

## Performance of viruses and bacteriophages for fecal source determination in a multi-laboratory, comparative study.

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### ABSTRACT

An inter-laboratory study of the accuracy of microbial source tracking (MST) methods was conducted using challenge fecal and sewage samples that were spiked into artificial freshwater and provided as unknowns (blind test samples) to the laboratories. The results of the Source Identification Protocol Project (SIPP) are presented in a series of papers that cover 41 MST methods. This contribution details the results of the virus and bacteriophage methods targeting human fecal or sewage contamination. Human viruses used as source identifiers included adenoviruses (HAdV), enteroviruses (EV), norovirus Groups I and II (NoVI and NoVII), and polyomaviruses (HPyVs). Bacteriophages were also employed, including somatic coliphages and F-specific RNA bacteriophages (FRNAPH) as general indicators of fecal contamination. Bacteriophage methods targeting human fecal sources included genotyping of FRNAPH isolates and plaque formation on bacterial hosts *Enterococcus faecium* MB-55, *Bacteroides* HB-73 and *Bacteroides* GB-124. The use of small sample volumes ( $\leq 50$  ml) resulted in relatively insensitive theoretical limits of detection (10-50 gene copies or plaques  $\times 50$  ml<sup>-1</sup>) which, coupled with low virus concentrations in samples, resulted in high false-negative rates, low sensitivity, and low negative predictive values. On the other hand, the specificity of the human virus methods was generally close to 100% and positive predictive values were ~40-70% with the exception of NoVs, which were not detected. The bacteriophage methods were generally much less specific toward human sewage than virus methods, although FRNAPH II genotyping was relatively successful, with 18% sensitivity and 85% specificity. While the specificity of the human virus methods engenders great confidence in a positive result, better concentration methods and larger sample volumes must be utilized for greater accuracy of negative results, i.e. the

prediction that a human contamination source is absent.

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