J Med Microb Diagn 2016, 5:4(Suppl)
http://dx.doi.org/10.4172/2161-0703.C1.003

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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Management of cystic fibrosis patient colonization using culture plate digital imaging technologies: Early growth detection of small colony variants and morphotype recognition by classical digital imaging and direct bacterial identification by hyperspectral imaging

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Microbiological monitoring of pulmonary colonization is essential for Cystic Fibrosis (CF) patient management. However, many factors make microbiological culture analysis especially difficult for CF patients: Variants with atypical morphology and several morphotypes of the same species can coexist. Colonization with *Pseudomonas aeruginosa* and the conversion into a mucoid phenotype and into Small Colony Variants (SCVs) are critical steps in the course of the disease. *Staphylococcus aureus* SCVs must be recognized in order to start appropriate antibiotic therapy. The first goal of the study was to demonstrate that "classical" digital imaging allows for an earlier detection of the SCVs and also for recognition of the different colony morphotypes. However classical digital imaging still limits itself to what can currently be observed by the naked eye. The second goal was to show that hyperspectral imaging gives additional means to observe more characteristics linked to the construction of colonies and could lead to an optical species-specific fingerprint, enabling direct bacterial identification. Digital imaging technologies ensure early detection of any colonization with SCVs of *P. aeruginosa* and *S. aureus* and authorize the recognition and identification of morphotypes thus providing a powerful tool to track the different morphotypes that colonize CF lung. Therefore, digital imaging technologies convey a real medical added value for diagnosis and management of CF patients.

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