

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

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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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