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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 cross-reacted 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

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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.

1. Introduction

Beach water quality monitoring is based on measurement of fecal indicator bacteria (FIB), which are used as surrogates for human pathogens because they are relatively easy to measure and have been found to correlate with human health outcomes (Pruss, 1998; Wade et al., 2003). However, FIB can originate from numerous pollution sources, such as human sewage, manure from livestock operations, wildlife, and urban runoff. In addition, non-fecal FIB sources have been well documented (Hardina and Fujioka, 1991; Byappanahalli et al., 2003; Yamahara et al., 2007). Effective beach management requires knowledge of the dominant FIB sources and their potential influences on water quality. Source identification also allows prioritization of watersheds for remediation based on predicted human health risks; risks will differ depending on the host source from which the FIB originated (Soller et al., 2010).

Numerous microbial source tracking (MST) methods intended to discriminate between human and non-human fecal sources have been developed, with some methods designed to differentiate among animal sources. The field was historically dominated by library-dependent methods that match genetic or phenotypic patterns of FIB isolates from a known source to that of isolates in an ambient sample. More recently, genetic markers associated with particular animal feces have gained favor because they do not require building costly isolate libraries, which have been found to be geographically (Wiggins et al., 2003; Ebdon and Taylor, 2006) and temporally (Jenkins et al., 2003; Hansen et al., 2009) specific. Several other classes of methods, including viruses specific to human fecal material (Noble et al., 2003; Noble and McQuaig, 2011), chemical (Hagedorn and Weisberg, 2009), community-based (Cao et al., 2011a), and metagenomic methods (Unno et al., 2010), are also used.

A few large studies to assess efficacy of these methods have been conducted (Griffith et al., 2003; Stoeckel et al., 2004), but they were conducted prior to development of many library-independent methods. Methods developed since that time have been mostly evaluated within the research laboratories in which they were developed, making it difficult to assess their geographical stability (Stoeckel and Harwood, 2007). Moreover, most evaluations have focused on a small number of candidate sources, limiting the ability to assess cross-reactivity that has been reported in some studies (Layton et al., 2006; Kildare et al., 2007; McLain et al., 2009; Van De Werfhorst et al., 2011). Studies that have investigated a large number of candidate sources (Shanks et al., 2010a,b) were performed in a single laboratory, resulting in no information on potential influence of inter-laboratory variability.

Here we provide results from a study (the Source Identification Protocol Project, SIPP) in which 41 MST methods implemented by 27 laboratories (Table 1) were challenged with 12 possible fecal sources in 64 blind samples. A number of papers in this journal issue are dedicated to presenting results from SIPP. This paper describes the study design and provides a broad overview of the results.

2. Materials and methods

A global call for participating laboratories was distributed by email. All laboratories that indicated they would like to participate were accommodated; this included those who wished to test newly developed assays as well as older assays gaining popularity in the MST field. Below we outline the

Table 1 – List of participating laboratories.

Principal investigator	Affiliation
C. Sinigalliano	National Oceanic and Atmospheric Administration
J. Lee	Ohio State Univ.
W. Meijer	Univ. College Dublin
J. Rose	Michigan State Univ.
M. Byappanahalli	U. S. Geological Survey
J. Stewart	Univ. North Carolina
M. Sadowsky	Univ. Minnesota
J. Ebdon & H. Taylor	Univ. Brighton
S. Wuertz	Univ. California Davis
J. Jay	Univ. California Los Angeles
R. Noble	Univ. North Carolina
S. Reynolds	Environmental Canine Services LLC
K. Vijayavel & D. Kashian	Wayne State Univ./Ottowa County
J. Griffith	Southern California Coastal Water Research Project
M. Gourmelon	French Research Institute for Exploration of the Sea
T. Fong	TetraTech
K. Goodwin	National Oceanic and Atmospheric Administration
A. Farnleitner	Vienna University of Technology
J. Santo Domingo	U.S. Environmental Protection Agency
D. Diston & M. Wicki	Federal Office of Public Health, Switzerland
J. Fuhrman	Univ. Southern California
A. Boehm	Stanford
O. Shanks	U.S. Environmental Protection Agency
P. Holden	Univ. California Santa Barbara
R. Rodrigues & J. Brandão	National Institute of Health, Portugal
T. Madi	Source Molecular
G. Andersen	Lawrence Berkeley National Laboratory