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Evaluation of ELISA and qPCR for detection of *Mycoplasma bovis*

The bacterium *Mycoplasma bovis* infects cows and is difficult to treat. Infected cows risk causing large economic consequences for the farmer. To avoid this, it is very important to have a satisfying screening method available.

Two commonly used screening methods are available; qPCR and ELISA. The qPCR tests for presence of DNA in the cow's milk, meaning that *Mycoplasma bovis* (*M. bovis*) bacteria are secreted in the milk. The ELISA tests for the presence of antibodies targeting the *M. bovis*, the antibodies are extremely specific, and their presence is triggered by the *M. bovis* infection. Since the assays are testing for different analytes, it is more difficult to compare their performance. However, it is due to this difference that it is my recommendation to use the qPCR and the ELISA in parallel. The qPCR is the best choice when confirming the identity of a cultured bacteria, since this is what the qPCR tests for. While the ELISA is a good choice when testing a cow that are displaying symptoms, since symptoms appear roughly at the same time as antibodies have been produced.

Eurofins Sweden AB, a section of Eurofins Scientific, performs screenings of cow's milk to detect *Mycoplasma bovis* (*M. bovis*) and they wanted to know if they could replace the qPCR that they use today with an ELISA method. When choosing which method to use a lot of factors need to be considered. Since the antibodies are so specific it takes up to two weeks for the body to produce them, making the ELISA unable to detect an infection prior to this. However, after they have been produced, they stay in the milk for a long period of time. The qPCR is able to detect the infection earlier than this, but there is a risk that the cow stops secreting the bacteria in the milk if the infection is chronic. This means that the ELISA might be a better detection method when the infection is in a later stage. In addition, the ELISA is cheaper per run, making it attractive for the farmers. Besides these factors, the methods were experimentally evaluated with regards to a number of performance characteristics.

The qPCR and ELISA were tested regarding their LOD, repeatability and intermediate precision, selectivity and matrix effects. The tests were performed using two positive milk samples. The qPCR performed the best regarding the LOD, since it was able to detect *M. bovis* DNA in a sample that was diluted about a hundred times more than the sample that was detected by the ELISA. However, the ELISA performed better regarding the repeatability and intermediate precision since it was more consistent when measuring the same sample several times. It is important that the assays are selective enough to distinguish between *M. bovis* and other *Mycoplasma species* (*M. spp.*). Both the qPCR and the ELISA were able to detect samples containing other *M. spp.* as negative. The sample background is called the matrix, and this include all milk components except for the DNA/antibodies which is the target for the analysis. Depending on the sample, there is a risk that the matrix can affect the outcome of the test by masking or intensifying the target. The milk components didn't affect the qPCR result, but the ELISA result was amplified by them. This means that the qPCR performed better concerning the matrix effects. Overall, the qPCR performed better than the ELISA experimentally, but the price and the user friendliness of the ELISA still makes it an attractive method to use for *M. bovis* detection.

Abreviations:
ELISA = Enzyme-linked immunosorbent assay
qPCR = quantitative polymerase chain reaction
LOD = limit of detection