Q-469

UV Inactivation of Coxsackievirus, Echovirus, and Poliovirus Quantified Using an Integrated Cell Culture-qRTPCR Assay

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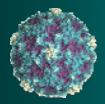
Motivation

The simultaneous promulgation of the Environmental Protection Agency's (EPA) Disinfectant and Disinfection Byproduct Rule and Enhanced Surface Water Treatment Rule emphasized the importance of balancing the risks posed by microbial and chemical contamination of drinking water. Ultraviolet (UV) disinfection produces no known disinfection byproducts, and as such, has generated interest as an alternative disinfectant for drinking water treatment. Accordingly, it is important to understand the efficacy of UV disinfection for microbial inactivation, particularly emerging pathogens such as those on the EPA's Contaminant Candidate List (CCL). The traditional infectivity assays required for such studies are time- and material-intensive, which limits understanding of UV efficacy. In particular, a better understanding of the effect of UV on emerging microbes, such as coxsackievirus and echovirus (which are currently included on the CCL), is needed. This study demonstrates the applicability of integrated cell culture-quantitative RTPCR (ICC-qRTPCR) for the simultaneous quantification of infectious coxsackieviruses, echoviruses, and polioviruses and validates its use in UV disinfection studies.

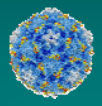
Background

Study Viruses

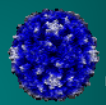
- Coxsackievirus B6, Echovirus type 12, and poliovirus type 1 were simultaneously quantified in this study.
- As members of the enterovirus genus, they are small (24–30 nm), non-enveloped, positive strand RNA viruses.
- Although poliovirus is perhaps the most well-studied of all viruses, less is known regarding the occurrence, health effects, treatment, etc. of other enteroviruses, which led to the inclusion of coxsackievirus and echovirus on the EPA's Contaminant Candidate List.
- Enterovirus infections are common (annually 30–50 million in the U.S.), but are often manifested subclinically. However, a range of clinical outcomes is possible, including colds and fevers, hand-foot-and-mouth disease, conjunctivitis, meningitis, and poliomyelitis.



Coxsackievirus Type B6 Host: BGM cells



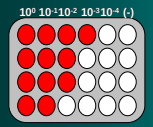
Echovirus Type 12 Host: BGM cells



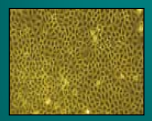
Poliovirus Type 1, Strain Lsc-2ab Host: BGM cells

Standard Infectivity Assay for Enteroviruses : In-Vitro Cell Culture:

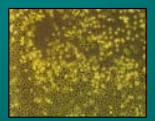
- Depends on visual differentiation between infected host cells demonstrating cytopathic (CPE) effects and healthy, uninfected cells.
- For the $TCID_{50}$ assay, a single sample may require 24 wells.
- For the $TCID_{50}$ assay, cells are observed for up to 14 days and statistical methods are used to estimate the concentration of the original sample based on the number of positive and negative replicates.
- Method is time- and cost-intensive, and somewhat subjective.



24-Well Tray Per Sample



Non-Infected BGM Cells



Infected BGM Cells Showing CPE

Plaque Assay Infection

Quantitative Real-Time qPCR using Taqman Probes:

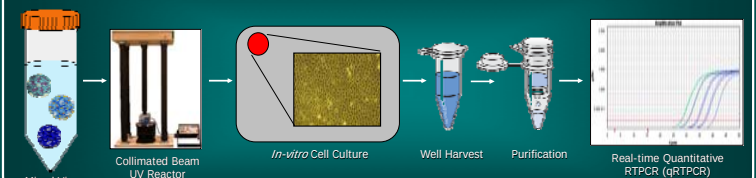
Primer and Probe Annealing

Primer Extension

Probe Cleavage and Fluorescence

- Reverse transcription at 48ºC for 30 min; RT inactivation and initial denaturation at 95ºC for 10 min; 40 cycles of denaturation at 95ºC for 15 sec, annealing and extension at 60ºC for 30 sec.

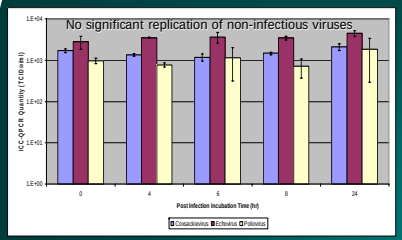
Experimental Approach



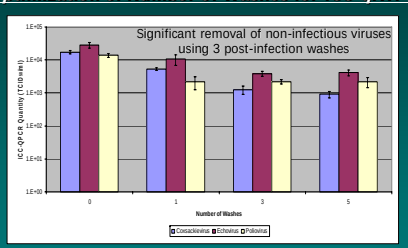
Results

ICC-qRTPCR Method Development and Optimization

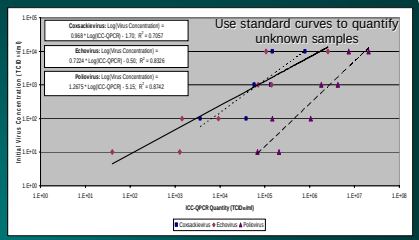
Non-Infectious Virus ICC-qRTPCR Control



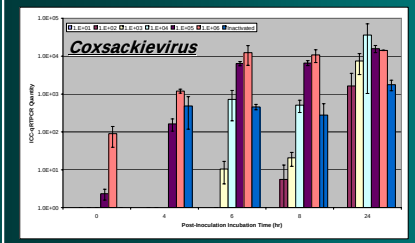
Optimization of Number of Washes for ICC-qRTPCR

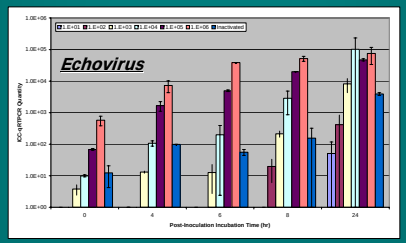


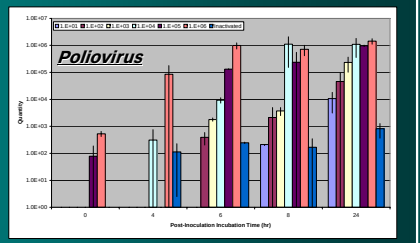
ICC-qPCR Standard Curves for 24 h.p.i.



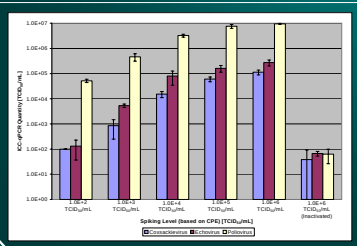
Incubation Time Optimization experiments were performed to determine the optimal incubation time for quantification of the mixed enteroviruses. Serial dilutions (10^6 through 10^1 $TCID_{50}$/ml) were applied to cells and harvested after different incubation times (0, 4, 6, 8, and 24 hours post inoculation). A time of 24 hours post inoculation was identified as optimal based on consistent and significant replication of viruses at different initial concentrations. Samples with high virus concentrations (low inactivation) should be diluted to avoid cell capacity limitations.



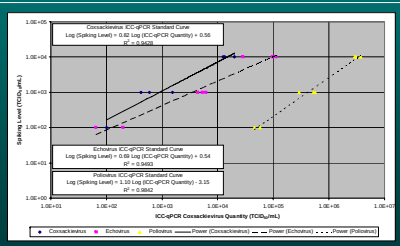




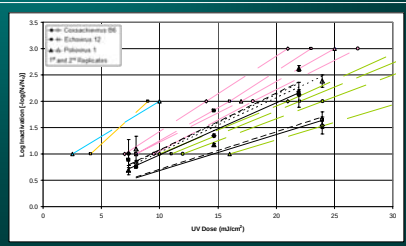
UV Disinfection Quantified Simultaneously using ICC-qRTPCR



ICC-qRTPCR Validation at 24 h.p.i. for UV Experiment



ICC-qRTPCR Standard Curve for UV Experiment



UV Dose-Response Curves

Black lines and symbols represent experimental data; colors signify reports from the literature.

Summary

- The integrated cell culture-quantitative RTPCR (ICC-qPCR) assay was successfully applied for the simultaneous quantification of coxsackievirus, echovirus, and poliovirus.
- The assay provides significant time and material savings and offers a practical alternative to traditional methods for the quantification of infectious viruses.
- Assay optimization demonstrated that 24 h.p.i. and 3 post-infection washes was optimal for the simultaneous quantification of coxsackievirus, echovirus, and poliovirus.
- Three-log inactivation was achieved at UV doses of 30–44, 28–42, and 28–29 mJ/cm^2 for coxsackievirus B6, echovirus 12, and poliovirus 1, respectively, which is similar to values reported using conventional methods.

Acknowledgements

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