

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

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Introduction

Clinical and subclinical mastitis remains the most costly infectious disease and the most frequent cause of antimicrobial use on commercial dairy farms. 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 strategic treatment decisions by producers. The objectives of this study were to perform an in-lab validation of the Minnesota Easy Culture System II bi-plate method as compared to standardized laboratory culture methods and to describe inter-reader agreement for bi-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. The samples were plated on a Minnesota Easy Culture System II bi-plate 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 μ 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 bi-plate, using standard laboratory procedures previously described for the subclinical mastitis study.

Plates cultured using the standard laboratory procedures were read, and results were recorded by Laboratory for Udder Health technicians. The bi-plates were read independently by two students using the Minnesota Easy Culture System II handbook to identify bacterial growth. The bi-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 bi-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-) and Treat/Don't Treat (T/DT). The T/DT classification was used to assess the potential action taken by a producer in treating the mastitis case: treat was suggested for G+ growth while DT was assigned to results of G- growth or NG.

Result

Subclinical Mastitis Study

When evaluated for G/NG, the bi-plate culture system had a SE of 81.1%, SP of 69.4%, PPV of 75%, NPV of 76.4%, and inter-reader simple agreement of 93.3% (Kappa 86.2%). When evaluated for G+/G- growth, the bi-plate culture system had a SE of 100%, SP of 92.3%, PPV of 98.9%, NPV of 100% and an inter-reader simple agreement of 98.4% (Kappa 92.9%). When considering the T/DT decision, the bi-plate culture system had a SE of 83.2%, SP of 74.5%, PPV of 77.4%, NPV of 80.9% and an inter-reader simple agreement of 93.3% (Kappa 86.4%).

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).

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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

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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