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

Alexander Schriewer¹, Kelly D. Goodwin², Christopher D. Sinigalliano², Annie M. Cox³,¹²,¹³, David Wanless⁴, Jakob Bartkowiak⁴, Darcy L. Ebentier⁵, Kaitlyn T. Hanley¹,⁵, Jared Ervin⁶, Louise A. Deering⁷, Orin C. Shanks⁸, Lindsay A. Peet⁸, Wim G. Meijer⁷, John F. Griffith⁹, Jorge SantoDomingo¹⁰, Jennifer A. Jay⁵, Patricia A. Holden⁶, Stefan Wuertz¹,¹¹

¹Department of Civil and Environmental Engineering, University of California Davis, One Shields Ave, Davis, CA, USA
²National Oceanic and Atmospheric Administration, Atlantic Oceanographic and Meteorological Laboratory, Miami, FL, USA
³National Oceanic and Atmospheric Administration, Northwest Fisheries Science Center Laboratory, Seattle, WA, USA
⁴Cooperative Institute for Marine and Atmospheric Studies, University of Miami, Miami, FL, USA
⁵Department of Civil and Environmental Engineering, University of California Los Angeles, Los Angeles, CA, USA
⁶Bren School of Environmental Science & Management and Earth Research Institute, University of California, Santa Barbara, CA, USA
⁷UCD School of Biomolecular and Biomedical Science, University College Dublin, Ireland
⁸U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Cincinnati, OH, USA
⁹Southern California Coastal Water Research Project, Costa Mesa, CA, USA
¹⁰U.S Environmental Protection Agency, Microbial Contaminants Control Branch, National Risk Management Research Laboratory, Cincinnati, OH, USA
¹¹Singapore Centre on Environmental Life Sciences Engineering (SCELSE), School of Biological Sciences and School of Civil and Environmental Engineering, Nanyang Technological University, Singapore
¹²Stationed at NOAA/SWFSC, La Jolla, CA, USA.
¹³Current address: Department of Microbiology and Immunology, Life Sciences Center, University of British Columbia, 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.

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