

Q-099 Applicability of Quantitative PCR for the Determination of Removal Efficacy of Enteric Viruses and *Cryptosporidium* by Water Treatment Processes



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A number of data points are needed for evaluating treatability of enteric viruses and *Cryptosporidium* using physicochemical treatment processes such as coagulation and filtration

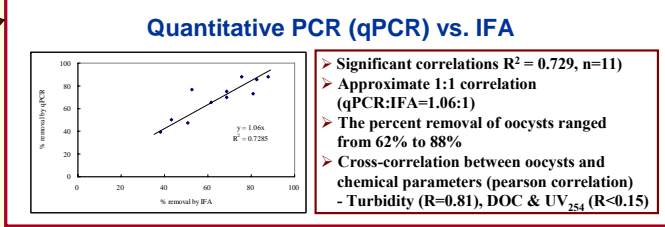
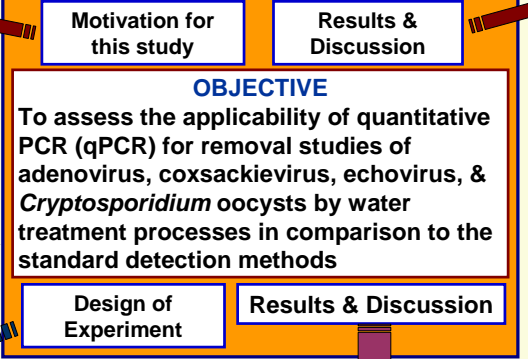
Cell culture assays for enteric viruses

- Time consuming
- Expensive
- Subjective (cytopathogenic effects)
- Not a user-friendly technique

An immunofluorescence assay (IFA) for *Cryptosporidium* oocysts

Epifluorescence microscopy should not be performed for more than 4 hr/day nor more than 5 consecutive days/week for an analyst

Limiting the feasible number of samples

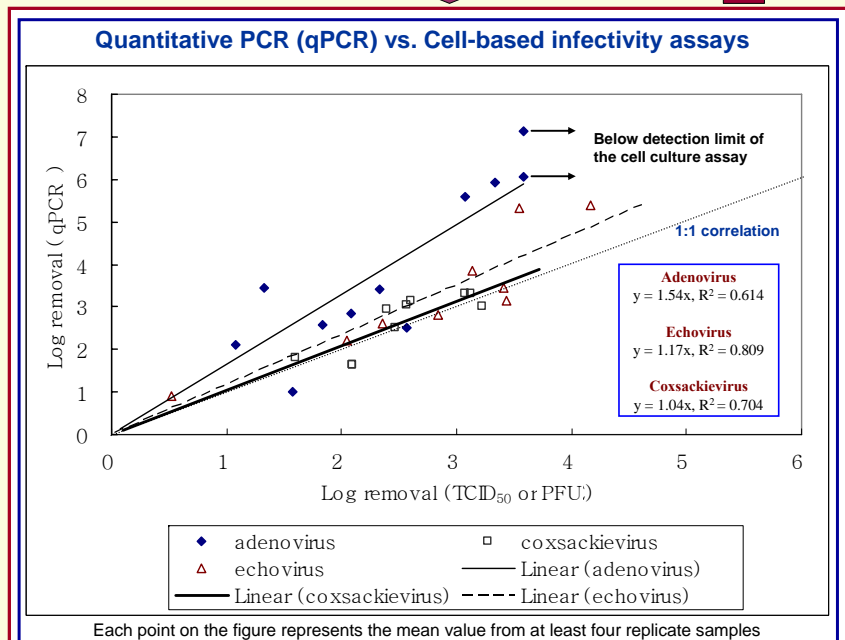
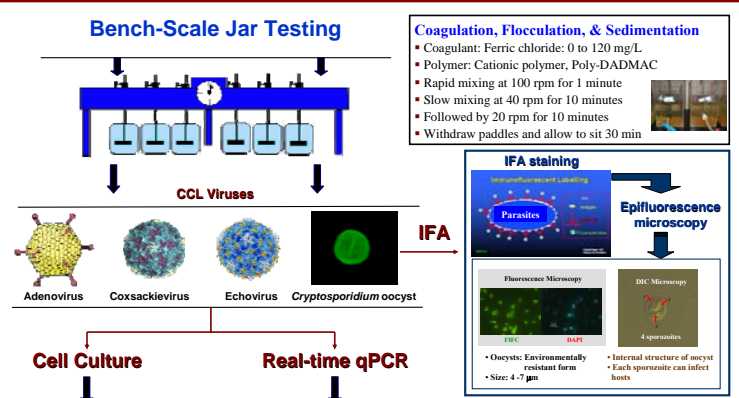


SUMMARY

qPCR is an alternative method to cell culture assays for microbial removal studies.

- Significant correlations between the two methods for log removal of tested microorganisms 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.
- Amplification of the target virus without cross-reactivity
- qPCR is desirable tool for more rigorous investigation of physical removal of microorganisms at the higher removal range.
- Due to the 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 and *Cryptosporidium* oocysts.



Significant correlations between the two methods for log removal of tested viruses was observed.

- For adenovirus, the ratio was 1.54:1 possibly due to the relatively conservative determination of CPE for adenovirus compared to a PFU assay for the other viruses, resulting in underestimation of log removal. Further study is needed to elucidate the difference between the two methods for adenovirus.
- 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.
- The physical removal of coxsackievirus and echovirus (Mayer et al., ES&T 2008) and adenovirus (Abbaszadegan et al., ES&T 2007) using enhanced coagulation was presented in the companion studies.

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