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Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring *Enterococcus* in environmental waters

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ABSTRACT

Draft criteria for the optional use of qPCR for recreational water quality monitoring have been published in the US. One concern is that inhibition of the qPCR assay can lead to false negative results and potentially inadequate public health protection. In this study we evaluated the effectiveness of strategies for minimizing the impact of inhibition. Five qPCR method permutations for measuring *Enterococcus* were challenged with 133 potentially inhibitory fresh and marine water samples. Serial dilutions were conducted to assess *Enterococcus* target assay inhibition, to which inhibition identified using four internal controls (IC) was compared. The frequency and magnitude of inhibition varied considerably among qPCR methods, with the permutation using a proprietary environmental master mix performing substantially better. Five-fold dilution was also effective at reducing inhibition in most samples (>78%). ICs were variable and often ineffective, with 54 to 85% agreement between ICs and serial dilution. The current IC methods appear not to accurately predict *Enterococcus* inhibition and should be used with caution; 5-fold dilution and the use of reagents designed for environmental sample analysis (more robust qPCR chemistry) may be preferable. Suitable approaches for defining, detecting, and reducing inhibition will improve implementation of qPCR for water monitoring.

Full Text

ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2012AnnualReport/ar12_287_298.pdf