

# Interpretation of results from milk samples tested for mastitis bacteria with Mastit 4 qPCR test from DNA Diagnostic

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## Abstract

The use of Real-time PCR tests to identify mastitis pathogens are growing, as they are faster and more sensitive than conventional bacteriological culturing, especially for Mycoplasma detection. Since 2014 a quantitative real-time PCR test kit (Mastit 4, DNA Diagnostic) has been commercially available. The objective of this study was to investigate the correlation between Ct values of the Mastit 4 PCR test kit and bacterial colony forming units (CFU) in fresh milk samples for the 11 bacteria that can be detected by the Mastit 4 BDF, PCR test kit.

For each bacteria six different isolates were tested. Tenfold dilutions were made of an overnight culture of the bacteria using fresh quarter milk initially tested negative for all the studied bacteria. Culture was performed according to NMC guidelines. For each isolate CFU/ml in the initial samples were calculated from the duplicate plates of diluted samples containing between 10 and 300 colonies, and thereby the CFU/ in all the different 10-fold dilutions was calculated. All dilutions of all isolates of all bacteria milk samples, were tested by qPCR in the DNA Diagnostic laboratory with the Mastit 4 test using the M4BDF kit. The expected bacterial count and pathogen was blinded to the laboratory.

The dilution curves for *S. aureus*, *Strep. agalactiae*, *Strep. dysgalactiae*, *Strep. uberis*, CNS, *Mycoplasma bovis*, Mycoplasma species, *E. coli*, Klebsiella, Prototheca and Enterococcus incl. *Lactococcus lactis ssp lactis* were produced.

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 one in the examined sample volume. Furthermore samples with negative PCR-results (Ct $\geq$ 40) are plotted but not included in the correlation line.

The correlation line shows 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.

The correlation lines gives a very good impression of the CFU/½ ml that can be expected for a measurement of a Ct-value for the different bacteria. It also gives a good impression of the Ct-value that can be expected at the detection limit of 100 CFU/ml for culture. This will correspond to a Ct value of 27-28 for the Streptococci and Ct-value 34 for *S. aureus*.

For *S. aureus*, CNS, *Mycoplasma bovis* and Mycoplasma species 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 the 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.

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Keywords: *katholm*, *qpcr*, *mastit 4*, *mastitis bacteria*, *correlation*, *cfu/ml*, *ct-value*.