

TEST CHARACTERISTICS OF THE QPCR TEST MASTIT 4 TO IDENTIFY MAJOR PATHOGENS IN SPIKED AND ORIGINALLY INFECTED MILK SAMPLES

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Introduction

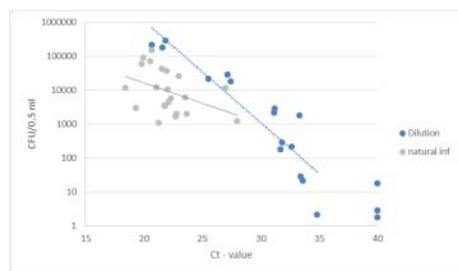
Real-time PCR tests are increasingly used to identify mastitis pathogens as they are faster and more sensitive than conventional bacteriological culturing. Recently a new commercially available quantitative real-time PCR test kit (Mastit 4, DNA diagnostic) was introduced in Denmark and is mainly used to test cows prior to dry-off for subclinical intramammary infections or in herd screenings for eradication of *Streptococcus agalactiae*. The objective of our study was to investigate the association between Ct values of the Mastit 4 PCR test kit for and bacterial colony forming units in fresh milk samples from quarters without clinical mastitis for *Streptococcus agalactiae*, *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae*.

Materials and Methods

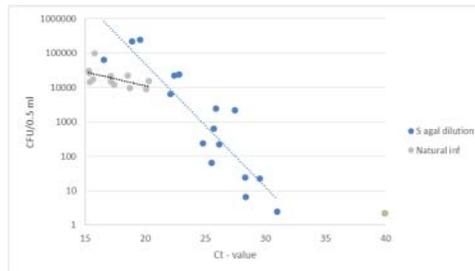
Three different isolates of each bacterial species were selected. Tenfold dilutions were made of an overnight culture of the bacterias using fresh quarter milk initially tested negative for all the studied bacteria. Quarter milk samples from natural infections were divided into two samples; one preserved with bronopol and tested with the Mastit-4 PCR test, the other was cultured according to NMC guidelines. 25 quarters with *S. aureus* and 14 quarters with *S. agalactiae* were included. The colonies for calculating CFU/ml were counted from duplicate plates containing between 10 and 300 colonies. All milk samples were analyzed by DNA Diagnostic with the MASTIT 4 test using the M4BD kit. The expected bacterial count and pathogen was blinded to the laboratory.

Results

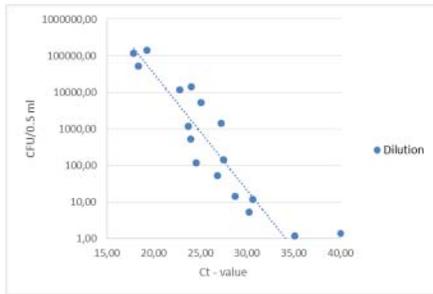
The dilution curves for *S. aureus*, *Strep. agalactiae*, *Strep. dysgalactiae*, *Strep. uberis* and CNS are shown in Figure 1. For *S. aureus* and *Strep. agalactiae* the results for the samples from natural infections are also plotted in figure 1(a) and 1(b).



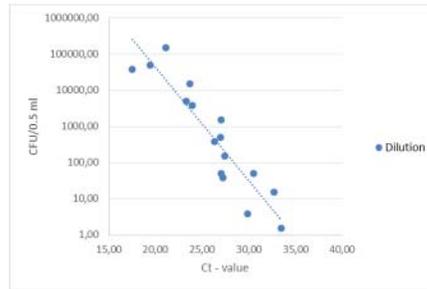
(a) *S. aureus*



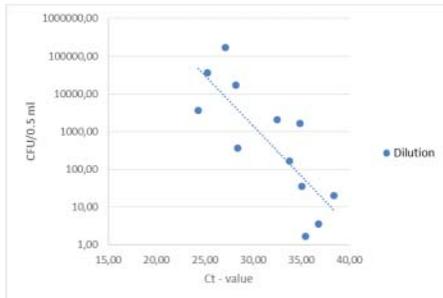
(b) *Strep. agalactiae*



(c) *Strep. dysgalactiae*



(d) *Strep.uberis*



(e) CNS

Figure 1a-e. Correlation of CFU/ml (log scale) and Ct values for diluted samples and test results for natural infected quarters for *S. aureus*, *Strep. agalactiae*, *Strep. dysgalactiae*, *Strep. uberis* and CNS.

For each data series an exponential correlation curve has been added. Dilutions with a concentration of less than 1 CFU /0.5 ml have been excluded, since the expected number for bacteria would be less than 1 in the examined sample volume. Furthermore samples with negative PCR-results ($Ct \geq 40$) are plotted but not included in the correlation line.

Discussion

The figures show an exponential relation between the Ct-value and the CFU. This was expected since the Ct value is an expression of the multiplication of bacterial DNA in the PCR-process. For *S. aureus* and CNS a concentration of 1 CFU/0.5 ml results in a Ct-value close to 40 whereas the corresponding Ct-value for the three streptococcal species at 1 CFU/ 0.5 ml lies between 33-34. As a consequence results for streptococci with a Ct value 33 to 40 indicate a concentration of less than 1 CFU/0.5 ml. Therefore true positive samples with a bacterial concentration of less than 1 CFU/0.5 ml cannot be expected to test positive in repeated tests of the same sample. The proportion of samples with this low bacterial concentration will affect the apparent sensitivity of the test. The detection limit of bacteriological culture where a volume of 0.01 ml is used is equivalent to 100 CFU/ml and will thus correspond to a Ct value of 27-28 for streptococci and Ct-value 34 in for *S. aureus*. For the natural infections all results except one showed Ct-values below the curve of the dilution experiment. This might indicate the presence of DNA from dead bacteria or that the bacteria in natural infections are more difficult to culture (intracellular *S. aureus*).

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