COMPARISON OF REAL-TIME PCR AND CONVENTIONAL BACTERIAL CULTURING IN BOVINE MASTITIS TESTING

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Reliable identification of bacteria responsible for intramammary infection (IMI) is one of the cornerstones for controlling mastitis and targeting antimicrobial therapy. Bacterial culture-based methods have long served as the golden standard for mastitis testing. However, the conventional methods are time consuming, routinely requiring 48 hours to complete. Culture-based identification of many mastitis-causing species also requires extensive experience from the operator, causing differences in the reliability between different testing laboratories. For example, based on a European proficiency testing program, it was reported that the overall rate of erroneously identified mastitis bacteria ranged from 9%-37% across the different laboratories (Pitkälä et al., 2005). An additional drawback is that approximately 25%-40% of milk samples from clinical and subclinical mastitis show no bacterial growth in conventional culturing.

Real-time Polymerase Chain Reaction (real-time PCR) has become a mainstay technology in diagnostics, and, due to its undisputed benefits, is currently considered as the 'golden standard method' in many clinical microbial testing applications. A real-time PCR assay, identifying a total of 11 mastitis-causing bacterial species or species groups, and the staphylococcal penicillin resistance gene, became commercially available in March 2008 ('PathoProof Mastitis PCR Assay', Finnzymes Oy, Espoo, Finland). The assay uses raw milk as a starting material, does not require bacterial culturing, takes 3-4 hours from start-to-finish, and includes all necessary reagents for DNA extraction and real-time PCR. The analytical specificity and sensitivity of the test was reported to be excellent, based on a large collection of culture isolates originating from Europe and North-America (Koskinen et al., 2008). The assay has also been shown to yield clinically relevant positive results in more than 40% of samples providing no growth in bacterial culturing (Taponen et al., 2008). Due to the benefits of the assay, several European countries have already implemented the assay into routine mastitis testing programs (see www.vhlgenetics.com/vhl/pdf/20081001.pdf). For veterinarians, test laboratories, and dairy producers using this new technology in routine mastitis testing, it is now important to compare the performance of the PathoProof Mastitis PCR Assay, against the conventional culture test, in bacterial identification from mastitic milk samples. In this study, we report a field trial validation of the real-time PCR assay against the conventional culture test from mastitic milk samples.

Quarter milk samples were collected into sterile vials using aseptic sampling techniques from 676 dairy cows having clinical mastitis. The samples were tested for mastitis pathogens using conventional methods, i.e. 0.01ml of milk was streaked on blood agar and incubated at +37°C for 18-24 hours and then controlling possible growth for 24 hours more. The same samples were analyzed with PathoProof Mastitis PCR Assay. It can identify these bacterial species and groups: 1. *Staph. aureus*; 2. *Staph.* sp., including *Staph. aureus*, *Staph. intermedius* and all mastitis-causing CNS; 3. *Streptococcus agalactiae*; 4. *Str. dysgalactiae*; 5. *Str. uberis*; 6. *Escherichia coli*; 7. *Enterococcus* sp., including *E. faecalis* and *E. faecium*; 8. *Klebsiella* sp., including *K.*

oxytoca and *K. pneumoniae*; 9. *Corynebacterium bovis*; 10. *Arcanobacterium pyogenes* and *Peptoniphilus indolicus*; 11. *Serratia marcescens*; and 12. ß-lactamase penicillin resistance gene.

Of the milk samples, 496 were positive for one or more species in bacterial culture, whereas 180 samples (27%) gave culture negative results. In 56 of the 496 positive cases, bacterial culture identified the samples to contain two species, while 60 samples included more than two species, and were considered contaminated and uninformative. The percentages of the different mastitis bacteria observed in the 676 samples, based on the culture test, were: CNS 22.6%, *Staph. aureus* 11.6%, *Str. uberis* 10.7%, *Str. dysgalactiae* 10.1%, *E. coli* 5.8%, *C. bovis* 5.0%, *A. pyogenes* 1.8%, *Enterococcus* sp. 1.3%, *Klebsiella* sp. 1.0%, *Serratia* sp. 0.3% and *Str. agalactiae* 0.1%.

The real-time PCR assay yielded positive results for 592 samples and negative results for only 84 samples (12%). Of the positive samples, 283 contained one species only and 309 contained two or more species. The percentages of the different mastitis bacteria observed in the 676 samples, based on the real-time PCR test, were as follows: *C. bovis* 41.8% CNS 41.4%, *Str. uberis* 25.4%, *Str. dysgalactiae* 17.6%, Staph. *aureus* 17.2%, *E. coli* 9.5%, *A. pyogenes* 8.9%, *Enterococcus* sp. 5.2%, *Klebsiella* sp. 2.4%, *Str. agalactiae* 0.6% and *Serratia* sp. 0.3%.

When comparing conventional bacterial culture test and real-time PCR results for the samples providing positive results with both methods, there was a high level of concordance. For example, 87 samples contained *Staph. aureus* in bacterial culturing, and 80 (92%) contained *Staph. aureus* also in real-time PCR. The real-time PCR assay can quantify the bacterial DNA amount present in a sample, separately for each target species. This was a major benefit in comparison to bacterial culture: real-time PCR could provide valuable information on species and their relative quantities even if two or more species were identified. We assessed the relative amounts of different bacterial species present in the 309 samples containing multiple bacteria. Many of the mixed species samples contained one primary species and other bacteria were present in minor quantities. In 196 of 309 samples (63.4%), one species represented more than 90% of total bacterial quantity; and in 98 of 309 samples (31.7%), one species represented more than 99% of total bacterial quantity. This presentation discusses the main differences (benefits) real-time PCR technology has over the conventional bacterial culture test and how its utilization can improve the efficacy of routine mastitis testing programs.

References

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