



Real-Time QPCR-based DNA Mastitis Analysis using the preserved DHIA sample

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Abstract

Real-Time PCR has dramatically improved the usefulness of the common preserved DHIA bovine milk sample. It allows for a quicker turn around time while utilizing a high-end technological test. This helps to identify many of the common mastitis causing bacteria without the need for a sterile sample. It dramatically cuts down the wait time from up to 7 days to down to 1 day. The need for better, more accurate results is a welcome change for the dairymen.

Keywords: preserved DHIA sample, Real-time PCR, mastitis, bacteria.

1.0 Introduction

For decades in the dairy industry producers have strived to supply higher quality milk. Lancaster DHIA has aided in this need by testing for individual somatic cell count through the DHIA samples. Today producers want to perform further testing to identify the source of infection that is contributing to a high SCC. Lancaster DHIA saw the need for this service and opened a culture lab to assist the producer in achieving a higher quality product.

Producers are increasingly aware of management practices, and have used new resources like the culture lab to decrease their average SCC. The average LDHIA rolling herd SCC for 2008 was 306,000; it has decreased in 2010 to an average of 267,000 SCC for 3,000 farms with 210,000 cows. Producers have discovered that by lowering their SCC they have not only increased their milk quality bonus but have also increased milk production. For example, if current SCC were decreased by half, there would be a gain of 1.5 pounds (.68 kilogram) of milk per cow, per day, on average. With an increase in milk production of this magnitude, the potential increased revenue is a welcome reward. In the current economic landscape, maximizing milk production and quality is of utmost importance for success. Lancaster DHIA has made it a priority to help our members, and the agriculture community, prosper while promoting a safe and abundant food supply. We started a new service, PathoProof Real-Time qPCR-based Mastitis Assay, in October 2009, which will provide more accurate test results and the ability to use DHIA preserved milk. This assay is provided by Finzymes Diagnostics of Finland.

1.1 Tests Provided

The real-time qPCR-based mastitis assay identifies and quantifies the DNA of 12 mastitis-causing species or groups and the β -lactamase to the producer, as well as their veterinarian. This will show the specific bacterial specie(s) along with semi-quantification. The semi-quantification will show -, +, ++, +++ for all 13 targets. The 12 bacterial targets are *Staphylococcus aureus*, *Staphylococcus sp.* (including all major coagulase-negative staphylococci), *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli*, *Enterococcus sp.* (including *E. faecalis* and *E. faecium*), *Klebsiella sp.* (including *K. oxytoca* and *K. pneumoniae*), *Serratia marcescens*, *Corynebacterium bovis*, *Arcanobacter pyogenes* and *Peptoniphilus (Peptostreptococcus) indolicus* and *Mycoplasma bovis*. Approximately 25-40% of conventional milk cultures result in no bacterial growth. The real-time qPCR-based mastitis assay does not rely on bacterial growth, and as a result has 100% sensitivity for bacterial identification. With the ability to test DHIA preserved samples and milk from animals treated with antimicrobials, sample collection is easier and results more reliable. Also, producers can choose to set up regular qPCR testing regimens that utilize DHIA preserved samples.

1.1.1 How it works

The first step is taken during the DHIA test day. The producer and field technician fill out the DNA lab form based on the needs of the dairy producer. Producers may specify which cows to test based on somatic cell count and clinical or sub clinical mastitis. Bulk tank analysis is also available as a screening method for contagious and environmental bacteria. The producer can pool samples to screen select groups of cows. Based on the pooled results, producers can determine which cows should be used for individual testing. Using this method, producers will reduce testing costs when trying to detect the contagious bacteria. Then the information is emailed to the veterinarian and a copy sent to the dairy producer. This information is analyzed for proper treatment and potential extended therapy treatment based on individual findings.

1.1.2 The results

A dairy producer has the ability to utilize several reports such as the report below showing the results of a sample pool for the Major-3 Contagious Analysis.

Sample	Bacterial finding	Quantity	Proportion
1	Negative	-	-
2	Negative	-	-
3	Negative	-	-
4	Negative	-	-
5	Staph. aureus	+	
6	Negative	-	-
7	Negative	-	-
8	Negative	-	-
9	Staph. aureus	+	
10	Negative	-	-
11	Negative	-	-

628,794,812,470,26
759,349,532,420,707
723,146,865,19,198
832,200,033,1007,566
37,705,547,88,144
4150,335,722,3134,123
589,452,789,668,782
1189,640,1100,97,473
2984,858,2808,701,713
733,074,2416,600,613
10,913,27,938,5

Next is an example of the results of the individual cows from the pool

Sample	Bacterial finding	Quantity	Proportion
37	Negative	-	-
705	Negative	-	-
547	Negative	-	-
88	Negative	-	-
144	Staph. aureus	+	
7984	Negative	-	-
858	Negative	-	-
2808	Negative	-	-
701	Staph. aureus	suspect	-
713	Negative	-	-
685	Negative	-	-
627	Negative	-	-
660	Negative	-	-
760	Staph. aureus	+	
1061	Negative	-	-

This is an example of the results from the Complete-12 Full Panel Analysis for their 10 highest SCC.

Sample	Bacterial finding	Quantity	Proportion
768	Enterococcus sp. (including faecalis and faecium)	++	
	Klebsiella sp. (including oxytoca and pneumonia)	++	
	E. coli	+	
	Staphylococcus sp.	+	
4063	A. pyogenes and P. indolicus	+	
	Enterococcus sp. (including faecalis and faecium)	+	
	Klebsiella sp. (including oxytoca and pneumonia)	+	
	Staphylococcus sp.	+	
410	Staphylococcus sp.	++	> 90%
	Enterococcus sp. (including faecalis and faecium)	+	
596	Enterococcus sp. (including faecalis and faecium)	+	
	Staphylococcus sp.	+	
5307	Staphylococcus sp.	+	
813	Beta-lactamase gene	+	
	Staphylococcus sp.	+	
913	A. pyogenes and P. indolicus	+	
	Enterococcus sp. (including faecalis and faecium)	+	
	Staphylococcus sp.	+	
4521	Beta-lactamase gene	+	
	Enterococcus sp. (including faecalis and faecium)	+	
	Staph. aureus	+	
	Staphylococcus sp.	+	
29	Enterococcus sp. (including faecalis and faecium)	+	
	Staphylococcus sp.	+	
676	Enterococcus sp. (including faecalis and faecium)	+	
	Staphylococcus sp.	+	

1.1.3 Conclusion

In conclusion, the real-time qPCR-based mastitis test is fast, reliable and accurate. This allows us to give the results to our dairymen in a timely manner. This allows them to make more effective treatment decisions leading to improvements in milk quality and milk production.

As shown in this example, the dairyman started using the qPCR mastitis at the end of 2009. This allowed him to identify the top cows and has even lowered his already low SCC to an impressive 3 month average of 39,000 for 73 cows.

DATE OF TEST	WT. AVG. ACTUAL SCC
MONTH DROPPED	108
4-23-09	122
6-09-09	195
7-09-09	86
8-27-09	103
10-21-09	72
12-09-09	101
1-22-10	53
2-27-10	25
3-27-10	41
AVERAGES	89

→ 39,000