

## OSU researcher develops test to determine fecal-pollution source

**N**ONPOINT SOURCE WATER pollution is one of the great conundrums of environmental management. You can clean it up, but if you don't know where it's coming from, how are you going to keep it from coming back?

In Oregon's Tillamook Bay—an area rich with dairy farms—fecal pollution poses significant human health risks and also threatens the area's shellfish industry. But it's difficult to tell whether the pollution is coming from cows, humans, or other animal sources, and controversy over that question has blocked efforts to correct the problem.

Is the source, for example, from cattle manure washing into the waterway from adjoining fields, or is it human waste spilled from a city's sewage plant or percolating through the groundwater from a failed septic system? A lot rides on the answer to that question, and either the dairy industry, city officials, or a specific rural resident in the example might face serious regulatory pressure.

With funding from Oregon Sea Grant, Katharine Field, a microbiologist at Oregon State University, has developed a test for the source of fecal contamination of water that is both faster and far more specific than traditional tests, which can reveal whether there's fecal contamination but not what animal it came from.



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The test developed by Field, an assistant professor specializing in environmental microbiology, molecular ecology, and antibiotic resistance, shows promise of being able to make that determination, and in far less time than the standard fecal coliform tests.

Instead of focusing on fecal coliform, it looks for different bacteria associated with feces.

Field chose *Bacteroides-Prevotella*, a group of anaerobic bacteria—bacteria that grow without oxygen—associated with feces. Using a process called polymerase chain reaction, she

creates millions of copies of a particular gene. Using those genes, she looks for specific markers, gene sequences unique to particular species.

“These bacteria are more diverse than coliforms, and there are more genetic markers,” she explained.

By isolating the genetic markers for different species, she can build a “library” of such indicators to compare with marker genes in samples taken from polluted water. The comparison should reveal the source.

Field has been working with the Tillamook Bay National Estuary Project to see whether her tests can show exactly where these fecal markers are turning up, and when.

“A lot of ecological and water quality studies have been done there, and it has a relatively small population in a relatively confined area,” qualities that make it ideal for the experiment, she said.

In another recent case, Field was approached to identify the source of polluted well water on a Eugene-area farm. The two possible suspects were a chicken-farming operation and composted human sewage that had been sprayed on a nearby field.

The tests came back negative for a human source, Field said. Because she had not yet found a *Bacteroides-Prevotella* marker for chickens, she could not immediately say whether chickens were the cause. But after going back to the site to do more testing, she was able to show conclusively that it was fowls that fouled the well.

The *Bacteroides-Prevotella* test is also much faster than other fecal source tests, another factor that could lead to its success. According to Field, other methods require growing large numbers of bacteria, testing them, then doing a fairly

complex statistical analysis. The turnaround time for these approaches is two weeks to a month, she said.

For the *Bacteroides-Prevotella* test, which is still under development, the turnaround is about a week.

“It should take a day, but life is never that easy,” she said.

While a patent application on the test is pending, Field continues to work on the project. She wants to standardize the procedure so that a simple kit can be developed containing everything needed to perform the test. She also wants to expand the “library” of genetic markers, eventually to include all the animals likely to be the source of contamination—everything from cows and pigs to other ruminants, marine mammals, and even dogs and cats. Dogs, she

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noted, are a major issue for urban nonpoint fecal pollution, and a recent article in the *Scientist* magazine suggested that flushable cat litter may be a culprit in a die-off of sea otters in northern California.

Russell T. Hill, an associate professor at the University of Maryland’s Center of Marine Biotechnology who reviewed Field’s work, said the expansion of Field’s work could be enormously important for efforts to control contamination of rivers and the ocean.

“If Katharine is successful, *Bacteroides* could emerge as the most useful single indicator of fecal pollution.... We have known the limitations of fecal coliform counting for many decades. It will be nice to have a solution at last,” he said.