Evaluation of the Petrifilm™ Culture System for the Identification of Mastitis Bacteria as Compared to Standard Bacteriological Methods

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Introduction

The largest cost associated with treating clinical mastitis during lactation is the discarded milk during and after therapy. To justify this cost, one should ensure that the present infection will respond to antibiotic therapy. Most intramammary antibiotics target gram-positive cocci organisms, and are not particularly effective against *Mycoplasma* spp, yeasts and mild infections caused by gram-negative pathogens. Similarly, research has demonstrated that other organisms, such as *Streptococcus uberis*, are highly responsive to intramammary antibiotics, or that the cure rates achieved by various intramammary treatments are not significantly better than no treatment. Thus, there has been an increasing demand for research towards the development of on-farm mastitis culturing media for early identification and accurate classification of bacterial species. Petrifilm™ plates are selective culture media products, which are used for rapid bacteriological isolation and enumeration from food products. The 3M Petrifilm™ products are small, playing card size, sample-ready plates that will allow users to easily and efficiently perform on-site microbial identification. Petriflim™ plates that are potentially useful for mastitis diagnoses are the Aerobic Count Plates, Coliform Count Plates, and Staph Express Count Plates, due to their ease of use, and the ability to reach a diagnosis in as little as 24 hours. The usefulness of the Petrifilm™ culture system for the diagnosis of mastitis has been previously reported.[1] The objective of the current project was to determine the test characteristics of Petrifilm™ to characterize the causative organism in producer-defined cases of clinical mastitis.

Materials and Methods

The evaluation of Petriflim™ media was performed using milk samples from 156 cases of mastitis identified in 10 commercial herds in southwestern Ontario. The participating producers were instructed to take duplicate milk samples from cows identified as having clinical mastitis at the time of milking. Milk samples were refrigerated and stored on farm until they were picked up regularly by a technician of the veterinary clinic. Once received at the veterinary clinic, the first milk sample was plated onto three Petriflim™ media plates: Aerobic Count, Coliform Count, and Staph Express. The second milk sample from each cow was frozen at -328°F (-200°C) and later transferred to the Mastitis Research Laboratory, University of Guelph, for standard microbiological culture. Each culture media plate was interpreted using guidelines provided by 3M Canada. The results from each different type of plate were interpreted together to record an outcome for each clinical mastitis sample. The outcome categories utilized were: bacterial growth, gram-positive growth, coliform growth, *Staphylococcus* spp growth, and *Streptococcus* spp growth. The standard laboratory microbiology results were interpreted to provide a similar outcome. For this study, the definition of "bacterial growth" from Petrifilm™ was based on a positive sample on the Aerobic Count Plate. The definition of "coliform growth" was based on growth on both the Aerobic Count and Coliform Count Plates and no growth on the Staph Express Plate. A positive result of "staph spp" was made based on growth in the Aerobic Count and Staph Express Plates, and no growth on the Coliform Count. Interpretation of the indicator dye in the Staph Express Plate to differentiate *Staphylococcus aureus* was not used. A "strep spp" Petrifilm™ result was made with growth on the Aerobic Count Plate, and no growth on either of the Coliform Count or Staph Express Plates. Finally, a result of "gram-positive growth" was recorded with a positive Aerobic Count Plate and negative Coliform Count Plate, coupled with either positive or negative growth on the Staph Express.

Results

From the 156 samples collected, 52 (33%) had no growth recorded in the microbiology laboratory. For each culture result category, the sensitivity, specificity and predictive values were calculated. There was excellent

sensitivity (> 85%) for all categories, with the exception of those classified as *Streptococcus* spp. There was a corresponding good specificity, indicating low numbers of false positive results, except for the category of bacterial growth (27%). Specifically, the Sn/Sp calculated for each category were: bacterial growth (93%/27%), gram-positive growth (92%/73%), coliforms (93%/86%), *Staphylococcus* spp (86%/82%) and *Streptococcus* spp (58%/91%). Closer examination of the raw data revealed that of all samples evaluated, there were 38 (24%) that were positive for growth on Petrifilm™ which the mastitis laboratory recorded as having no bacterial growth. In this study, Petriflim™ was observed to have an excellent sensitivity and specificity for detecting coliforms. It is noteworthy that 18 of 44 Petrifilm™ plates were false positive for coliform organisms. The positive predictive value was decreased due to these samples that were positive Petrifilm™, but did not result in any coliform growth when shipped to the mastitis laboratory. One reason for this may be due to the freezing of the samples destined for the laboratory, whereas all Petrifilm™ samples were plated as refrigerated fresh samples. Studies examining the effect of freezing in the handling of milk samples have reported that freezing is an important consideration for loss of coliforms, as well as increased recovery of *S. aureus*. The low sensitivity of the Petrifilm™ plates for environmental streptococci was not totally unexpected, as there is no specific Petrifilm™ plate to differentiate streptococci. Furthermore, in this study, a very liberal definition of strep spp was used based on interpretation of three specific Petrifilm™ plates. The comparison between the two tests to detect the broad category of bacterial growth, revealed Petrifilm™ suffered from a low specificity (27%). Thirty-eight out of 52 positive plates were false positive for growth. This again could be due in part to the potential effect freezing had on coliforms. In addition, there is a large difference in the volume of milk used between the two tests.

Significance

The core principle of mastitis treatment protocols begins with differentiating mastitis-causing organisms into either gram-positive or gram-negative categories. User-friendly culture systems that can accurately make this distinction in a short enough time to delay commencement of therapy, will result in reduced amount of antibiotics used on farm, reduced discarded milk from clinical mastitis cases and ultimately reduce the cost of the disease. Petrifilm™ media plates represent a very user-friendly culture system, which can confirm growth of coliform bacteria within 24 hours. Compared to routine laboratory confirmation, Petrifilm™ had an excellent sensitivity and specificity for diagnosing coliform pathogens as the cause of producer-defined cases of clinical mastitis. Petrifilm™ also performed favorably for detection of gram-positive growth and staph spp growth. This on-farm diagnostic tool offers considerable potential for the implementation of therapy protocols, which could guide appropriate treatment of clinical mastitis cases.

Reference

1. Silva B, Caraviello D, Rodrigues A, Ruegg P: Use of Petrifilm™ for mastitis diagnosis and treatment protocols. *Proc 43rd Ann Meet Nat Mast Counc*, 2004, pp 52-59.