Laboratory Evaluation of Two Potential On-farm Culture Systems for Clinical Mastitis Cases

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Introduction

In Canada, mastitis treatment accounts for more than half of all antibiotics used by dairy producers. Treatment decisions are often made without knowledge of the pathogen, therefore, researchers have called for a more targeted approach to antibiotic therapy for clinical mastitis. The overall hypothesis of this project is that the use of rapid on-farm tools for the identification of mastitis pathogens will result in therapy decisions that will decrease antibiotic use, without compromising short or long term cow or herd mastitis outcomes. The objective of this study was to determine test characteristics and compare two potential on-farm culture systems, the Minnesota Easy Culture System II Bi-plate (University of Minnesota, St. Paul, MN) and 3M Petrifilm™ (3M Microbiology, London, ON).

Materials and Methods

Clinical mastitis samples (N= 282) were collected on twenty Prince Edward Island dairy herds over a six month period. For Gold Standard (GS) comparison, samples were evaluated using standard laboratory techniques. Bi-plates were evaluated by swabbing plates and classifying growth as Gram Positive (GP), Gram Negative (GN) or No Growth (NG) based on which side of the plate the colonies grew on. For Petrifilm™, to create the same categories of GP, GN or NG, two types of Petrifilms™ were used, Aerobic Count (AC) and Coliform Count (CC). Petrifilms™ were inoculated with 100ul of milk in 900ml of sterile diluent. Both Bi-plates and Petrifilms™ were read after 24 hours of incubation by a trained microbiology technologist and five blinded individuals with limited microbiology experience for an inter-reader comparison. The tests were evaluated to determine their ability to differentiate the appropriate treatment groups (GP, GN and NG). For Bi-plates, growth was classified as positive or negative on each media. Analysis was conducted at various cut points for the Petrifilm™ test system. The combination of Petrifilms™ using a CC cut-off of 20 colonies and an AC cut-off of 5 colonies had the highest sensitivity. With these criteria, samples were classed as GN if there were ≥ 20 colonies on CC; GP if there were < 20 colonies on the CC and ≥ 5 colonies AC and NG if there were < 20 colonies on CC and < 5 colonies on AC.

Results

The Bi-plate had a sensitivity of 97.9% and a specificity of 68.6%. In this group of samples the Bi-plates had a negative predictive value of 96.4% and a positive predictive value of 79.0%. The Petrifilm™ test system had a sensitivity of 93.8% and a specificity of 70.1%. The negative predictive value was 89.7% and the positive predictive value was 80.3% using the Petrifilms™. Kappa values for the five readers were 0.75 for Bi-plates and 0.84 and 0.86 for AC and CC Petrifilms™, respectively. These values are considered very good to excellent agreement.

Significance

In a targeted mastitis treatment protocol, the ability to identify GP bacterial infections is an important bench mark. A low false negative rate limits the number of animals that go untreated that may have benefited from treatment. Both of the tests evaluated in this laboratory study performed well and have potential as on-farm diagnostic tools.