

## Design of Internal Amplification Control for PCR Detection of Mycoplasma Spp

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**Background & Objectives:** Mycoplasma is one of the smallest alive microorganism, having the ability of transmitting from microbiological filters makes it to be a severe and important contaminant for cell culture, biological and biotechnological products. Using a rapid and sensitive Methods for diagnosing Mycoplasma's infection is the first priority. This Methods should be able to discover typical Mycoplasma's infection that PCR is, but for meeting the standard we need to produce the internal control which is the main aim of the project.

**Methods:** By using of special primers for IS6110 target, PCR test was optimized. Besides, the composit primers for IC- M. spp were designed thence the PCR was optimized. The IC- M. spp which amplified in nonstringent condition, was ligated in pTZ57R plasmid, transformed in *E.coli* JM107 and was cloned. Specificity and sensitivity of test were determined.

**Results:** PCR amplicon for Mycoplasma spp and IC- M. spp with special primers were 272bp and 660bp respectively, so there was a significant difference between their size.

**Conclusion:** Despite of high speed and accuracy of PCR, false positive and negative results which are caused because of PCR inhibitors, are the important problems of this technique that can reduce its efficiency. Using another DNA as an internal control can detect these inhibitors. Indeed, amplification of this DNA shows correct amplification and detection steps.

**Keywords:** Mycoplasma Spp; PCR