

### *Subclinical Mastitis Study*

When evaluated for G/NG, the bi-plate culture system had a SE of 76.9%, SP of 63.3%, PPV of 69.0%, NPV of 72.1% and an inter-reader simple agreement of 93.2% (Kappa 83.7%). When evaluated for G+/G- growth, the bi-plate culture system had a SE of 96.8%, SP of 81.8%, PPV of 93.8%, NPV of 90.0% and an inter-reader simple agreement of 98.3% (Kappa 96.1%). When considering the T/DT decision, the bi-plate culture system had a SE of 76.9%, SP of 77.8%, PPV of 68.2%, NPV of 84.5% and an inter-reader simple agreement of 90.2% (Kappa 80.1%).

### **Significance**

Results seen in both the subclinical and clinical mastitis studies proved similar. The moderate-to-high

diagnostic SE, SP and predictive values for G/NG suggest that the bi-plate culture system has a reasonably good ability to correctly identify the presence of infection (G/NG). The high SE, SP and predictive values for G+/G- suggest that, for plates showing growth, the bi-plate is very accurate in categorizing infections into these two general classification groups (G+/G-). The very high Kappa values indicate that this culture system yielded very similar results when used by two different readers. Overall, these study results suggest that the Minnesota Easy Culture System II bi-plate is an accurate tool that may be useful to guide strategic mastitis treatment decisions on farms. However, the efficacy and cost-benefit of incorporating this test into a mastitis control program will not be fully known until results of its use are described in a controlled field study on commercial dairy farms (study currently in progress).

## Validation of the Minnesota Easy Culture System II: Results from In-lab Tri-plate Culture versus Standard Laboratory Culture, and Tri-plate Inter-reader Agreement

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### **Introduction**

Regardless of intensive control efforts, mastitis remains the most costly infectious disease in dairy cows and is the most frequent cause for using antibiotics on commercial dairy farms. 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 between infections caused by *Streptococcus* and *Staphylococcus* spp, thus allowing producers the possibility of making strategic

treatment decisions based on the type of infection present. The objectives of this study were to perform an in-lab validation of the Minnesota Easy Culture System II tri-plate method as compared to standardized laboratory culture methods and to describe inter-reader agreement for tri-plate culture results.

### **Materials and Methods**

#### *Subclinical Mastitis Study*

Fresh cows from four Minnesota dairies were screened between one and three days post-calving by collecting quarter-milk samples and performing the California Mastitis Test (CMT) on each quarter. The

CMT result was recorded as negative, 1+, 2+ or 3+. Any cows freshening with clinical mastitis were excluded from the study. The quarter-milk samples were frozen and transported to the Laboratory for Udder Health at the University of Minnesota. At the lab, a total of 210 quarter-milk samples were randomly selected for the validation study and thawed at room temperature. These samples 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 (estimate 0.1 ml). The samples were also cultured and speciated using standardized laboratory methods after plating milk onto MacConkey and Factor agar media using a calibrated 10  $\mu$ l calibrated loop. Both plates were then incubated at 98.6° F (37° C) and read after 24 and 48 hours of incubation.

#### *Clinical Mastitis Study*

Cows presenting with clinical mastitis at four other Minnesota dairies were enrolled in the clinical mastitis study. The severity of mastitis was scored on a scale of 1 to 3, with 3 being systemically ill. Cows with grade 3 mastitis were excluded from the study and treated according to individual farm protocols. Milk samples collected from mastitic quarters were frozen and transported to the Laboratory for Udder Health at the University of Minnesota. At the lab, a total of 101 quarter samples were randomly selected for the validation, thawed and cultured on a Minnesota Easy Culture System II tri-plate using standard laboratory procedures previously described for the subclinical mastitis study.

Plates cultured using the standard laboratory procedures were read and results recorded by Laboratory for Udder Health technicians. The tri-plates were read independently by two students using the Minnesota Easy Culture System II handbook to identify bacterial growth. Tri-plate results recorded by the students were compared for inter-reader agreement beyond chance (Kappa). The results of one student were compared to the results attained using standardized laboratory culture methods to determine the diagnostic sensitivity (SE), diagnostic specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) of the tri-plate results as compared to the standard laboratory results (the gold standard). To do this, the results from the two culture methods were analyzed comparing Growth/No Growth (G/NG), Gram + growth/Gram – growth (G+/G-), Treat/Don't Treat (T/DT) and, when G+ growth was observed on both plates, *Staphylococcus* growth/*Streptococcus* growth (Staph/Strep). *Staphylococcus* growth on the tri-plate was considered when growth occurred on the Factor media only. *Streptococcus* growth on the tri-plate was considered when growth

occurred on the Modified TKT media. Finally, T/DT was used to assess the potential action taken by the producer in treating the mastitis case: Treat was suggested for G+ growth while DT was assigned to results of G-growth or NG.

## Results

### *Subclinical Mastitis Study*

When evaluated for G/NG the Minnesota Easy Culture System II tri-plate had a SE of 87.6%, SP of 65.7%, PPV of 71.9%, NPV of 84.1% and Kappa value (estimating inter-reader agreement beyond chance) of 93.0%. When evaluated for G+/G- growth, the tri-plate had a SE of 95.7%, SP of 73.9%, PPV of 93.6%, NPV of 81% and Kappa value of 94.7%. When evaluated for Staph/Strep growth, the tri-plate had a SE of 83.5%, SP of 74.3%, PPV of 88.8%, NPV of 65.0% and Kappa value of 85.1%. When considering the T/DT decision, the tri-plate had a SE of 87.1%, SP of 67.0%, PPV of 71%, NPV of 85.0% and Kappa value of 93.1%.

### *Clinical Mastitis Study*

When evaluated for G/NG, the Minnesota Easy Culture System II tri-plate had a SE of 76.9%, SP of 68.8%, PPV of 72.7%, NPV of 73.3% and Kappa value of 83.8%. When evaluated for G+/G- growth, the tri-plate had a SE of 94.1%, SP of 100%, PPV of 100%, NPV of 77.8% and Kappa value of 89.3%. When evaluated for Staph/Strep growth, the tri-plate had a SE of 78.3%, SP of 66.7%, PPV of 75.0%, NPV of 70.6% and a Kappa value of 81.3%. When considering the T/DT decision, the tri-plate had a SE of 78.0%, SP of 78.3%, PPV of 71.1%, NPV of 83.9% and Kappa value of 90.0%.

## Significance

The moderate-to-high diagnostic SE, SP and predictive values for G/NG, G+/G- growth and for Staph/Strep growth indicates that the tri-plate culture system has a good ability to correctly identify and categorize mastitis pathogens into these general classification groups for both clinical and subclinical infections. The very high Kappa values indicate that this culture system yielded very similar results when used by two different readers. Overall, these study results suggest that the Minnesota Easy Culture System II tri-plate is an accurate tool that may be useful in guiding strategic mastitis treatment decisions. However, the efficacy and cost-benefit of incorporating this culture method into a mastitis control and treatment program will not be fully known until the results of its use are described in a controlled field study on commercial dairy farms.