

# *Milk Mycoplasma*

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As mentioned elsewhere in this newsletter, the Integrated Livestock Management (ILM) program at CSU is developing a Quality Milk Program (QMP). The goal of this program is to help Colorado dairy producers produce the highest quality milk possible by providing a reliable milk culture lab, consultative services, and pertinent research in milk quality issues.

Currently one of our major concerns is the accuracy of our milk mycoplasma culturing technique. The investigation of laboratory test validity is not straight-forward but understanding the steps of the investigation will give our customers a better understanding of the complexities of the issue. The following article explains the means by which we have evaluated our technique.

In conjunction with the QMP, the Colorado State University Veterinary Diagnostic Laboratories has been analyzing the milk mycoplasma culturing technique. This summer a study was performed to compare the results of our culturing technique to that of 3 other laboratories. We identified 211 samples from our laboratory or that had been shipped to us from other laboratories and split each into four separate samples. These were then shipped to three other laboratories for mycoplasma culture. Comparison of the results of all 4 laboratories (CSU Dx Lab and the 3 outside labs) analyzing the same samples revealed that the CSU Dx Lab identified fewer milk samples positive for Mycoplasma. In laboratory lingo, the “sensitivity” of our technique was less than that of the other 3 laboratories. Even though one could conclude that other labs reported false-positive results due to inability to distinguish mycoplasmas from similar non-pathogenic species, we decided to assume we were reporting false-negative results and resolved to modify our techniques to improve our lab sensitivity.

The CSU culturing process had been designed to be the best available anywhere because it used the best growth medium and an extra enriching step. These features add cost and time to the process but were included to optimize results. At CSU we make our own media with fetal bovine serum. Published scientific literature reports that this is a preferred supplement, but is rarely used because of expense. The other 3 laboratories in our comparison use horse serum in the growth media.

To evaluate the contribution of the difference of the media supplements (fetal bovine serum and horse serum), we re-cultured samples from which other laboratories had isolated mycoplasma, and we had not, as well as some that we had reported as positive for mycoplasma.

However, our most startling finding was unexpected. It appears that the cause of our lower sensitivity is related to the other procedural step conducted only by the CSU lab that was intended to increase sensitivity of the culture technique. At CSU samples are enriched in broth media and then filtered before plating. It appears that the filtering step intended to decrease contamination has actually decreased the likelihood of culturing Mycoplasma! These findings seemed illogical, since we are enriching the sample to encourage the growth of mycoplasma! Unfortunately, an occasional side effect of enrichment is increased growth of contaminating background organisms, and thus the enriched culture broth must be filtered before the final plating. It appears that the mycoplasma organisms are being trapped in the filter along with the other microorganisms and are not available for plating. This indicates to us that it is not our media that is a concern, but perhaps the final filtering step.

At this stage we plan to apply the milk from all individual cow samples directly to plates without the intervening broth enrichment procedure. A benefit of this direct plating procedure is decreased turnaround time for results: We will perform an initial reading at 72 hours and a final reading at 7 days. Additionally, we will be performing another blinded culture study on mycoplasma positive and negative samples in the coming month comparing our sensitivity with the new method and old method to another laboratory's results. Stay tuned for an update when we complete this new set of samples!