**Título:** EMA-PCR: Detection only viable pathogen on cell culture by Real-Time PCR

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**Resumo:** Classical microbiological methods for detecting microorganisms in food is time consuming and labor intensive, and can take up to a week to detect pathogens in contaminated food. The real time PCR (qPCR) technology can shorten the detection time for one day, with more specificity and sensibility. As this methodology detects pathogens based on the presence of their DNA, false positive signals can be generated by not differentiate if this DNA is from a viable or dead pathogen. To overcome this obstacle were used an DNA intercalating dye, ethidium bromide monoazide (EMA) (Molecular Probes Europe BV) which is able to bind to double stranded DNA but do not have the capability to pass through the plasma membrane of viable cells with the intact membrane. These two features ensure that this dye prevents the amplification of DNA of unviable cells during qPCR. To validate this methodology were tested two bacteria, *Salmonella* spp. and *Staphylococcus aureus*, in isolated cultures. After cultivating, these microorganisms were divided into aliquots, and half of them was subjected to a heat treatment in boiling water for 15 minutes to unable the cells. After the heat treatment, all samples, viable and unviable were subjected to a treatment with EMA. This treatment consists of adding the dye (50 µg/mL), incubation in the dark for 5 minutes, and exposition to halogen light for 5 minutes. Then it was performed the DNA extraction using DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany). The qPCR assays were performed with TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The required probes and primers were designed from sequences described by Piknová et al. (2005) and Shortle (1983). Analysis of these assays were performed based on cycle threshold values. The values of Ct showed a difference of 11 amplification cycles between unviable samples treated or not with EMA, that was considered statistically significant by t test ($p < 0.05$). Neither was difference between unviable sample without EMA treatment and viable samples with and without addition of the intercalating dye. Dead cells treated with EMA have the highest Ct values. With these results it is concluded that this dye is capable of preventing amplification of the DNA of non-viable cells, unlikely viable samples. Therefore, EMA is capable of detecting the presence of viable cells of *Salmonella* spp. and *S. aureus* by qPCR technique.

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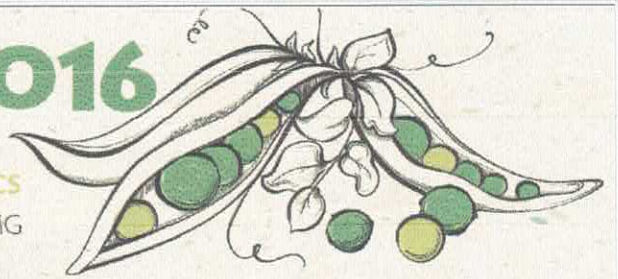

Certificamos que FELIPE DE OLIVEIRA VIEIRA apresentou o trabalho entitulado EMA-PCR: DETECTION ONLY VIABLE PATHOGEN ON CELL CULTURE BY REAL-TIME PCR de autoria de VIEIRA,FO, MENDONÇA,JFM, EONSECA,I, ARCURI,EF, RIBEIRO,JB, MARTINS,MF , no 62° CONGRESSO BRASILEIRO DE GENÉTICA, no período de 11 a 14 de setembro de 2016, em Caxambu, MG , na área de GENÉTICA DE MICROORGANISMOS.

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