A Comparison of the Multiple-Tube Fermentation Method and the Colitag Method for the Detection of Waterborne Coliform Bacteria

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Abstract Recreational water quality is an important issue in public health because of problems, including diarrhea, that are caused by fecal contamination. Constant monitoring for *Escherichia coli* (*E. coli*), an indicator organism for such contamination, is a way to reduce human exposure. This research project was designed to determine if the Colitag method for detecting *E. coli* is as reliable as the Multiple-Tube Fermentation (MTF) method, which is currently the technique approved by the U.S. Environmental Protection Agency. From September 2000 to March 2001, identical water samples were taken, approximately once a week, from Strawberry and Codornices Creeks, in Berkeley, California, to perform both tests simultaneously. The Colitag method was found to be as sensitive as the MTF method in detecting fecal coliforms in recreational water. Statistical analysis of parallel test results showed a strong linear correlation of 0.87 between the two methods, which held up well at both high and low fecal coliform counts. Because only 24 hours are required to obtain results from the Colitag method, compared to 96 hours for the MTF method, the former is preferable. Saving three days means that monitoring agencies could respond faster to sudden increases in *E. coli* and could therefore take immediate corrective action to ensure the public safety.

Introduction

Strawberry Creek, which runs through the campus of the university of California at Berkeley, represents an irreplaceable natural resource that is highly valued by both the university and the community at large. The riparian corridors provide essential places for educational, recreational, social, and individual activities (Charbonneau 1987). Therefore, the water quality of the creek is extremely important for human health, especially since the creek is used by many students for educational and recreational purposes, ranging from college students, who study it in Biology 1B at UC Berkeley (Moser 1999), to elementary school students, who come to play and picnic there (Charbonneau 1987).

In past years, the water quality of the creek has caused great concern among members of the university community and their neighbors. An article in the student newspaper, the *Daily Californian* (July 2, 1973, p. 6), reported that fecal bacteria were contaminating the north fork of the creek from sources in the north Berkeley hills. This caused concern in the community because fecal bacteria can cause human disease and illness (Baker and Herson 1999). Another article in the paper, eight years later (November 2, 1981, p. 1), reported that the creek was being used as a sewer, contaminated by urban runoff, chemicals, and sewage. With recent efforts to improve the water quality of the creek, the levels of fecal coliform and *Escherichia coli* (*E. coli*) detected in the creek have decreased dramatically.

Over the last century, scientists around the world have tried to come up with an easy, affordable, and accurate way of monitoring the quality of drinking and recreational water (Chang 2000, pers. comm.). One way to determine water quality is to measure the presence of *E. coli*, which is the main member of a large bacterial family Enterobacteriaceæ, which is composed of faculatively anaerobic Gram-negative rods that live in the gastrointestinal tract of most warm-blooded animals (Edberg *et al.* 1997). The presence of *E. coli* in normal human intestines and feces has led to using the bacteria in nature as an indicator of fecal pollution in water (Edberg *et al.* 1997). Most *E. coli* strains are harmless, but several cause diarrhea. In severe cases, people, especially children and the elderly, have died from ingesting water or food contaminated with *E. coli* (AWWA Research Committee 1999).

The current standard method for monitoring members of the coliform group in water is the Multiple-Tube Fermentation (MTF) technique, which is certified by the U.S. Environmental Protection Agency, USEPA (Clesceri *et al.* 1998). However, this method has the disadvantage of

requiring up to 96 hours to obtain results. When a large quantity of pathogens is present in recreational water, it would be ideal to inform the public of this as soon as possible. But since the MTF technique is so slow, people may become sick from contact with recreational water before test results are released to them. Another disadvantage of this technique is that it is not *E. coli*–specific, but instead measures *all* fecal coliforms. In 1986, USEPA recommended using *E. coli* as the indicator organisms instead of fecal coliforms for monitoring recreational water because the former give a more accurate measurement. However, this recommendation has not been widely accepted, since the MTF method has been around for almost 100 years as a way to measure fecal coliforms. When agencies are used to monitoring water with the same method for a long time, it is hard to get them to try new methods.

The Colitag technique (Chang 2000, pers. comm.), on the other hand, has neither of these disadvantages. It detects only *E. coli*, and it yields results in only 24 hours. The purpose of the present research project, therefore, is to compare the results obtained from these two different methods to see if the Colitag method works as well in detecting fecal coliforms in recreational water as the MTF technique.

Samples were taken not only from Strawberry Creek but also from Codornices Creek, which flows through UC Berkeley's Married Students' Housing Village, in Albany. The second creek was added to the study because many children of all ages play nearby and may be at risk from contacting the water (Lee and Lee 2000). Another reason for testing this creek was to increase the range of fecal coliform levels tested by both techniques, because Codornices Creek has historically had higher counts of fecal coliform than Strawberry Creek (Maranzana 2000, pers. comm.).

Methods

Strawberry Creek is divided into two main branches, the north and south forks. The confluence of the two forks is located in Eucalyptus Grove at the western edge of the central campus, about 400 feet east of Oxford Street (Figure 1).

Codornices Creek runs nearby UC Berkeley's Married Students' Housing Village in Albany between Ninth Street and Gilman Street (Figure 1). Exactly the same sampling methods were used at Codornices Creek as at Strawberry Creek. The two sites were chosen because UC Berkeley's office of environment, health, and safety already uses them for weekly monitoring.

Figure 1 Sampling Sites of Strawberry Creek at the confluence of North and South Forks and Codornices Creek at the intersection of Eighth and Gilman Streets

Approximately every week between September 11, 2000 and March 5, 2001, two 100 ml samples of water were collected simultaneously in sterilized containers from each fork of Strawberry Creek (Figure 1). The samples were collected with this frequency in order to get a good variety of coliform counts from dry and wet seasons. In the past, high rainfall had usually been associated with high coliform counts in the creek water.

The four samples were taken back to the Office of Environmental Health and Safety at UC Berkeley, which forwarded one north fork and one south fork sample to a commercial lab, Cerco Analytical Inc., in Pleasanton, California, to be analyzed by the MTF method (Clesceri *et al.* 1998, numbers 9221B [total coliforms] and 9221E [fecal coliforms]). The other two samples were analyzed by the Colitag method (fecal coliforms and *E. coli*).

The controls used for the Colitag method were SaLT2, ECATCC 25922, ECTC 219, KpTC 249 and a blank, which consisted only of Colitag medium at 6.20 pH (Sloat & Ziel 1991). Colitag, which is an enzymatic indicator-based medium, contains o-nitrophenyl- ß-d-galactopyranoside (ONPG), 4-methylumbelliferyl-ß-D-glucuronide (MUG), and other selective ingredients that are specific to coliforms with little interference from high heterotrophic bacteria counts. The fecal coliforms produced an enzyme to cleave the ONPG and release the indicator. A positive test for fecal coliforms was indicated by a yellow color. Failure to cleave with ONPG with little or no growth constituted a negative reaction and thus had a color similar to that of the

blank. *E. coli* cleaves MUG and produces a blue fluorescence under long-wave ultraviolet (UV) light. Therefore, a UV lamp was used to read the results. An Indole test for *E. coli* was also conducted with the use of Kovac's reagent. A positive test was produced when a reddish surface layer appeared with a few drops of the Kovac's reagent due to *E. coli* cleaving with the chemicals.

Medium (16 mm) and large (18 mm) sterile tubes were used for the experiment. There were five serial dilutions: 1, 10, 100, 1,000, and 10,000. The large tubes were used to perform dilution series for the test, while the medium tubes were used for the five-tube most probable number (MPN) test. Each medium sterile tube contained 4.5 ml of Colitag medium and 0.5 ml of diluted creek water. Each large sterile tube contained 4.5 ml of saline solution and 0.5 ml of creek water at five different dilution series. There were five medium sterile tubes per dilution, hence a five-tube MPN. The 0.5 ml of diluted creek water in each of the sterile medium tubes was from the dilution series in the large sterile tubes. After the initial four hours of incubation at 35º C, the rack with a total of 55 tubes was transferred into a water bath at 44.5º C for an additional 20 hours.

A five-tube (MPN) dilution series was performed for each fork to analyze the total count of pathogens and other fecal coliforms in the creek. The results from the Colitag method were then compared with the results from the commercial lab. According to Clesceri *et al.* (1998), as long as the two results fall into a 95% confidence interval corresponding to the MPN table, the two results are considered consistent with each other.

Results

Between the months of September 2000 and March 2001, 20 samples were collected from Strawberry Creek and 4 samples from Codornices Creek to be analyzed using both the Colitag and the MTF methods. Table 1 shows the results of the two different methods for both dry and wet conditions. According to Federal Water Pollution Control Administration's (1968) regulations, less than 2,000 most probable numbers (MPNs) of fecal coliforms per 100 ml of water is the standard for fecal contamination in non-contact recreational water. In this study, he numbers in Strawberry and Codornices creeks ranged from 40 to 160,000 MPNs per 100 ml.

Sample Date	Colitag Method **North Fork Fecal Coliform (MPN / 100 ml)	MTF Method **North Fork Fecal Coliform (MPN / 100 ml)	Colitag Method **South Fork Fecal Coliform (MPN / 100 ml)	MTF Method **South Fork Fecal Coliform (MPN / 100 ml)
09/11/00	260	500	220	230
09/18/00	600	500	2,600	5,000
09/25/00	600	170	1,000	800
10/16/00	220	110	1,000	800
10/23/00	1,000	800	160	230
10/30/00	26,000	13,000	48,000	160,000
11/13/00	600	1,300	80	40
12/13/00	6,000	17,000	600	800
01/08/01	6,000	1,300	10,000	5,000
02/05/01	*10,000	*3000		
02/20/01	10,000	11,000	2,600	3,500
03/05/01	*600	*1300		

Table 1 A comparison of raw data between the Colitag Method and the Multiple–Tube Fermentation (MTF) Method
* Codornices Creek data ** North and South Forks refer to Strawberry Creek

Although the numbers of coliforms per 100 ml were not exactly the same in the results for both tests, they showed a strong linear correlation of $R^2 = 0.87$ (Figure 2). The association between high coliform counts (>2,000) yielded a linear correlation of $R^2 = 0.55$, while the low coliform counts (≤2,000) yielded a linear correlation of $R^2 = 0.67$.

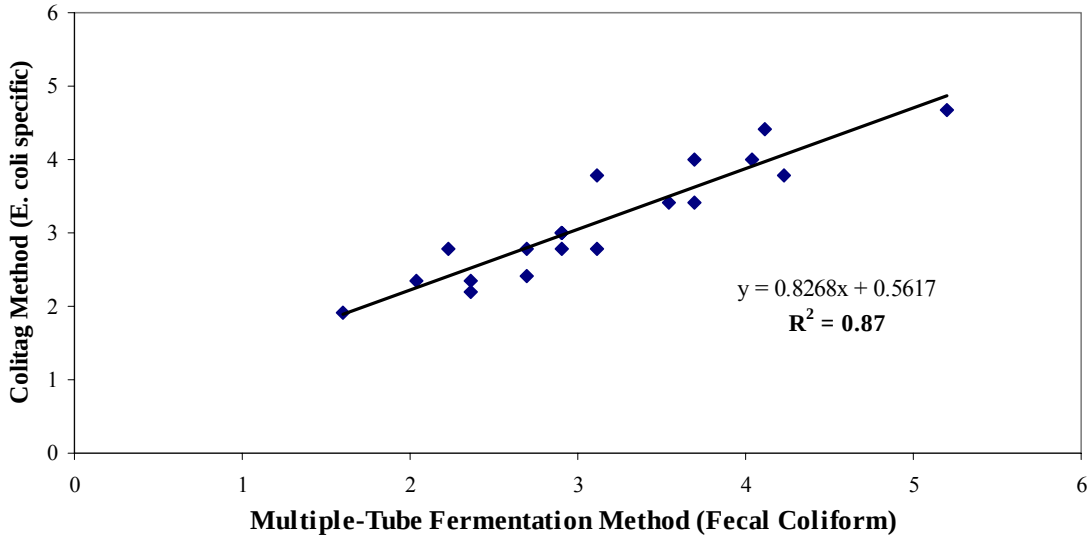

Figure 2 Linear correlation between the Colitag Method (*E. coli* specific) and the Multiple -Tube Fermentation Method (fecal coliform)

Discussion

The analysis of parallel test results showed a strong linear correlation of 0.87 between the Colitag method and the MTF method, which held up well at both high and low fecal coliform counts. This demonstrates that the Colitag method works as well as the MTF method for detecting fecal coliforms. The wide range (from 40 to 160,000) of the most probable numbers (MPNs) of fecal coliforms per 100 ml of water showed that the Colitag technique is capable of detecting fecal coliforms at any level.

One reason that the Colitag method is preferable to the MTF method is that it only takes 24 hours to obtain the results, whereas the MTF method takes up to 96 hours. The fewer hours required to obtain the results will allow agencies to conduct more frequent monitoring of recreational waters while screening for potential public health hazards. Beside the time-efficient factor, the Colitag method has one other advantage over the MTF method, since it only requires one medium, Colitag, whereas the MTF method requires three different media to obtain the final results (Clesceri *et al.* 1998).

A basic principle of water testing, according to the World Health Organization (WHO 1993), is that frequent testing by a simple method is preferable to less frequent testing by a complex method or series of methods. The Colitag technique is less complicated than the MTF technique, since it does not require a series of tests to obtain the final data. Thus, the Colitag technique not only saves time in critical situations but also minimizes experimental errors. Another advantage of the Colitag method is the relatively low cost of running experiments. According to Chang and Maranzana (2000, pers. comm.), the Colitag method is tenfold cheaper than the MTF method. This would be beneficial for water monitoring agencies with low budgets that wish to conduct frequent samplings.

The USEPA (1986) recommends that all water monitoring agencies use *E. coli*, rather then fecal coliforms, as the primary indicator organisms for detecting fecal pollution in recreational water, because the former is specifically associated with fecal matter. *E. coli* is the only member of the coliform group that is an inhabitant of every human intestinal tract. Hence, it has come to be the definitive organism for demonstrating the fecal pollution of water (Edberg *et al.* 1997).

The Colitag technique, which is designed to be *E. coli*–specific, is able to detect many different strains of *E. coli* (Chang 2000, pers. comm.). The MUG and the Indole tests, which are part of the Colitag method for detecting *E. coli*, complement each other by catching different

strains of *E. coli*. On the other hand, the MTF technique only catches some strains with the MUG test, which results in higher false positive counts of fecal coliforms than are found by the Colitag technique. Thus, the Colitag method clearly works better than the MTF method for detecting *E. coli*. Since the Colitag method specifically tests for *E. coli*, while the MTF method tests for fecal coliforms, this accounts for the 0.13 discrepancies in the regression analysis.

The risks to human health from fecal contamination of recreational water can be reduced not only by constant monitoring of the water but also by identifying the source of the contamination. One way to accomplish this is by conducting a dye test. After placing the organic dye fluorescein in sewer system pipelines, one can search for the presence of the dye in nearby creeks, rivers, or lakes. If fluorescein is found, that indicates possible leakage from the sewer system.

But this kind of test is generally hard to conduct because of many uncertainty factors. For example, it may take a long time for the dye to travel through soils into the creeks, rivers, or lakes. Also, accurate sewer maps may be unavailable or nonexistent, making it difficult or impossible to determine the distance between the potential pipe leak and the contaminated site.
Another method suitable for finding the source of fecal contamination is Polymerase Chain Reaction (PCR) testing for *E. coli* strains, as was done, for example, by the city of San Diego (URS Greiner Woodward Clyde 1999). However, it is very hard to conduct such studies successfully at the present time because of the incompleteness of the *E. coli* DNA library. Thus, for example, it is not presently possible to distinguish *E. coli* that comes from, say, coyotes from *E. coli* that comes from other wild animals or even humans. In the near future, when the *E. coli* DNA library is more complete, such distinctions will be possible, enabling public health officials and water monitoring agencies to identify the source of *E. coli* contamination in a relatively brief time.

The Colitag method offers several advantages over the USEPA's standard method for detecting fecal contamination in recreational water. Of primary importance is the public health benefit of shortened analysis and response time should fecal coliforms or *E. coli* be present in water. The elimination of the confirmation steps involved in the MTF method saves potentially 72 hours. This can speed up corrective measures when contamination is found to be present, and it can also speed up the removal of social prohibitions, such as preventing children from playing in the water, when contamination is found to be absent. The Colitag method is also laboratory friendly, since the test is easy to use, and it permits public health officials to test for

contamination more frequently. Because of all these advantages of the Colitag method over the MTF method, UC Berkeley's Office of Environment, Health, and Safety has decided to adopt the former for internally monitoring both sources of recreational water on the university campus, Strawberry and Codornices creeks.

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