Experiences with a new PCR-assay for diagnosis of bacterial infections in calves

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This project is the first part from the GUDP project - Diagnostic tests for veterinary practice (VetDiagnostics).

In this part, we have developed a fast qPCR test also to be used in private veterinary practice, for detection of pathogens associate with pneumonia in calf.

We have designed primers and probes for detection of *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. We tested these primers for respectively 15, 16, 10, and 3 different target isolates and all turned out to be correct positive. Then we tested all primers for 135 different non-target isolates. All were negative, among these isolates we also tested very related subtypes of *Mannheimia* and *Pasteurella*.

We tested different extraction procedures. It turned out that the extraction procedure developed by DNA-Diagnostic for the Mastit 4 kit also was the best for the tracheal fluid samples. We named this qPCR test Pneumo 4.

In a validation trial, we collected 65 tracheal fluid samples from clinical sick and control calves in 10 farms. From each calf, we collected 15-30 ml of tracheal fluid. This was transported on the same day to DNA Diagnostic in Risskov, where the Pneumo 4 qPCR test was performed on the same day. At the same day, 3 ml of sample was shipped to Copenhagen University for culture and 2 ml to DTU-vet for *Mycoplasma bovis* and virus detection. The rest of the sample was stored at DNA Diagnostic for further development of a qPCR for the virus detection.

**Results**

Of the 65 samples, we found 20 positives for *M. haemolytica* by Pneumo 4; only 4 of these were positive by culture. Similarly, 34 samples were positive for *P. multocida* by Pneumo 4; 19 of these were positive by culture. The corresponding results for *H. somni* were 4 positive by Pneumo 4 and 0 positive in culture for, and for *M. bovis* 30 positive by Pneumo 4 and 16 positive in the PCR DTU-vet test.

The efficiency of Pneumo 4 was evaluated on a quantified genomic DNA ten-fold dilution to estimate the copy number of nucleic acids. The sensitivity of Pneumo 4 test was found to be accurate in detecting target nucleic acids down to 10 copy number per PCR reaction.

The correlation between Ct values of the qPCR test and bacterial colony forming units (CFU) of target bacteria has been tested in triplicates on three different isolates for each target. The CFU/0.5 ml in the diluted samples was calculated from the plates containing between 10 and 300 colonies. The correlation curves for all four targets show an acceptable correlation between CFU/0.5 ml and Ct value.