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Inter-laboratory comparison of real-time PCR protocols for quantification of general fecal indicator bacteria

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ABSTRACT

The application of quantitative real-time PCR (qPCR) technologies for the rapid identification of fecal bacteria in environmental waters is being considered for use as a national water quality metric in the United States. The transition from research tool to a standardized protocol requires information on the reproducibility and sources of variation associated with qPCR methodology across laboratories. This study examines inter-laboratory variability in the measurement of enterococci and *Bacteroidales* concentrations from standardized, spiked, and environmental sources of DNA using the Entero1a and GenBac3 qPCR methods, respectively. Comparisons are based on data generated from eight different research facilities. Special attention was placed on the influence of the DNA isolation step and effect of simplex and multiplex amplification approaches on inter-laboratory variability. Results suggest that a crude lysate is sufficient for DNA isolation unless environmental samples contain substances that can inhibit qPCR amplification. No appreciable difference was observed between simplex and multiplex amplification approaches. Overall, inter-laboratory variability levels remained low (<10% coefficient of variation) regardless of qPCR protocol.

Full Text

ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2012AnnualReport/ar12_273_286.pdf