

# Third Rock Investigators Use New Methodologies for Tracking *and* Identifying Sources of Pathogen Contamination in Surface Waters

Waterborne disease has long been a public health issue. According to one recent report, more than five million people die each year from water-related disease and the leading cause of child death worldwide is diarrhea. The World Health Organization estimates that unsafe water is the cause of 80% of all sickness in the world.

Add to these statistics the millions of dollars lost when recreational and commercial waters must close and the ecological impact of fish and shellfish die-off and it is readily apparent that microbial pollution in our waterways represents a significant problem.

In Kentucky, the US EPA's 1998 303(d) list indicates that 20,964 acres of wetland and lake as well as 1,289 miles of stream and river exceed pathogen standards. Thus, controlling microbial (or fecal) pollution is a local issue as well as a national and international one.

To date, methods for remediating microbial pollution have been generally unreliable. Traditionally, the answer has been to monitor concentrations of fecal indicator organisms, like total coliform and *E. coli*. *However, simply monitoring the organisms doesn't identify the source and the potential for disease outbreak and environmental impact.*

An additional set of tools is required to answer these more difficult questions. Microbial source tracking (MST) methods provide the means by which biologists and engineers can identify fecal sources actually impacting a watershed.

Currently, there are several methods available offering differing degrees of source discrimination and reliability. All source tracking tools operate on the assumptions that the bacteria or viruses of interest are geographical and temporally stable, host specific, and found representatively in water or soil sampling.

Although there is no "perfect" method applicable to every situation, knowledgeable and informed investigators such as the biologists and environmental engineers at Third Rock Consultants, LLC can identify and employ the most appropriate methodology to achieve the objectives for a watershed. Some of the most promising methods

include rep-PCR, ribotyping, host specific PCR, and F<sup>+</sup> RNA coliphage.

**Rep-PCR and Ribotyping** are both labor intensive, genotypic methods that are best used when looking to discriminate down to the species or individual animal level for a watershed. Both methodologies involved building a library of genetic fingerprints of fecal bacteria from known potential pollution sources in the area. Bacteria are then isolated from the water samples and categorized into one of the source groups based on the similarity of their genetic fingerprints. In this way, a relative contribution of each source may be accessed.

When looking to broadly distinguish between human and animal fecal pollution sources, the ratio subgroups of **F<sup>+</sup> RNA coliphage** is an inexpensive, useful microbial source indicator. According to EPA methodology, the F<sup>+</sup> RNA coliphage virus may be isolated and then sub-typed into one of four groups that show which are generally specific to either human or non-human sources.

By targeting host specific genetic sequences in indicator organisms, **host specific PCR** methods are fast and inexpensive tools to indicate the presence of human, cattle, or wildlife source material in environmental samples. If the relative contribution of each of these groups is desired, real-time PCR can provide that answer as well.

The tools are available and effective in the hands of a consultant who knows the most appropriate application. At Third Rock, we have invested heavily in researching current MST methods and utilizing MST as a tool for watershed planning. We can work with you to identify the appropriate method for your situation and conduct the requisite sampling and analysis employing that method.



For more information on MST and its applications, contact Third Rock's Steven Evans at [sevans@thirdrockconsultants.com](mailto:sevans@thirdrockconsultants.com) or call 859.977.2000 to register for a free MST workshop hosted by Third Rock on Tuesday, December 5, 2006.