA, *Streptococcus agalactiae*, *Mycoplasma bovis*, and *Prototheca*. DHI samples are composite samples. It is possible for a cow to have a SA infection in one quarter and to also have an infection with another pathogen in another quarter. When SA or another contagious pathogen is identified in the milk sample but the history and signalment in the cow differs from what you expected, it may not be appropriate to assume that the contagious bacteria was the only pathogen in that sample. If mastitis pathogens other than the three included in the Mastitis 4 are present in the sample they will not be identified. All cows with clinical mastitis should be fully assessed (CMT or other tests, clinical exam etc) and handled in an appropriate manner following the specific farm protocols.

4 - Can I test for other pathogens with DHI samples?

The Mastitis 4 PCR Assay was originally developed to identify a wide spectrum of bacteria in aseptically collected milk samples in a diagnostic laboratory setting, not for direct use on milk samples collected via meters. The decision to investigate its use for testing DHI milk samples was made only after investigation into the reliability of the results. When one of the three contagious pathogens (SA, *Streptococcus agalactiae* or *Mycoplasma bovis*) is identified by the PCR test in a DHI metered sample, research has shown there is a high probability that the bacterial pathogen originated in the cow's quarter. However, as DHI samples are collected in meters, after milk travels through milking units and hoses, the samples are not aseptically collected. If the full Mastitis 4 Assay were applied, DNA from many more bacteria would be detected. It would be impossible to tell whether these additional bacteria had originated from the cow's udder or had entered the milk sample from skin surfaces, hoses, meters, equipment, or other sources after leaving the teat end. Therefore, we strongly believe it is inappropriate to apply the full range of the Mastitis 4 Assay for testing of DHI metered milk samples. The results would be very difficult to interpret and likely misleading.

5 - Is there risk of “carry-over” of milk and DNA in the milking meters?

Carry-over of a small amount of milk from one DHI cow sample to the next at the time of collection is possible due to residual milk in the milking unit, milk meter or milk sampler. It could also occur in the laboratory if small amounts of milk were carried from one sample to the next on the pipetting equipment used to process the DHI milk samples. However, given the degree of mechanization involved in both sample collection and testing, there is a very small possibility that any carry-over occurs. Based on several investigations carried out in the field and in the DHI laboratory, we are confident that carry-over is unlikely. If it were to occur, the volume of sample potentially carried forward, either in the field or in the laboratory, is so small that dilution would markedly decrease the concentration of DNA to below the PCR detection limits. Therefore, false positive tests on the Mastitis 4 PCR are unlikely.

6 - Why was a cow’s PCR result positive one month and negative the next?

A cow may be PCR positive at one point in time and PCR negative at the next test, regardless of whether that next test is days, weeks or months later, for several reasons. Cows infected with SA may shed SA in the milk in low numbers and intermittently, thus the amount of DNA in the milk from an infected cow can vary dramatically from day to day, and may fluctuate above and below the detection limit of the PCR test.

The second reason for a negative test following a positive test is that it is possible for cows to cure SA infection either spontaneously or following antibiotic therapy. While we know that cure rates for well established SA infections are very, very low, it is possible for some infections to be only temporary. These SA infections resolve on their own or as a result of antibiotic therapy.

Reference:

Olde Riekerink RGM, HW Barkema, DF Kelton and DT Scholl. ,
Incidence Rate of Clinical Mastitis on Canadian Dairy Farms. J Dairy Sci 2008. Vol 91 Iss 4 pp 1366-1377.

Table 1. Comparison of bacteriological culture and Mastitis 4 PCR testing of cow milk samples

	Bacteriological Culture of Milk	Mastitis 4 PCR Testing of Milk
Sample Type	<p>Hand stripped sample of one or more (composite usually all 4) quarters.</p> <p>Sterile sample – alcohol prep of teat ends required.</p> <p>Foremilk only. Collected after milking prep, alcohol prep of teat ends and before milking unit applied.</p>	<p>Collected by meter at one milking.</p> <p>Very clean but non-sterile sample</p> <p>Composite samples. Collected from all quarters milking and throughout milk flow.</p>
Sample Collection	<p>Collected specifically for culture (only use). Stringent sample collection requirements.</p>	<p>Routinely collected as part of the regular DHI milk recording and testing service.</p>
Sample Handling	<p>Samples must be cooled and arrive at the laboratory within 24 hours of collection to prevent bacterial multiplication and overgrowth of non-target bacteria.</p>	<p>Preservative added at time of sample collection so bacterial multiplication is arrested. Samples are robust to storage and handling conditions.</p>
Volume of Sample Tested	<p>0.1 ml (100 µl) of milk is streaked on the culture plate(s).</p>	<p>0.35 ml (350 µL) of milk is used for DNA extraction and testing.</p>
Pathogen Detection	<p>Bacteria are grown on culture media and identified using a standard protocol for colony identification. Additional tests applied as needed.</p>	<p>Bacterial DNA extracted and identified using real-time PCR.</p>
Range of Pathogens Identified	<p>Broad (all bacteria in milk) – but does not include all mastitis pathogens (i.e. <i>Mycoplasma bovis</i> requires specific media for growth and identification).</p>	<p>Mastitis 4 is a very specific test. The test detects only <i>Staph. aureus</i>, <i>Strep. agalactiae</i> and <i>Mycoplasma bovis</i>.</p> <p>Even if other mastitis bacteria are present they will not be identified and the test will not be positive.</p>