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Evaluation of the repeatability and reproducibilityof a suite of qPCR-based microbial source tracking methods

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ABSTRACT

Many PCR-based methods for microbial source tracking (MST) have been developed and validated within individual research laboratories. Inter-laboratory validation of these methods, however, has been minimal, and the effects of protocol standardization regimes. have not been thoroughly evaluated. Knowledge of factors influencing PCR in different laboratories is vital to future technology transfer for use of MST methods as a tool for water quality management. In this study, a blinded set of 64 filters (containing 32 duplicate samples generated from 12 composite fecal sources) were analyzed by three to five core laboratories with a suite of PCR-based methods utilizing standardized reagents and protocols. Repeatability (intra-laboratory variability) and reproducibility (inter-laboratory variability) of observed results were assessed. When standardized methodologies were used, intra- and inter-laboratory %CVs were generally low (median %CV 0.1e3.3% and 1.9 e7.1%, respectively) and comparable to those observed in similar inter-laboratory validation studies performed on other methods of quantifying fecal indicator bacteria (FIB) in environmental samples. ANOVA of %CV values found three human-associated methods (BsteriF1, BacHum, and HF183Taqman) to be similarly reproducible ($p > 0.05$) and significantly more reproducible ($p < 0.05$) than HumM2. This was attributed to the increased variability associated with low target concentrations detected by HumM2 (approximately 1 e2 log10copies/filter

lower) compared to other human-associated methods. Cow-associated methods (BacCow and CowM2) were similarly reproducible ($p > 0.05$). When using standardized protocols, variance component analysis indicated sample type (fecal source and concentration) to be the major contributor to total variability with that from replicate filters and inter-laboratory analysis to be within the same order of magnitude but larger than inherent intra-laboratory variability. However, when reagents and protocols were not standardized, inter-laboratory %CV generally increased with a corresponding decline in reproducibility. Overall, these findings verify the repeatability and reproducibility of these MST methods and highlight the need for standardization of protocols and consumables prior to implementation of larger scale MST studies involving multiple laboratories.