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

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**ABSTRACT**

Here we report results from a multi-laboratory (n = 11) evaluation of four different PCR methods targeting the 16S rRNA gene of *Catellicoccus 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.

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