SCCWRP Annual Report 2013

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

Darcy L. Ebentier[1], Kaitlyn T. Hanley[1,2], Yiping Cao[3], Brian D. Badgley[4], Alexandria B. Boehm[5], Jared S. Ervin[6,7], Kelly D. Goodwin[8], Michèle Gourmelon[9], John F. Griffith[3], Patricia A. Holden[6,7], Catherine A. Kelty[10], Solen Lozach[9], Charles McGee[11], Lindsay A. Peed[10], Meredith Raith[3], Hodon Ryu[10], Michael J. Sadowsky[4], Elizabeth A. Scott[3], Jorge Santo Domingo[10], Alexander Schriewer[2], Christopher D. Sinigalliano[8], Orin C. Shanks[10], Laurie C. Van De Werfhorst[6,7], Dan Wang[5], Stefan Wuertz[2,12] and Jennifer A. Jay[1]

[1]University of California, Department of Civil and Environmental Engineering, Los Angeles, CA

[2]University of California, Department of Civil and Environmental Engineering, Davis, CA

[3]Southern California Coastal Water Research Project, Costa Mesa, CA

[4]University of Minnesota, BioTechnology Institute and Department for Soil, Water and Climate, St. Paul, MN

[5]Stanford University, Department of Civil and Environmental Engineering, Environmental and Water Studies, Stanford, CA

[6]University of California, Bren School of Environmental Science and Management, Santa Barbara, CA

[7]University of California, Earth Research Institute, Santa Barbara, CA

[8]National Oceanic and Atmospheric Administration, Atlantic Oceanographic & Meteorological Laboratory, Miami, FL (stationed at SWFSC, La Jolla, CA)

[9]Ifremer, Laboratoire de Microbiologie, MIC/LNR, Département Ressources Biologiques et Environnement, Unité Environnement, Microbiologie et Phycotoxines, Plouzané, France

[10]US Envrionmental Protection Agency, National Risk Management Research Laboratory, Cincinnati, OH

[11]Orange County Sanitation District, 10844 Ellis Ave, Fountain Valley, CA

[12]Nanyang Technological University, Singapore Centre on Environmental Life Sciences Engineering, School of Biological Sciences, and School of Civil and Environmental Engineering, Singapore, Singapor

ABSTRACT

Many PCR-based methods for microbial source tracking (MST) have been developed and validated within individual research laboratories. Interlaboratory 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 the 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 0.1 - 3.3% and 1.9 - 7.1%, 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 (Bsteri, 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 - 2 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.

Full Text

http://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2013AnnualReport/ar13_433_444.pdf