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Research Article

Evaluation of propidium monoazide and long-amplicon qPCR as an infectivity assay for coliphage

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A B S T R A C T

Standardized and rapid assays for viable viral pathogens are needed to inform human health risk assessments. Conventional qPCR is designed to enumerate the gene copies of an organism in a sample, but does not identify those that originated from a viable pathogen. This study was undertaken to evaluate modified qPCR methods as infectivity assays for the enumeration of infectious MS2 coliphage. Propidium monoazide (PMA) treatment coupled with long-amplicon qPCR assays were assessed for their ability to quantify infectious MS2 in pure cultures and following inactivation by a range of UV light exposures and chlorine doses. The qPCR results were compared to the plaque assay, which was used as the standard to indicate the level of infectious MS2 in each sample. For pure cultures, PMA-qPCR results were not significantly different from the plaque assay ($p > 0.05$). At >4 log inactivation, combined PMA and long-amplicon qPCR assays overestimated the level of infectious MS2 remaining ($p < 0.05$). The most accurate long-amplicon qPCR infectivity assay targeted a 624-bp region at the 5′ end of the genome. Modified qPCR approaches may be useful tools to monitor the loss of infectivity as a result of disinfection processes.

1. Introduction

Standardized methods for the quantification of infectious enteric viruses are needed to inform human health risk assessments. The ideal method should be rapid, simple, sensitive, specific, affordable, suitable for use in a range of matrices, and provide an indication of infectivity. Cell culture and molecular based approaches are often used when the detection of pathogenic viruses in water is deemed necessary (APHA, 1998; Cashdollar et al., 2013; Fong and Lipp, 2005). While cell culture methods may provide an indication of viral infectivity, they are not available for all human viruses; are labor- and time-intensive; costly; and susceptible to interfering compounds that may cause false-positive or false-negative results (Hamza et al., 2011). Molecular-based methods, such as real-time PCR (qPCR), overcome several of these shortfalls (Gensberger and Kostić, 2013). Advantages of qPCR assays are that the results can be collected within hours, while cell culture results generally require weeks; the methods are specific and becoming more affordable than cell culture; and the assays can be multiplexed to detect a suite of pathogens within a single amplification protocol.

A common criticism of conventional qPCR assays is that they do not provide an indication of viral infectivity (Girones et al., 2010; Sherchan et al., 2014). Various modifications to molecular detection methods have been proposed to allow for the quantification of infectious pathogens by qPCR. The general goal is to minimize the amplification of gene targets from viruses that are not infectious and from “free” viral genetic material not within an intact viral capsid. Common approaches include the use of DNA/RNA intercalating dyes or enzymes prior to the genomic extraction process, and long-amplicon DNA targets during qPCR amplification (Fig. 1). The use of dye treatments, including propidium monoazide (PMA) or ethidium monoazide (EMA), to minimize qPCR amplification of free gene targets and of damaged viruses and bacteriophages has become of interest in the last 5 years (Coudray-Meunier et al., 2013; Fittipaldi et al., 2010; Graiver et al., 2010; Kim and Ko, 2012; Sánchez et al., 2012). PMA dye intercalates with accessible DNA and RNA upon exposure to light prior to genome extraction and subsequently inhibits amplification during qPCR. PMA-qPCR as an assay for infectious viruses assumes that protein capsid integrity is the sole differentiating factor between infectious and inactivated viruses (Graiver et al., 2010). PMA-qPCR could fail to quantify infectious viruses in a given sample if the organism can sustain capsid damage yet still cause infection, or if the virus is inactivated by minor damage to the genome yet retains an intact capsid (e.g. UV light; Fig. 1a). Nucleases and proteinases that degrade genomic material and proteins present similar limitations in their ability to eliminate amplification of genes from inactivated viruses

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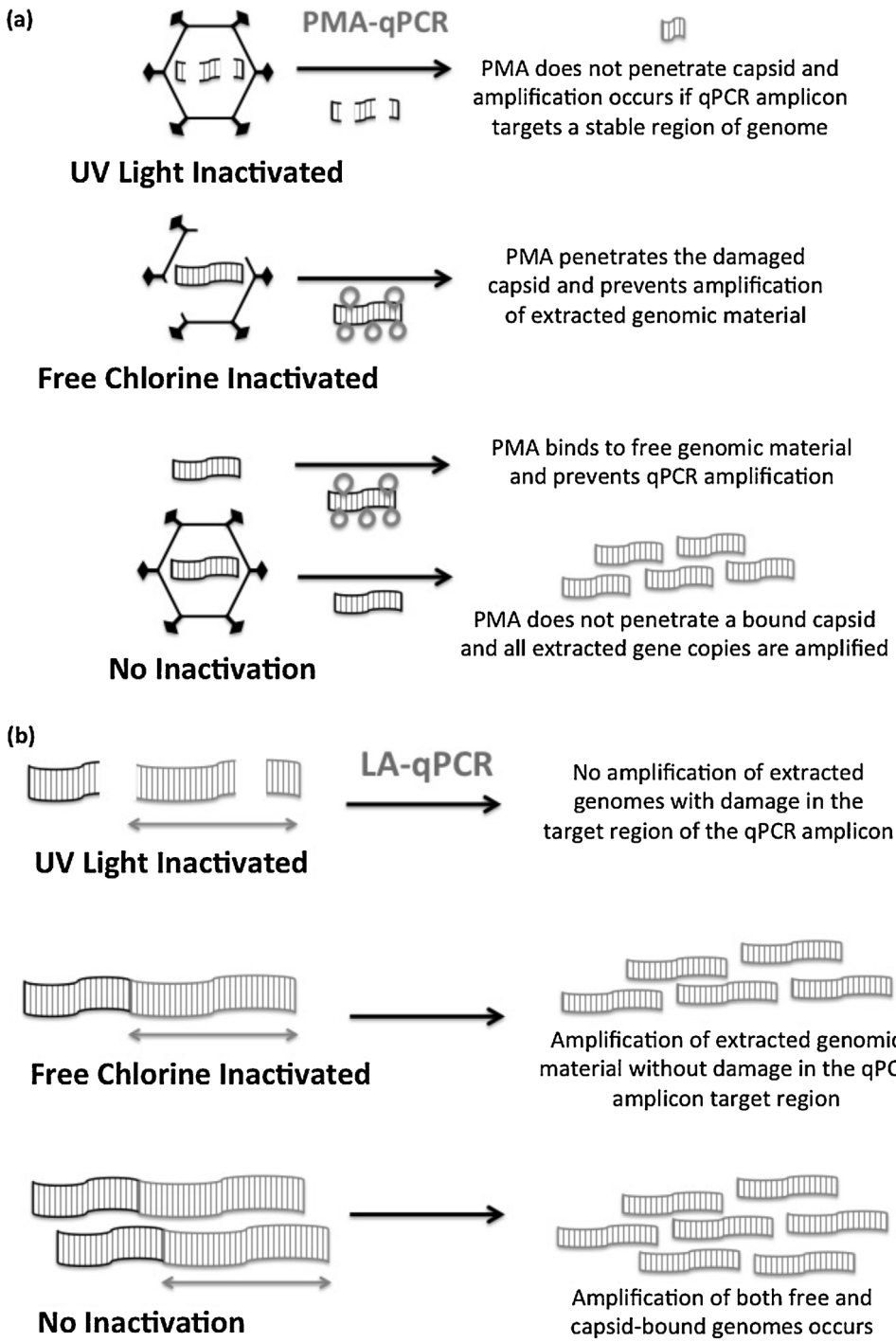

Fig. 1. Conceptual model of theoretical damage by UV light and free chlorine to a virus, and detection by (a) PMA-qPCR and (b) LA-qPCR (following genome extraction); grey arrows and grey DNA segments indicate amplification targets and products about >400 bp.

during qPCR and present similar limitations as infectivity assays (Nuanualsuwan and Cliver, 2002; Pecson et al., 2009). For example, Pecson et al. (2009) found that proteinase K and RNase treatment coupled with qPCR underestimated the inactivation of MS2 following heat, singlet oxygen and UV light treatments when compared to plaque assay results.

Another approach to quantify viral infectivity involves the use of long-amplicon targets for qPCR (LA-qPCR). LA-qPCR (also known as long-range [lo-qPCR] and long-template [LT-qPCR]) has been used to improve molecular detection of infectious viruses (Simonet and Gantzer, 2006; Rodríguez et al., 2009; Wolf et al., 2009). Typical qPCR assays target genomic regions between 60 and 100 bp long, while LA targets are generally >400 bp long (Pecson et al., 2011). LA-qPCR may reduce amplification of viral genomes that have been damaged; effectively minimizing detection of inactivated viruses (Fig. 1b).

A combined assay using PMA and LA-qPCR (PMA-LA-qPCR) has been validated for the quantification of heat- and UV-killed bacteria *Campylobacter jejuni* and *Salmonella enterica* (Banihashemi Jahromi et al., 2012). However, the combined approach of PMA-LA-qPCR has yet to be trialed as an infectivity assay for viruses or bacteriophages that have been inactivated by UV light and chlorination. The process