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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

Aims: Draft criteria for the optional use of qPCR for recreational water quality monitoring have been published in the United States. One concern is that inhibition of the qPCR assay can lead to false-negative results and potentially inadequate public health protection. We evaluate the effectiveness of strategies for minimizing the impact of inhibition.

Methods and Results: 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 an environmental master mix performing substantially better. Fivefold dilution was also effective at reducing inhibition in most samples (>78%). ICs were variable and somewhat ineffective, with 54–85% agreement between ICs and serial dilution.

Conclusions: The current IC methods appear to not accurately predict Enterococcus inhibition and should be used with caution; fivefold dilution and the use of reagents designed for environmental sample analysis (i.e. more robust qPCR chemistry) may be preferable.

Significance and Impact of the Study: Suitable approaches for defining, detecting and reducing inhibition will improve implementation of qPCR for water monitoring.