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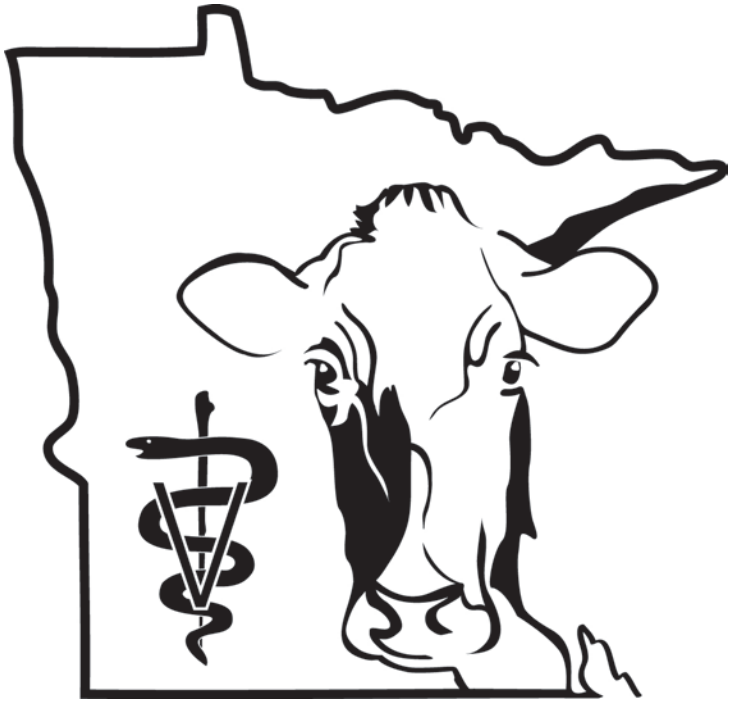

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Validation of the Minnesota Easy Culture System II: Results from On-farm Bi-plate and In-lab Tri-plate Culture vs. Standard Laboratory Culture

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Introduction

Despite continued progress in mastitis control research, mastitis remains the most costly infectious disease, and the most frequent cause of antibacterial use, on commercial dairy farms. As such, research should continue on the development and validation of new management tools that will help reduce the health and economic impact of this disease, while at the same time promoting the judicious and strategic use of antimicrobials on dairy farms. Accordingly, there is increasing adoption of on farm culture systems for selective and/or strategic treatment of clinical mastitis cases. Similarly, there may be an opportunity for using on farm culture systems for the diagnosis and selective and/or strategic treatment of subclinical intramammary infections in fresh cows.

The Minnesota Easy Culture System II bi-plate is an on-farm culture system that uses a selective media to diagnose and differentiate between gram-negative versus gram-positive infections. This on-farm culture system may be a useful tool to guide selective treatment decisions by producers.

Treatment and management decisions for clinical and subclinical intramammary infections may vary depending on the pathogen isolated. The Minnesota Easy Culture System II tri-plate is an on-farm culture system designed to assist producers in diagnosing and differentiating between gram-negative vs. gram-positive infections, and identifying the gram-positive bacteria as *Streptococcus* or *Staphylococcus* spp.

A multi-site, multi-herd, 3-year, controlled field study has been designed to validate the efficacy, and to quantify the cost-benefit, of incorporating on-farm culture systems into both clinical and subclinical mastitis monitoring and treatment programs. This manuscript outlines the methodology followed, and presents the preliminary results of one of the objectives of the project, which is the validation of an on-farm culture system (Minnesota Easy Culture System II).

Methods

Farm personnel were trained to aseptically collect milk samples from clinical mastitis quarters upon detection, excluding cows with severe mastitis; and from fresh cow quarters that test positive using the Californian Mastitis Test (CMT) within the first three days after calving, excluding cows freshening with clinical mastitis. The fresh samples were then plated, on farm, by dipping a sterile cotton swab into the milk sample, plating it onto one half of the on-farm culture media bi-plate, redipping the swab, and applying to the other half (Minnesota Easy Culture System II). Milk samples were then placed in the farm freezer to be transported to the lab. Plates were placed in an incubator overnight and the next morning the plate was read and interpreted according to the culture system guidelines. Results were recorded as "no growth" when bacteria did not grow in either half of the bi-plate, or "gram-positive" or "gram-negative" depending if growth was on the Factor or the MacConkey media half of the bi-plate respectively. A sample was considered contaminated when

bacteria grow in both halves of the bi-plate. If the result was "no growth" the plate was returned to the incubator and re-read approximately 24 hours later. With "no growth" or "gram-negative" culture results, the quarter did not undergo intramammary treatment. However, with "gram-positive" culture result, intramammary therapy with Cephapirin Sodium (Cefa-Lak®, Fort Dodge, IA) was initiated according to the product label.

After transporting frozen samples to the University of Minnesota Udder Health Laboratory, the samples were thawed and cultured using identification procedures recommended by the National Mastitis Council (NMC, 1999), and using standardized among university labs at all participating sites. Study technicians also randomly selected a total of 101 clinical mastitis and 210 fresh cow quarter milk samples, which were then plated on a Minnesota Easy Culture System II tri-plate (contains MacConkey, Factor, and Modified TKT agar media) using a sterile cotton swab. The tri-plates were then incubated at 37 °C and then read after 24 and 48 hours of incubation. Results of the tri-plate were recorded as "no growth" when bacteria did not grow in either section of the tri-plate, or "gram-negative" when bacteria grew in the MacConkey media section, and the "gram positive" were differentiated in *Staphylococcus* growth when growth occurred on the Factor media only, or *Streptococcus* growth when growth occurred also on the Modified TKT media of the tri-plate.

In order to describe the ability of the on-farm bi-plate culture results to differentiate a gram-positive infection (positive test result) versus a gram-negative infection or no growth (negative test result) - Treat/Don't Treat (T/DT), the sensitivity, specificity, and predictive values of the bi-plate results as compared to the in-lab culture, our gold standard, were calculated. The validation of the tri-plate culture results was also based on the Treat/Don't Treat (T/DT) decision and, when gram-positive growth was observed on both plates, *Staphylococcus* growth vs. *Streptococcus* growth (Staph/Strep) was compared between the two culture methods. The test characteristics were determined separately for clinical and subclinical mastitis samples. Preliminary analysis did not include cultures that were classified as contaminated on-farm, or samples from which more than one bacterial isolate was isolated in the lab.

Preliminary Results and Conclusions

On-farm Bi-plate Culture versus Standard Laboratory Culture

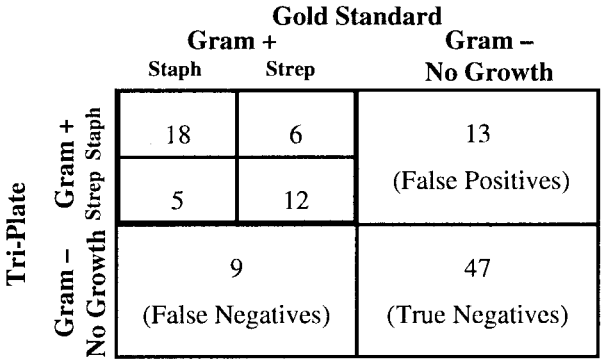
At this stage of the study, on farm bi-plate culture results and corresponding in lab results are available from 80 quarter cases of clinical mastitis, and from 87 fresh cows with CMT positive quarters.

Clinical Mastitis Quarter Cultures
Using the on-farm culture method for clinical mastitis cases, producers were able to detect 83% of the gram-positive cases (sensitivity), and classified correctly about 90% of the gram-negative cases or cases where bacteria was not present (specificity). Consequently, 83% of the treated cases were truly gram-positive (predictive value of a positive test; PV+), and 90% of the not treated cases were truly uninfected or gram-negative (predictive value of a negative test; PV-).

Bi-Plate	Gold Standard: Gram +	Gold Standard: Gram – No Growth
Gram +	25 (True Positives)	5 (False Positives)
Gram – No Growth	5 (False Negatives)	45 (True Negatives)

Bi-Plate	Gold Standard: Gram +	Gold Standard: Gram – No Growth
Gram +	44 (True Positives)	11 (False Positives)
Gram – No Growth	6 (False Negatives)	26 (True Negatives)

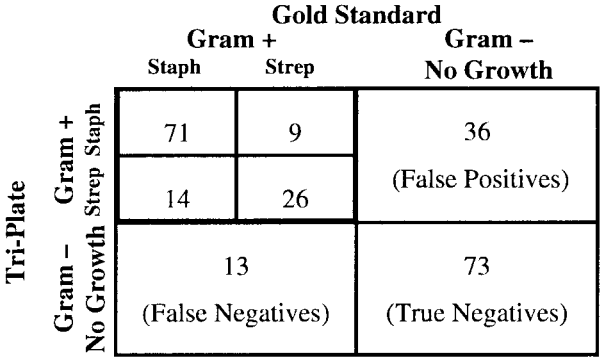
CMT Positive Fresh Cow Quarter Cultures
For fresh cow CMT positive quarters, the sensitivity of the on-farm culture to detect gram-positive quarters was 88%, and the specificity was 70%. Accordingly, 80% of the treated cases were truly gram-positive (PV+), and 81% of the not treated cases were truly uninfected or gram-negative (PV-).



<u>In-lab Tri-plate Culture versus Standard Laboratory Culture</u>

Clinical Mastitis Quarter Cultures
When considering the T/DT decision, the tri-plate had a Se of 82%, Sp of 78%, PV+ of 76 %, and PV- of 84%. For those quarter samples that gram-positive growth was observed on both culture methods, when evaluated for Staph vs. Strep growth, the tri-plate had a Se of 78%, Sp of 67%, PV+ of 75%, and PV- of 71% for identifying *Staphylococcus* growth.

CMT Positive Fresh Cow Quarter Cultures
The tri-plate had a Se of 90%, Sp of 67%, PV+ of 77%, and PV- of 85% for the T/DT decision. When the Staph vs. Strep differentiation was done for the gram-positive growth, the tri-plate had a Se of 84%, Sp of 74%, PV+ of 89%, and PV- of 65% for identifying *Staphylococcus* growth.



Predictive values of the on-farm bi-plate versus the lab standard procedures were moderately high. However, the test will not be fully validated until the conclusion of the study, when the cost of missing a false negative or the cost of treating a false positive has been quantified. The moderate-to-high diagnostic Se, Sp and predictive values to differentiate a gram-positive infection versus a gram-negative infection or no growth, and to classify gram-positive in Staph/Strep growth indicates that the tri-plate culture system has a good ability to correctly identify and classify mastitis pathogens into general categories for both clinical and subclinical infections. The bi-plate and tri-plate test characteristics to detect and classify bacteria growth appear not be different between milk samples from fresh cows and clinical cases.

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