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Multi-laboratory survey of qPCR enterococci analysis method performance in U.S. coastal and inland surface waters

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ABSTRACT

Quantitative polymerase chain reaction (qPCR) has become a frequently used technique for quantifying enterococci in recreational surface waters, but there are several methodological options. Here we evaluated how three method permutations, type of mastermix, sample extract dilution and use of controls in results calculation, affect method reliability among multiple laboratories with respect to sample interference. Multiple samples from each of 22 sites representing an array of habitat types were analyzed using EPA Method 1611 and 1609 reagents with full strength and five-fold diluted extracts. The presence of interference was assessed three ways: using sample processing and PCR amplifications controls; consistency of results across extract dilutions; and relative recovery of target genes from spiked enterococci in water sample compared to control matrices with acceptable recovery defined as 50 to 200%. Method 1609, which is based on an environmental mastermix, was found to be superior to Method 1611, which is based on a universal mastermix. Method 1611 had over a 40% control assay failure rate with undiluted extracts and a 6% failure rate with diluted extracts. Method 1609 failed in only 11% and 3% of undiluted and diluted extracts analyses. Use of sample processing control assay results in the delta–delta Ct method for calculating relative target gene recoveries increased the number of acceptable recovery results. Delta–delta tended to bias recoveries from apparent partially inhibitory samples on the high side which could help in avoiding potential underestimates of enterococci — an important consideration in a public health context. Control assay and delta–delta recovery results were largely consistent across the range of habitats sampled, and among laboratories. The methodological option that best balanced acceptable estimated target gene recoveries with method sensitivity and avoidance of underestimated enterococci densities was Method 1609 without extract dilution and using the delta–delta calculation method. The applicability of this method can be extended by the analysis of diluted extracts to sites where interference is indicated but, particularly in these instances, should be confirmed by augmenting the control assays with analyses for target gene recoveries from spiked target organisms.