Analysis of Molecular- Marker- Based Microbial Source Tracking for Fecal Load Estimation

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Introduction

The current state of microbial source tracking science is that fecal sources can be detected, but not quantified. Quantification of loads from specific sources will require one of the following technologies:

1. The ability to accurately classify fecal-indicator bacteria isolates to source (library dependent).
2. The ability to quantify a host-specific marker in the environmental matrix and relate that quantity to fecal load.

The inability of library-dependent methods to accurately classify fecal-indicator bacteria to source has been established (Griffith et al., 2003; Stoeckel et al., 2004; Moore et al., 2005). Thus, quantitative MST will require detection of markers (by qPCR, MPN, or other quantitative approaches) and analysis of the marker density to fecal load.

Objectives:

To use measured error rates in a generalized model to estimate confidence intervals about MST marker concentration in water samples, and conversion to fecal concentration.

The section below includes descriptions of error sources in analysis of environmental samples, data regarding the extent of error, and development of processes that may reduce those sources of error.

Results

Steps at which error is introduced to the process of measuring target-sequence copy number in environmental waters were identified. Published and unpublished error rates at each step were compiled (see presentation of errors, below). Refinements to several steps were identified.

The expected qPCR measurement (C(t) value) was calculated for a hypothetical sample containing 0.25 mg/L human waste and 0.75 mg/L ruminant manure based on current knowledge of marker distribution in various hosts (Layton and others, 2006; Seurinck and others, 2005). The qPCR measurement of C(t) was then used to estimate both the concentration of fecal material from the detected source, and the confidence interval of that concentration.

The calculation was done using 1) generalized assumptions (extraction efficiency not measured, sample inhibition not corrected, standard curve not done with each run, replicate analyses not done), 2) incorporation of extraction efficiency measurement and 3) both extraction efficiency and run-specific standard curves.

Results were plotted as the distribution of confidence intervals at decadal values of alpha (type I error) for three cases. Artificial extended standard curves were generated with reduced R² values. Regression equations were within 0.2% of the more precise single-run standard curve.

Interpretations and Conclusions

Error is measurable at each step of sample processing.

Some sources of error are correctable (sample inhibition, extraction efficiency). Others can be measured and minimized to reduce calculated uncertainty in the final result (Detection and Quantification variability). Still others are inherent and cannot be minimized (distribution of markers among source population), but must be accounted for.

Cumulative uncertainty can mask true differences in fecal contribution at the level of half-log difference when converting laboratory analysis results (C(t) values) to level of contamination in water (mg feces per L water).

Based on current estimates of uncertainty from the authors’ original work and data from published literature, current procedures are inadequate to estimate the proportional fecal contribution to a stream from various host sources. When error is carefully measured and controlled, ranking of contributions by various sources may be feasible.

Incorporation of internal controls in processing steps allows 1) simultaneous evaluation of the steps involved in the source tracking process 2) assessment of intraassay and interassay variability 3) calibration to correct for losses due to inefficiency 4) evaluation of where error needs to be reduced.

Concentration

Detection

Quantitation

Data