Q-329 Comparison of Quantitative PCR and Cell Culture assay for Determination of Removal Efficacy of Adenovirus, Coxsackievirus, & Echovirus by Water Treatment Processes

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A number of data points are needed for evaluating virus treatability using physicochemical treatment processes such as coagulation and filtration

Cell culture assays for enteric viruses
- Time consuming
- Expensive
- Subjective (cytopathogenic effects)
- Not a user-friendly technique

Any other rapid and quantitative technique?

Motivation for this study | Data presentation | Design of Experiment | Results & Discussion

OBJECTIVE

To assess the applicability of real time quantitative PCR (qPCR) for removal studies of CCL viruses by water treatment processes

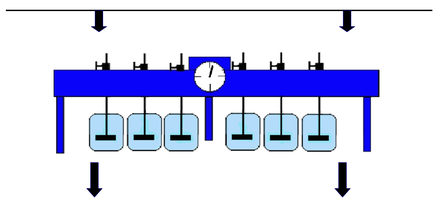
$$L = -\log_{10}\left(\frac{N_d}{N_0}\right)$$

- L: Log removal of viruses by treatment processes
- N_0: Initial concentration of viruses
- N_d: Concentration of viruses after a specified coagulant dose

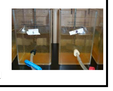
$$P = -k \times C$$

- P: The log removal values calculated by Q-PCR
- k: The approximate coefficient
- C: The log removal values calculated by cell culture assays

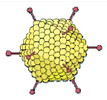
Bench-Scale Jar Testing



Coagulation, Flocculation, & Sedimentation
- Coagulant: Ferric chloride: 0 to 120 mg/L
- Polymer: Cationic polymer, Poly-DADMAC
- Rapid mixing at 100 rpm for 1 minute
- Slow mixing at 40 rpm for 10 minutes
- Followed by 20 rpm for 10 minutes
- Withdraw paddles and allow to sit 30 min



CCL Viruses



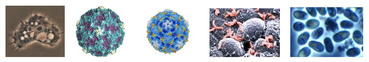
Adenovirus

Coxsackievirus

Echovirus

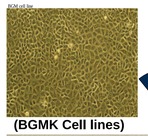
Emerging Microbial Pathogens - EPA CCL Microbes -

- *Acanthamoeba (removed)*
- Adenovirus
- *Aeromonas hydrophila*
- Calicivirus (norovirus)
- Coxsackievirus
- Cyanobacteria, algae, and toxins
- Echovirus
- *Helicobacter pylori*
- Microsporidia
- *Mycobacterium avium* complex (MAC)



Cell Culture | **Real-time qPCR**

Plaque Forming Unit Assay

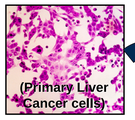


(BGMK Cell lines)

Coxsackievirus B6
Echovirus type 12

Quantification

Cytopathogenic Effects Assay



(Primary Liver Cancer cells)

Adenovirus type 4

Quantification

The Karber TCID50 method

$$TCID_{50} = 10^{-(\Delta - \alpha(S - 0.5))}$$

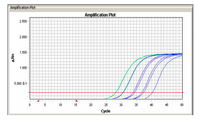
Δ = the $\log_{10}$ of the most diluted sample with 100% infectivity
α = the log dilution factor
S = the summation of the normalized positive infections, including both the last 100% and the first 0% infectious dilutions

Oligonucleotide primers and probes for TaqMan qPCR

Enteric viruses	Primer or probe/ position (5' – 3')	Sequence	T_m (°C)
Echovirus type 12	Forward primer (2523 – 2542) Reverse primer (2606 – 2587) Probe (2580 – 2555)	5'-GAGCATCCCCGCACTAACAG-3' 5'-TTCACATGACGGGTTTGCAT-3' 5' FAM-CCCAGGAACCACCTGAGATGTATGCC-TAMRA 3'	59 58 68
Coxsackievirus B6	Forward primer (2455 – 2472) Reverse primer (2566 – 2549) Probe (2500 – 2476)	5'-TGGAGGGCGCCATAGAGA-3' 5'-GAGGTGTGGCCCGTTTCC-3' 5' FAM-ATAGTGTCAGCGACCCGTGCAATGG-TAMRA 3'	59 60 69
Adenovirus type 4	Forward primer (19794 – 19816) Reverse primer (19866 – 19849) Probe (19818 – 19835)	5'-CATGGACAACGTAAATCCCTTCA-3' 5'-GTTGCCCAGGAGCATGGA-3' 5' FAM-CCACCACCGCAATGCGGG-TAMRA 3'	59 60 68

Real time PCR with TaqMan probe → **DNA extraction and purification**

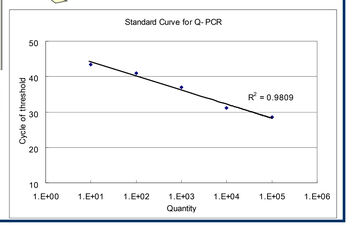
Qiagen QIAamp DNA/RNA kits



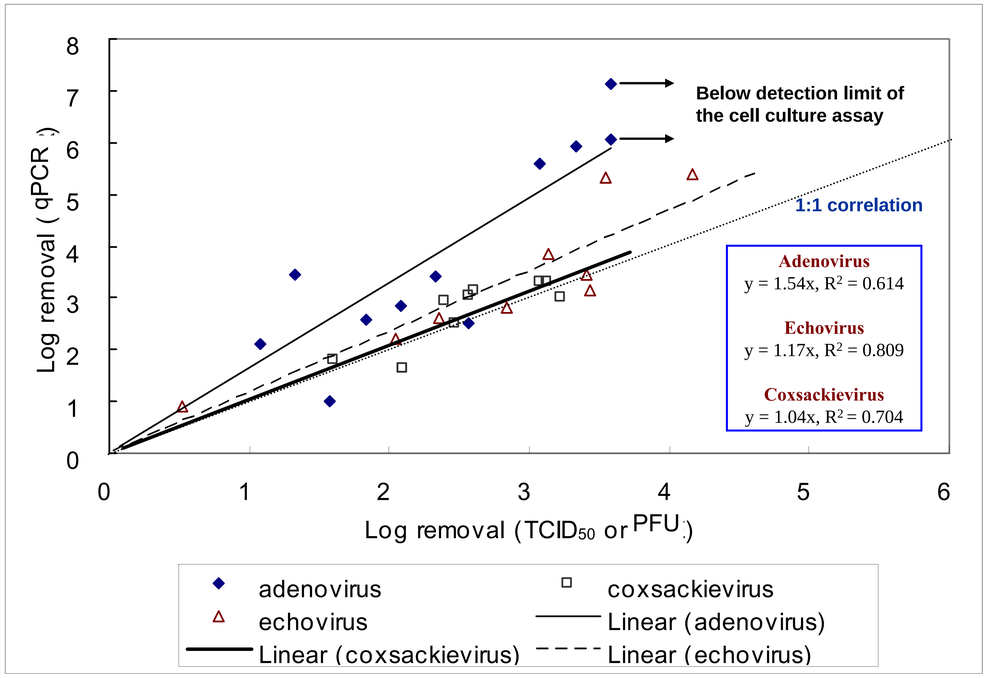
1. Primer Extension
2. Probe Cleavage
3. Fluorescent Signal

Probe intact, reporter is quenched

Generating Standard Curve



Quantitative PCR (qPCR) vs. Cell-based infectivity assays



Each point on the figure represents the mean value from at least four replicate samples

- **Significant correlations between the two methods for log removal of tested viruses was observed.**
  - **adenovirus (R^2 = 0.614, n=11), coxsackievirus (R^2 = 0.704, n=9), and echovirus (R^2 = 0.809, n=9)**
- **The correlation was 1:1 ratio between qPCR and cell culture**
  - **coxsackievirus (1.04:1) and echovirus (1.17:1)**
  - **For adenovirus the ratio was 1.54:1 possibly due to using cell culture assay for the determination of viral concentration, which is a conservative method compared to a PFU assay, resulting in underestimation of log removal.**
- **TaqMan probes designed for this study resulted in no cross-reactivity by amplifying only the target genome (data not shown), indicating that qPCR enables simultaneous quantification of several enteric viruses, thereby enhancing its applicability for virus removal studies.**

SUMMARY

- **qPCR is an alternative method to cell culture assays for viral removal studies.**
  - **Significant correlations between the two methods for log removal of tested viruses was observed**
  - **The removal efficacies determined by two methods showed an approximate 1:1 correlation**
  - **Further study is needed for a better removal calculation of adenovirus**
- **The applicability of qPCR for the simultaneous quantification of coxsackievirus, echovirus, and poliovirus was demonstrated.**
  - **Only the target virus was amplified with no cross-reactivity**
- **qPCR is desirable tool for more rigorous investigation of physical removal of viruses in higher removal range.**
  - **Due to higher sensitivity and accuracy of qPCR than TCID50, 4 orders of magnitude higher titer can be measured**
- **In addition to the ease of processing high number of samples, the method can be used for extreme water conditions such as low pH and high chemical concentrations, where microbes are treated by dual physical and chemical processes.**

The results of this study clearly support the applicability of qPCR assay for bench- or pilot-scale experiments for measuring microbial removal in drinking water including the CCL viruses.

ACKNOWLEDGMENT

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