Performance evaluation of canine-associated Bacteroidales assays in a multi-laboratory comparison study

Alexander Schriewer[1], Kelly D. Goodwin[2,*], Christopher D. Sinigalliano[2], Annie M. Cox[3,*,**], David Wanless[4], Jakob Bartkowiak[4], Darcy L. Ebentier[5], Kaitlyn T. Hanley[1,5], Jared Ervin[6], Louise A. Deering[7], Orin C. Shanks[8], Lindsay A. Peed[8], Wim G. Meijer[7], John F. Griffith[9], Jorge SantoDomingo[10], Jennifer A. Jay[5], Patricia A. Holden[6] and Stefan Wuertz[1,11]

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

[2]*National Oceanic and Atmospheric Administration, Atlantic Oceanographic and Meteorological Laboratory, Miami, FL*

[3]*National Oceanic and Atmospheric Administration, Northwest Fisheries Science Center Laboratory, Seattle, WA*

[4]*University of Miami, Cooperative Institute for Marine and Atmospheric Studies, Miami, FL*

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

[6]*University of California, Bren School of Environmental Science & Management and Earth Research Institute, Santa Barbara, CA*

[7]*University College Dublin, School of Biomolecular and Biomedical Science, Dublin, Ireland*

[8]*US Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Cincinnati, OH*

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

[10]*US Environmental Protection Agency, Microbial Contaminants Control Branch, National Risk Management Research Laboratory, Cincinnati, OH*

[11]*Nanyang Technological University, Singapore Centre on Environmental Life Sciences Engineering (SCELSE), School of Biological Sciences, and School of Civil and Environmental Engineering, Singapore*

Stationed at NOAA/SWFSC, La Jolla, CA

***Currently at: University of British Columbia, Life Sciences Center, Department of Microbiology and Immunology, Vancouver, Canada*

ABSTRACT

The contribution of fecal pollution from dogs in urbanized areas can be significant and is an often underestimated problem. Microbial source tracking methods (MST) utilizing quantitative PCR of dog-associated gene sequences encoding 16S rRNA of Bacteroidales are a useful tool to estimate these contributions. However, data about the performance of available assays are scarce. The results of a multi-laboratory study testing two assays for the determination of dog-associated Bacteroidales (DogBact and BacCan-UCD) on 64 single and mixed fecal source samples created from pooled fecal samples collected in California are presented here. Standardization of qPCR data treatment lowered inter-laboratory variability of sensitivity and specificity results. Both assays exhibited 100% sensitivity. Normalization methods are presented that eliminated random and confirmed non-target responses. The combination of standardized qPCR data treatment, use of normalization via a non-target specific Bacteroidales assay (GenBac3), and application of threshold criteria improved the calculated specificity significantly for both assays. Such measures would reasonably improve MST data interpretation not only for canine-associated assays, but for all qPCR assays used in identifying and monitoring fecal pollution in the environment.

Full Text