

Multi-laboratory evaluations of the performance of *Catellibacterium marimammalium* PCR assays developed to target gull fecal sources

Christopher D. Sinigalliano¹, Jared S. Ervin^{2,3}, Laurie C. Van De Werfhorst^{2,3}, Brian D. Badgley^{4,18}, Elisenda Balleste⁵, Jakob Bartkowiak^{1,6}, Alexandria B. Boehm⁷, Muruleedhara Byappanahalli⁸, Kelly D. Goodwin¹, Michelle Gourmelon⁹, John Griffith¹⁰, Patricia A. Holden^{2,3}, Jenny Jay¹¹, Blythe Layton¹⁰, Cheonghoon Lee¹², Jiyoung Lee^{12,13}, Wim G. Meijer⁵, Rachel Noble¹⁴, Meredith Raith¹⁰, Hodon Ryu¹⁵, Michael J. Sadowsky⁴, Alexander Schriewer¹⁶, Dan Wang⁷, David Wanless^{1,6}, Richard Whitman⁷, Stefan Wuertz^{16,17}, Jorge W. Santo Domingo¹⁵

¹National Oceanic and Atmospheric Administration, Atlantic Oceanographic and Meteorological Laboratory, Miami, FL, USA

²Bren School of Environmental Science & Management, University of California, Santa Barbara, CA, USA

³Earth Research Institute, University of California, Santa Barbara, CA, USA

⁴BioTechnology Institute, University of Minnesota, St. Paul, MN, USA

⁵Conway Institute, UCD School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland

⁶Cooperative Institute for Marine and Atmospheric Studies, University of Miami, Miami, FL, USA

⁷Environmental and Water Studies, Department of Civil and Environmental Engineering, Stanford University, Stanford, CA, USA

⁸U.S. Geological Survey Great Lakes Science Center, Lake Michigan Ecological Research Station, Porter, IN, USA

⁹Laboratoire de Microbiologie, MIC/LNR, Département Ressources Biologiques et Environnement, Unite Environnement, Microbiologie et Phycotoxines, Ifremer, ZI Pointe du diable, Plouzane, France

¹⁰Southern California Coastal Water Research Project, Costa Mesa, CA, USA

¹¹Department of Civil and Environmental Engineering, University of California Los Angeles, Los Angeles, CA, USA

¹²Division of Environmental Health Sciences, College of Public Health, Ohio State University, Columbus, OH, USA

¹³Department of Food Science & Technology, Ohio State University, Columbus, OH, USA

¹⁴Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC, USA

¹⁵U.S. Environmental Protection Agency, Microbial Contaminants Control Branch, National Risk Management Research Laboratory, Cincinnati, OH, USA

¹⁶Department of Civil and Environmental Engineering, University of California Davis, Davis, CA, USA

¹⁷Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University, Singapore

¹⁸Department of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, VA, USA

ABSTRACT

Here we report results from a multi-laboratory (n = 11) evaluation of four different PCR methods targeting the 16S rRNA gene of *Catellibacterium marimammalium* originally developed to detect gull fecal contamination in coastal environments. The methods included a conventional end-point PCR method, a SYBR® Green qPCR method, and two TaqMan® qPCR methods. Different techniques for data normalization and analysis were tested. Data analysis methods had a pronounced impact on assay sensitivity and specificity calculations. Across-laboratory standardization of metrics including the lower limit of quantification (LLOQ), target detected but not quantifiable (DNQ), and target not detected (ND) significantly improved results compared to results submitted by individual laboratories prior to definition standardization. The unit of measure used for data normalization also had a pronounced effect on measured assay performance. Data normalization to DNA mass improved quantitative method performance as compared to enterococcus normalization. The MST methods tested here were originally designed for gulls but were found in this study to also detect feces from other birds, particularly feces composited from pigeons. Sequencing efforts showed that some pigeon feces from California contained sequences similar to *C. marimammalium* found in gull feces. These data suggest that the prevalence,

geographic scope, and ecology of *C. marimammalium* in host birds other than gulls require further investigation. This study represents an important first step in the multi-laboratory assessment of these methods and highlights the need to broaden and standardize additional evaluations, including environmentally relevant target concentrations in ambient waters from diverse geographic regions.

Due to distribution restrictions, the full-text version of this article is available by request only.

Please contact pubrequest@sccwrp.org to request a copy.