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Preliminary Results from Validation of the Minnesota Easy Culture System II Bi-Plate and Tri-Plate

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On-farm culture systems, such as the Minnesota Easy Culture System (University of Minnesota, Saint Paul, MN), have proven themselves to be useful tools to guide strategic treatment decisions for clinical mastitis on farms. These systems use selective culture media to differentiate Gram-positive (GP) from Gram-negative (GN) organisms (Bi-plate and Tri-plate) and to differentiate Staphylococcal from Streptococcal infections (Tri-plate). When previously evaluated using clinical mastitis samples, Lago (PhD Thesis. U of MN. 2009) reported that the diagnostic sensitivity (SN) of the Bi-plate to correctly diagnose no bacterial growth, GP growth, and GN growth was of approximately 87%, 78% and 73%, respectively. In the same study, the specificity (SP) of the Bi-plate for these three diagnostic classifications was 55%, 83% and 87%, respectively. However, studies are lacking to similarly describe the diagnostic test characteristics of the Bi-plate and Tri-plate if the reader tries to use colony morphology characteristics to attempt to identify individual bacterial species. The objective of this study was to validate the use of the Minnesota Easy Culture System II Bi-plate and Tri-plate systems to diagnose the presence of classes and species of bacteria in milk samples.

The study was conducted using 172 frozen milk samples, representing a mixture of previously frozen quarter and composite samples from individual cows, submitted to the Udder Health Laboratory (College of Veterinary Medicine, University of Minnesota) during spring, 2010. Milk samples were thawed to room temperature and then underwent routine Udder Health Laboratory microbial culture procedures (Gold standard test). At the same time, sterile cotton swabs were dipped into the milk sample and then plated onto both the Bi-plate and Tri-plate, respectively. The plates were incubated at 37^{o}C for 24 hours and were then read and interpreted by two different and independent readers. These two readers were untrained, blinded, and used the MN Easy Culture System guidelines, as would a dairy producer, to reach their diagnoses. If bacteria did not grow the plate was returned to the incubator and re-read approximately 24 hours later. Final results for each plate were recorded by each reader as a) No growth (NG), Gram-positive growth (GP), Gram-negative growth (GN), Staphylococcal species (Staph), Streptococcal species (Strep), and then the specific species isolated including *Streptococcus agalactiae*, *Streptococcus dysgalactiae, Streptococcus uberis, Staphylococcus aureus, Coagulase-negative Staphylococci* (CNS)*, Escherichia coli, Klebsiella spp*. or other.

Contaminated samples, wherein 3 or more different species isolated using routine laboratory methods, were excluded from the final analysis. Statistics were produced to describe, for both readers and for both the Bi-plate and the Tri-plate, the Kappa (K,

agreement with lab), and the diagnostic sensitivity (SN), specificity (SP), accuracy (AC), predictive value of a positive test (PPV) and predictive value of a negative test (NPV) for each of the possible classes and species of microbial culture results described above.

Results from laboratory culture of 172 samples used in the Bi-plate evaluation, that yielded either a no growth or a single species of bacteria (excluding 'mixed' results), showed that approximately 19.2%, 66.3%, 13.4% and 1.2% of samples yielded a NG, GP, GN or 'other organism' result. Coagulase-negative Staphylococci, *Streptococcus uberis*, *Staphylococcus aureus*, Enterococcus spp., *Streptococcus dysgalactiae*, other GP, *Escherichia coli, Klebsiella spp*., other GN, and other microbes (i.e. Protheca or Yeast) constituted 26.7%, 12.8%, 10.5%, 6.4%, 5.2%, 4.7%, 6.4%, 5.8%, 1.2% and 1.2% of laboratory culture findings, respectively. Laboratory culture results from 171 milk samples used in the Tri-plate evaluation yielded a similar distribution of microbe species.

Overall accuracy, SP and NPV estimates were generally high (> 75%) for both the Bi-plate and Tri-plate for all classes and species groups of bacteria evaluated. However, we consider the diagnostic SN estimates to be of greater importance when seeking to identify a specific bacterial class or species. For the Bi-plate evaluation, the Kappa and SN estimates were consistently high when the reader was trying to identify the presence of a GP IMI (Kappa > 60%, SN > 80%). These values were generally intermediate when trying to identify the presence of NG, GN, Staph., or *S. aureus* (Kappa > 50%; SN > 60%), and were inconsistent or generally low when trying to identify the presence of a specific organism including CNS, *Strep. uberis*, *Strep. dystgalactiae*, *E. coli* or *Klebsiella* spp.

For the Tri-plate evaluation, the Kappa and SN estimates were generally high when the reader was trying to identify presence of a GP IMI (Kappa > 60%, SN > 80%). These values were generally intermediate when trying to identify the presence of NG, GN, Staph., Strep., or *S. aureus* (Kappa > 50%; SN > 60%), and were inconsistent or generally low when trying to identify the presence of a specific organism including CNS, *Strep. uberis, Strep. dystgalactiae*, *E. coli* or *Klebsiella* spp.

These preliminary findings suggest that readers using the Bi-plate or Tri-plate systems on farms will get the most accurate and useful results when using this diagnostic tool to identify the presence of a GP IMI. Either tool has moderate ability to identify NG, GN and *Staphylococcus aureus* IMI. As expected, the Tri-plate is more accurate than the Bi-plate at identifying IMI caused by Strep. or Staph. classes of organisms. With the exception of *Staphylococcus aureus*, readers should refrain from trying to speciate the type of organism cultured using either the Bi-plate or Tri-plate.