

# Evaluation of a new qPCR test to specify reasons behind total bacterial count in bulk tank milk

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Keywords: tbc, btm, quality, qpcr

## Abstract

To ensure the quality of raw milk Total Bacterial Count (TBC) and Somatic Cell Count (SCC) is tested in samples from the Bulk Tank Milk (BTM). There has been a long tradition to use culture of BTM samples to identify different bacteria causing high SCC in the milk. Also qPCR tests ex. Mastit 4 can be used to detect mastitis bacteria in BTM.

TBC in BTM can be investigated further by preliminary incubation count, laboratory pasteurization count, and coliform count. Also a five-day test for spore forming bacteria is used. To our knowledge, no qPCR addressing the bacteria involved in TBC has been commercially introduced, to be used in BTM samples. The aim of this study was to evaluate a recently introduced three-hour qPCR test, TBC 4 (DNA Diagnostic, Risskov Denmark) that detects bacteria involved in the TBC.

BTM samples were measured for TBC by the routine BactoCount test at Eurofins laboratory (Vejen, Denmark) and the results were used for settlement. Milk samples from different TBC intervals were selected for qPCR test with TBC 4 and included in the study.

When the result of the TBC tests were known the samples were immediately transported on ice to the laboratory of DNA Diagnostic A/S, Risskov Denmark and were tested on the same day using the TBC 4 qPCR test.

The TBC 4 test gives possibilities to address high TBC in BTM to four different targets; *Pseudomonas*, *Streptococcie*, *Enterobacteriaceae/Enterococcus*, and *Bacillus/Clostridia*. These four targets can be addressed to issues on the farm related to cooling, mastitis, environment, and silage.

In total 346 BTM samples in the different intervals of TBC were tested by the TBC 4 test, respectively 53 samples  $\leq 5,000$ , 67 samples  $> 5,000$  and  $\leq 15,000$ , 73 samples  $> 15,000$  and  $\leq 30,000$ , 65 samples  $> 30,000$  and  $\leq 50,000$ , 52 samples  $> 50,000$  and  $\leq 100,000$ , and 36 samples  $> 100,000$  bacteria/ml.

In each of the different TBC intervals the average Ct value, percent positive samples with Ct  $< 30$  and % samples with Ct  $< 25$  were calculated for all 4 targets in the test, indicating the relative influence of respectively cooling, mastitis, environment, and silage for high TBC in Danish BTM samples.

Not all problems with high TBC is solved by optimizing the cooling and washing procedure. The TBC 4 gives possibilities to address high TBC in BTM to four different groups of problems.

The TBC 4 test showed to be useful in indicating the major causes to high TBC in Danish BTM samples. We expect the test to be a strong and fast tool for farmers, advisors and service technicians to address high TBC issues and to secure good milk quality to be delivered to the Dairy.