

OTR13 - Detection of *Mycoplasma sp.* and *Acholeplasma laidlawii* in biological products and culture media by real time PCR

Natália Pedra Gonçalves^{1*}; Pedro Augusto Alves¹; Érica Louro da Fonseca¹; Darcy Akemi Okama¹; Jaline Coutinho Silvério¹; Daniel da Silva Guedes Júnior¹.

1 - Bio-Manguinhos/ Fiocruz

Introduction:

Mycoplasma is the smallest less-wall prokaryotic organism and can that cause pneumonia and urogenital diseases in humans. Moreover, they frequently contaminate research and commercial laboratories involved in development and production of biological and pharmaceutical cell-derived products, enabling unreliable experimental results and unsafe biological products. The detection of *Mycoplasma sp.* and *Acholeplasma laidlawii* in biological products is a quality control test required by the European Pharmacopoeia (Eur. Ph.) and carried out by industries.

Objective:

To develop an in house real time PCR (qPCR) protocol for assessing *Mycoplasma sp.* and *A. laidlawii* in biological products and culture media for pharmaceutical industry.

Methodology:

The genome of eight species required by Eur. Ph. were selected in GenBank[®] for the 16S ribosomal locus and aligned using the online tool ClustalOmega in order to select a common region for primer design. To achieve the specificity criteria, were excluded the regions shared with other phylogenetic related bacteria as *Streptococcus*, *lostridium* and *Lactobacillus*' genera. The primer design was analyzed through the online tool IDT OligoAnalyzer[®] 3.1, and the PrepSEQ[®] kit was used for DNA extraction of ATCC pure cultures and biological products spiked with *Mycoplasma* and *Streptococcus bovis*, *Clostridium sporogenes* and *Lactobacillus acidophilus*, that are phylogenetically related to *Mycoplasma sp.* and *A. laidlawii*. The specificity and detection limit assays were made using SYBR[®] Green dye and the results were compared with the commercial kit for *Mycoplasma* detection MycoSEQ[™] and conventional PCR.

Results:

Four pairs of primers were designed and BLAST research revealed homology to 40 *Mycoplasma* species, including the species required by Eur. Ph. with 100% of identity. The qPCR tests using in house protocol demonstrated that the primers are specific for *Mycoplasma* genus and *A. laidlawii*, and the detection limit was less than 1UFC/mL using pure culture of *Mycoplasma*. These results were similar to the obtained with the MycoSEQ™ and more sensitive than conventional PCR. Preliminary qPCR tests with biological products spiked with *Mycoplasma* has shown that in house protocol are also suitable and specific for this bacterial detection.

Conclusion:

The in house protocol for detection of *Mycoplasma* sp. and *A. laidlawii* by qPCR has shown to be as specific as the commercial kit MycoSEQ™, with the ability to detect less than 1 UFC/mL of *Mycoplasma*. This represents lower costs and an alternative to the conventional PCR already established at the Quality Control Department – BioManguinhos, since qPCR presents advantages as higher sensitivity, reduced time process, greatest biosafety and robustness.

keywords: *Mycoplasma*, real time PCR, SYBR Green