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Direct application of loop mediated isothermal amplification assay for detection of *Mycoplasma bovis* in mastitic milk

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Mycoplasma mastitis is always difficult to control due to lack of rapid and accurate diagnostic tool. The diagnostic methods available are mostly time consuming due to laborious culturing requirement, expensive, non-specific and less sensitive like biochemical tests and conventional PCR assay. A loop mediated isothermal amplification (LAMP) assay was developed for detection of *Mycoplasma bovis* directly from clinical mastitic milk samples. The LAMP assay was developed and validated on clinical samples obtained from *M. bovis* and other mastitis-causing pathogens detected by MALDI-TOF. Three different set of primers were used targeting different gene regions of *M. bovis*. The genes selected were UvrC, 16S rRNA and GyrB region. LAMP conditions were optimized for each of these and the efficiency, sensitivity and specificity of these LAMP primers were evaluated and compared. The result of 16S rRNA primers was more sensitive while GyrB primers were more specific. To confirm the specificity of the developed assay, other bacterial strains used were *Mycoplasma agalactiae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*. No cross reactivity was observed in all of the primer sets. Results were also compared to conventional PCR assay with primers chosen from the same genes and confirmed by sequencing. For the evaluation of LAMP assay sensitivity, culture-positive milk samples were subjected to the assay. LAMP assay detected *M. bovis* in some of those milk samples which were PCR negative. In the present study we have developed, validated and evaluated LAMP assay for detection of *M. bovis* from mastitis milk samples. The assay is authentic, rapid and sensitive.

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Confirmatory immunological tests to detect antibodies against foot-and-mouth disease virus non-structural proteins

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Foot-and-mouth disease (FMD) is a highly contagious disease of domestic and wild cloven hoofed animals. FMD outbreaks in endemic countries are controlled by biannual vaccination whereas in FMD free countries stamping out of infected and contact susceptible animals along with zoo-sanitary measures with or without restricted emergency vaccination is advocated followed by a rigid sero-surveillance to substantiate freedom from the FMD infection. Vaccination elicits antibodies against structural proteins (SPs) of FMD virus and infection against both structural and non-structural proteins (NSPs). Therefore detection of FMDV NSP antibodies in vaccinated animals can be used for differentiating infected from vaccinated animals (DIVA). Currently the most widely used NSP ELISA is the PrioCHECK® FMDV NS test. However for the absolute detection of infection in vaccinated population with a high degree of confidence, the current screening test does need another confirmatory test. Therefore, in this study six indirect protein/peptide (2B, 2C, 3B, 3ABC, 3CD and 3D) ELISAs were developed and validated using bovine sera samples extracted from experimental vaccinated-subsequently infected animals, a well established serum panel, known clinically positive animals in field outbreak and large number of naive animals. The sensitivity and specificity of three ELISAs (2B, 3B and 3ABC) were comparable to the PrioCHECK® FMDV NS test. By combining these individual tests with the PrioCHECK® FMDV NS in parallel testing an increase of sensitivity up to 99% was achieved whereas in serial testing the specificity has gone up to 99.99%. A multiplex assay using 6 NSPs (2B, 3B, 3AB, 3ABC, 3D & 3CD) has been developed and validated with the above groups of serum samples. Out of these 6 proteins/peptides 2B, 3D and 3CD were recorded as outstanding and by joining 2B and 3CD protein tests in multiplex assay the best sensitivity and specificity was achieved and were found higher than or similar to the sensitivity and specificity of the PrioCHECK® FMDV NS test.

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