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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* (*M. 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. 3 different sets 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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Novel T-cell assays for the discrimination of active and latent tuberculosis infection: The diagnostic value of PPE family

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The diagnosis of active and latent tuberculosis remains a challenge. Although a new approach based on detecting *Mycobacterium tuberculosis*-specific T-cells has been introduced, it cannot distinguish between latent infection and active disease. The aim of this study was to evaluate the diagnostic potential of interleukin-2 (IL-2) as biomarker after specific antigen stimulation with PE35 and PPE68 for the discrimination of active and latent tuberculosis infection (LTBI). The production of IL-2 was measured in the antigen-stimulated whole-blood supernatants following stimulation with recombinant PE35 and PPE68. The discrimination performance (assessed by the area under ROC curve) for IL-2 following stimulation with recombinant PE35 and PPE68 between LTBI and patients with active TB were 0.837 [95% confidence interval (CI) 0.72-0.97] for LTBI diagnosis and 0.75 (95% CI 0.63-0.89) for active TB diagnosis, respectively. Applying the 6.4 pg/mL cut-off for IL-2 induced by PE35 in the present study population resulted in sensitivity of 78%, specificity of 83%, PPV of 83% and NPV of 78% for the discrimination of active TB and LTBI. In addition, a sensitivity of 81%, specificity of 71%, PPV of 68 and 83% of NPV was reported based on the 4.4 pg/mL cut-off for IL-2 induced by PPE68. This study confirms IL-2 induced by PE35 and PPE68 as a sensitive and specific biomarker and highlights IL-2 as new promising adjunct markers for discriminating of LTBI and active TB disease.

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