Some Lessons Learned Analyzing Nucleic Acids In Milk

T.M. Byrem & K.C. Haase

Antel BioSystems, NorthStar Cooperative, 4200 Forest Road, 48910, Lansing, USA
Byremt@antelbio.com *(Corresponding Author)*

Abstract

New assays for milk recorded samples add value both directly to dairy producers for cow management and indirectly through genetic and genomic evaluations for selection. Immuno- and molecular diagnostics are being used with greater frequency in milk recording laboratories. Molecular diagnostics includes technologies used to detect single nucleotides or specific nucleotide sequences of cell or cell-free origin to yield information about the cow's genome, milk microorganism content, and health and infection status. Experience applying molecular diagnostics on milk to determine mycobacterial infections (paratuberculosis and tuberculosis), sire verification and mastitis pathogen content has revealed some interesting observations for future consideration.

Lesson 1: Molecular diagnostic assays are very sensitive, and results are challenging traditional wisdom. Limits of detection as low as 2 and 10 genomes per real-time PCR assay were achieved for the detection of *Mycobacterium paratuberculosis* (IS900) and *bovis* (IS1810), respectively in bulk milk. When applied in field studies on infected and non-infected herds, the herd-level sensitivity and specificity for each bulk milk assay was 39.8% and 93.9%, and 38.7% and 97.9%, respectively. Interestingly, in one tuberculosis-infected herd that was positive by bulk milk analysis, the assay did not detect *M. bovis* in milk samples from 70 cows that reacted positively to the caudal fold tuberculosis test. What is the source of mycobacteria in bulk milk?

Lesson 2: Milk is a very suitable matrix for nucleic acid analysis. The suitability of DNA extracted from milk recorded samples was compared to tissue DNA for sire verification on Sequenom's 96-SNP parentage panel. Seventy four percent (34 of 46) of the paired analyses contained no discordant results and produced similar SNP call rates (90%, $P > 0.05$), effectively producing identical results for the two sample matrices. Discordant results at SNP sites in the 12 remaining analyses did not affect sire verification. Interestingly, in another study, average SNP call rates of 91% (n = 4) were achieved directly on pelleted milk somatic cells suspended in Tris-EDTA buffer without DNA extraction and purification. Is milk really a problematic matrix for molecular diagnostics?

Lesson 3: Carryover contamination during recording can be accounted for. The PathoProof Major 3 mastitis PCR assay was used to address high *Streptococcus agalactiae* bulk milk culture counts (>10,000 cfu/mL) in a 3000-cow dairy on DHI test day. The mean Ct value for *Streptococcus agalactiae* positive sample pools (5:1, n = 387) was 34.3 ± 1.2. Samples in pools with Ct values less than 31.9 (2 std dev below mean) were chosen for further analysis and yielded 127 cows with individual Ct values less than 31.9. Interestingly, one month after removing the positive cows from the milking herd, bulk milk *Streptococcus agalactiae* culture counts were still at 0 cfu/mL. Is carryover contamination only a number?

Traditionally, samples obtained during milk recording have been labeled unsuitable for molecular diagnostics because they are considered compositionally unacceptable and

contaminated. Experience has revealed the contrary, opening the door to new opportunities; possibly the analysis of the milk microbiome, microRNA and fetal DNA.

Keywords: molecular diagnostic, nucleic acid, milk, mycobacteria, parentage, contamination