Identification of viable and non-viable *Haemophilus influenzae* using qPCR.

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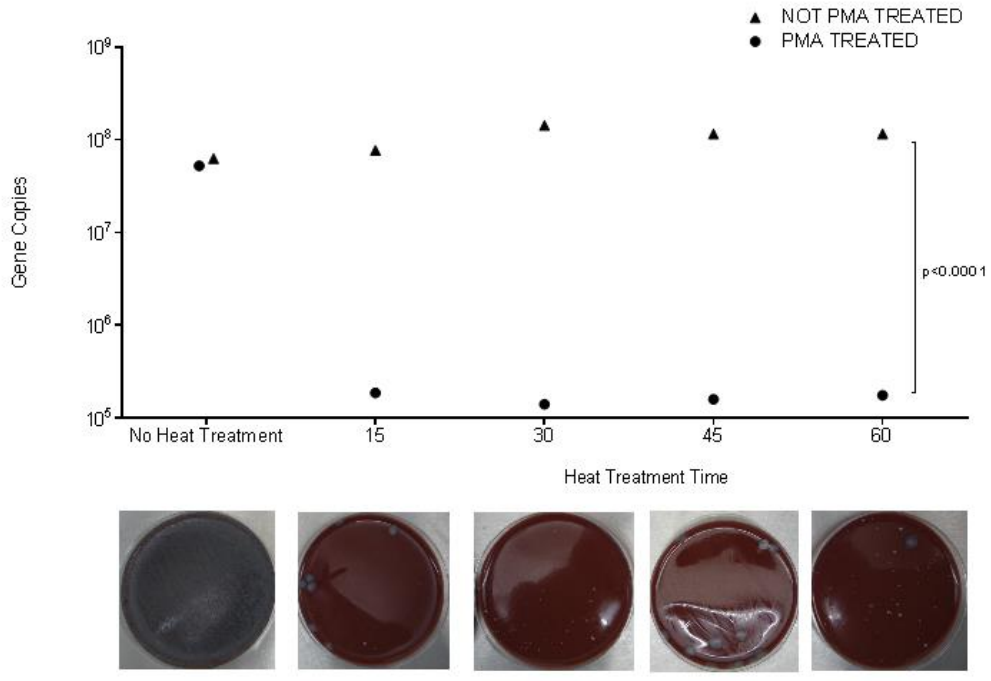
Introduction Conventional real-time PCR for the detection of bacteria is unable to distinguish between viable and non-viable cells within a sample. Propidium monoazide (PMA) is a photo-reactive dye that is cell membrane impermeable which interacts with non-viable cells ceasing amplification due to DNA modification. We investigated if PMA could distinguish between viable and non-viable *Haemophilus influenzae.*

Methods *H.influenzae* strain (NCTC11931) was grown in LB broth for 24hrs then subjected to 90°c heat. Half were treated with PMA and incubated in darkness for 5min, then subjected to an intense light source for 15min. Bacterial DNA was extracted from all samples, using a commercial assay and quantified using real time-PCR. Each sample was plated on a chocolate agar plate and incubated for 24hrs.

Results PMA treated and non-PMA treated samples not subjected to heat treatment had very similar values, geometric mean (95%CI) of 5.22 x 10^7(4.05 to 6.74 x 10^7) and 6.29 x 10^7 (5.09 to 7.78 x 10^7) gene copies respectively. Samples treated by heat and stained with PMA had a significantly lower amount of *H. influenzae* detected than samples not treated with PMA (p<0.001), Fig.1.

Conclusion PMA appears able to distinguish between viable and non-viable *H. influenzae*, but further studies are warranted.

Figure 1. Paired heat samples treated with PMA or not treated with PMA. Including two controls subjected to no heat treatment.



Characters - 1480

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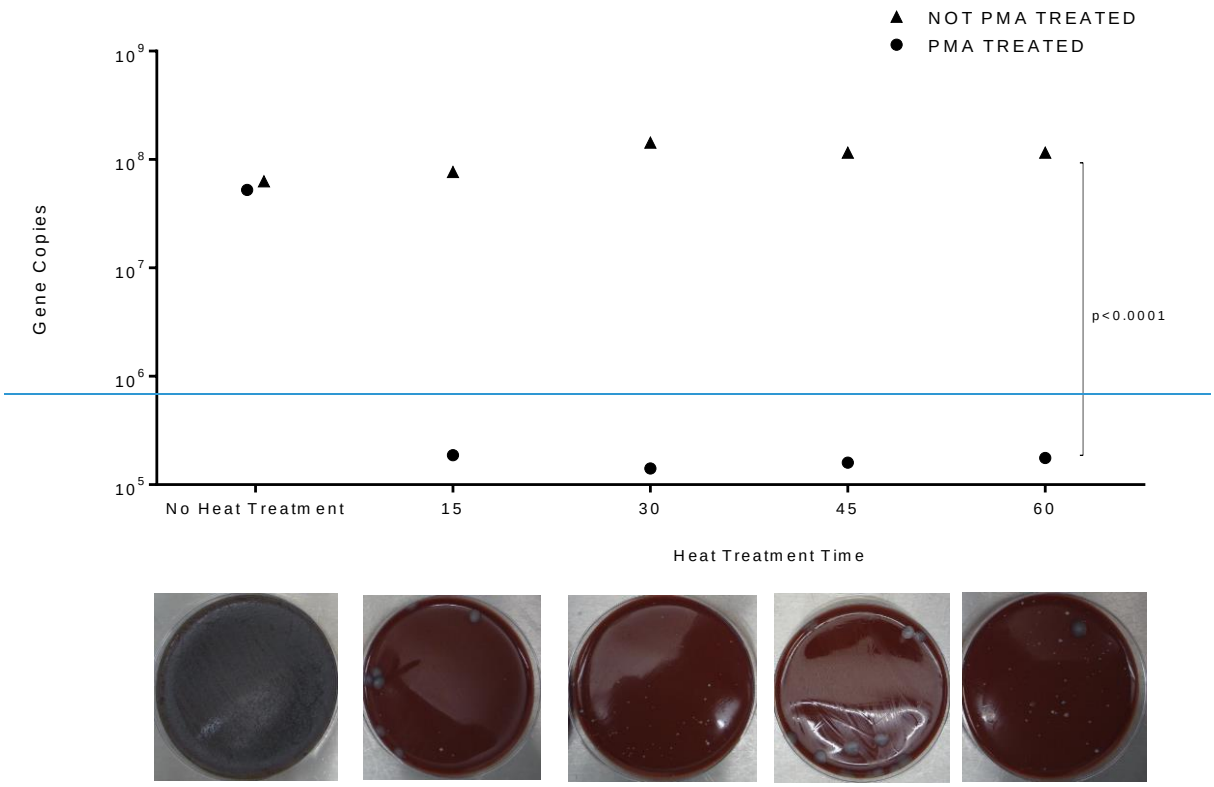

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