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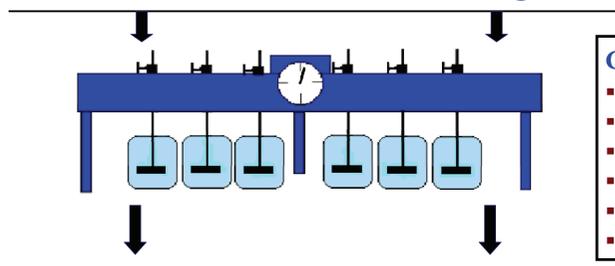
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Objectives

- 1) To assess the applicability of quantitative PCR for removal studies of *Cryptosporidium* oocysts by water treatment processes. An immunofluorescence assay (IFA) was used as a standard method for comparison.
- 2) To investigate oocyst removals using bench-scale enhanced ferric chloride (FeCl₃) coagulation.

Design of Experiments

Bench-Scale Jar Testing

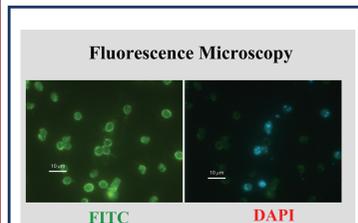


Coagulation, Flocculation, & Sedimentation

- Coagulant: Ferric chloride: 0 to 120 mg/L
- Polymer: Cationic polymer, Poly-DADMAC (0.4 mg/L)
- 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



Immunofluorescence assay (IFA)



DIC Microscopy



- Oocysts: Environmentally resistant form
- Size: 4 - 7 μm
- Each sporozoite can infect hosts

Real-time qPCR

Oligonucleotide primer and probe for TaqMan qPCR

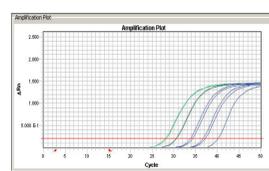
Cryptosporidium parvum hsp70 gene

Real time PCR with TaqMan probe

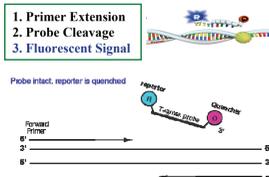
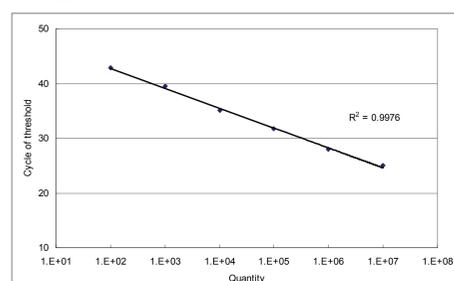
DNA extraction and purification

Qiagen QIAamp DNA kit

<ABI Prism 7900 HT Sequence Detection System>



Generating Standard Curve



Data Presentation

$$R = \left(\frac{N_d}{N_0} \right) * 100$$

- R: Percent removal of oocysts by treatment processes
- N₀: Initial concentration of oocysts
- N_d: Concentration of oocysts after a specified coagulant dose

Regression Analysis according to the following equation

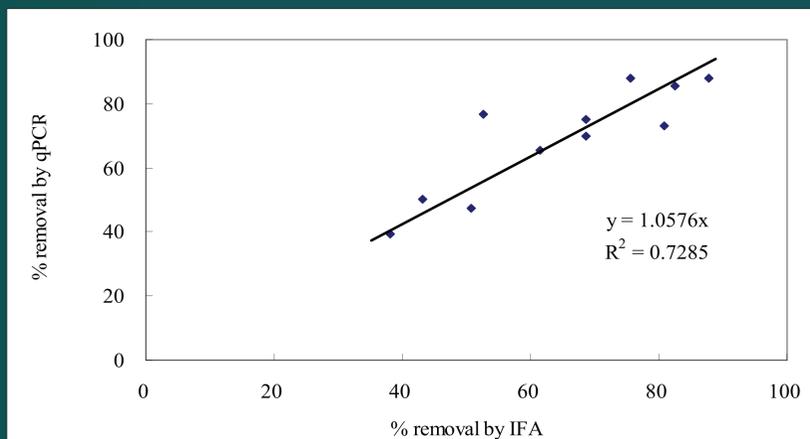
$$P = -k \times C$$

- P: The percent removal values calculated by qPCR
- k: The approximate coefficient
- C: The log removal values calculated by IFA

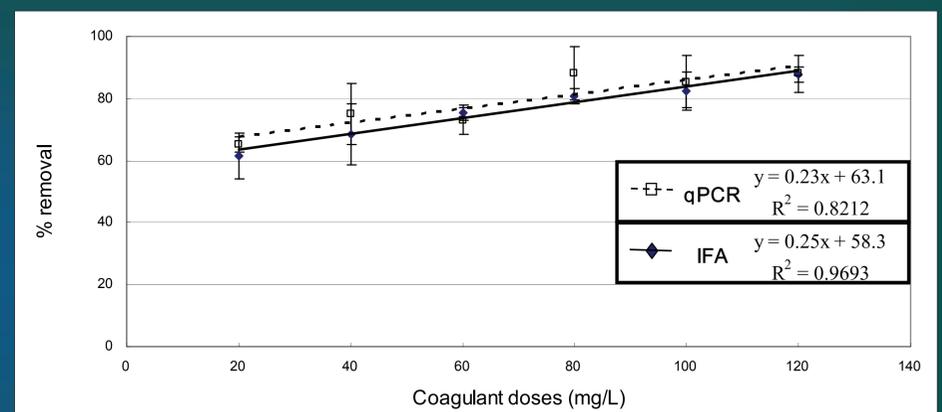
Statistical Analysis for cross-correlation

A Pearson correlation coefficient (R) with a two-tailed P value was calculated using the SPSS version 11.0 for cross-correlations between the removal of *C. parvum* oocysts, turbidity, DOC, and UV₂₅₄. P values of <0.05 were considered significant.

RESULTS AND DISCUSSION



- A significant correlation between percent removals calculated using qPCR and IFA was observed.
- An approximate coefficient k is 1.06.



- Oocyst removal ranged from 62% to 88%, as FeCl₃ concentration increased (main removal mechanism may be sweep flocculation).
- A strong correlation between the removal of oocysts and turbidity (R=0.81) (not shown in the figure).

SUMMARY AND SUGGESTIONS

- 1) The qPCR assay demonstrates that it could serve as an alternate to IFA for the quantification of oocysts and could be applied for removal studies using a relative quantification approach.
- 2) In coagulant dose optimization experiments, the United State EPA criteria was satisfied by removing more than 25% of DOC at coagulant doses of 40 mg/L or greater FeCl₃. Under these enhanced coagulation conditions, the percent removal of *C. parvum* oocysts ranged from 62% to 88%.
- 3) The pH optimization jar tests failed to meet the DOC removal criteria described by the USEPA guidelines for enhanced coagulation conditions, and resulted in relatively low levels of oocyst removal (38% to 53%).
- 4) The removal of turbidity showed a strong correlation with oocyst removal, suggesting that water turbidity measurement can be a useful tool to estimate the removal of oocysts by coagulation.

ACKNOWLEDGEMENTS

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