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# Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study

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## ABSTRACT

The last decade has seen development of numerous new microbial source tracking (MST) methodologies, but many of these have been tested in just a few laboratories with a limited number of fecal samples. This method evaluation study examined the specificity and sensitivity of 41 MST methodologies by analyzing data generated in 27 laboratories. MST methodologies that targeted human, cow, ruminant, dog, gull, pig, horse, and sheep were tested against sewage, septage, human, cow, dog, deer, pig, chicken, pigeon, gull, horse, and goose fecal samples. Each laboratory received 64 blind samples containing a single source (singletons) or two sources (doubletons), as well as diluted singleton samples to assess method sensitivity. Laboratories utilized their own protocols when performing the methods and data were deposited in a central database before samples were unblinded. Between one and seven laboratories tested each method. The most sensitive and specific assays, based on an analysis of presence/absence of each marker in target and non-target fecal samples, were HF183 endpoint and HF183SYBR (human), CF193 and Rum2Bac (ruminant), CowM2 and CowM3 (cow), BacCan (dog), Gull2SYBR and LeeSeaGull (gull), PF163 and pigmtDNA (pig), HoF597 (horse), PhyloChip (pig, horse, chicken, deer), Universal 16S TRFLP (deer), and Bacteroidales 16S TRFLP (pig, horse, chicken, deer); all had sensitivity and specificity higher than 80% in all or the majority of laboratories. When the abundance of MST markers in target and non-target fecal samples was examined, some assays that performed well in the binary analysis were found to not be sensitive enough as median concentrations fell below a minimum abundance criterion (set at 50 copies per colony forming units of enterococci) in target fecal samples. Similarly, some assays that crossreacted with non-target fecal sources in the binary analysis were found to perform well in a quantitative analysis because the cross-reaction occurred at very low levels. Based on a quantitative analysis, the best performing methods were HF183Taqman and BacH (human), Rum2Bac and BacR (ruminant), LeeSeaGull (gull), and Pig2Bac (pig); no cow or dog-specific assay met the quantitative specificity and sensitivity criteria. Some of the best performing assays in the study were run by just one laboratory so further testing of assay portability is needed. While this study evaluated the marker performance in defined samples, further field testing as well as development of frameworks for fecal source allocation and risk assessment are needed.