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Volunteer monitoring of *E. coli* in streams of the upper Midwestern United States: a comparison of methods

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Abstract Fecal contamination of water is a public health concern for those using the water for drinking or recreation. The EPA recommends using *Escherichia coli* to evaluate recreational freshwaters for fecal contamination. With limited resources available, states have recently focused on training volunteers to expand data collection and resource assessment. Several bacteria testing methods are available for use by the public; however, few studies have comprehensively evaluated their use by volunteers. This study evaluated two *E. coli* monitoring methods used by volunteers: Coliscan Easygel® and 3M™ Petrifilm™, incubated for 24 and 48 hours. The methods were assessed to determine how closely each matched results with EPA-approved laboratory analyses. Analysis of covariance results indicated that when used by volunteers to monitor surface water, 3M™ Petrifilm™ results were more similar to laboratory analyses than Coliscan Easygel®. Both test methods had similar overall accuracy of predicting if a sample exceeded or fell below the 235 cfu/100 mL EPA body contact standard for recreational surface waters. Two-thirds of volunteers preferred 3M™ Petrifilm™.

Keywords *E. coli* bacteria · Citizens · Volunteer monitoring · Citizen science · Methods comparison · Streams

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Introduction

Contamination of water by fecal matter threatens public health and is a concern to those using the water for drinking or recreational activities. Within the Great Lakes states in US Environmental Protection Agency (EPA) Region 5, 28% of

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the 5,673 impaired waters on the EPA's 303d list cite pathogens as one of the pollutants of concern (USEPA 2006a). Trying to detect pathogens in water can be expensive and difficult. Testing for pathogens requires large volumes of water, and the pathogens can often be difficult to isolate and grow in the laboratory. The EPA recommends *Escherichia coli* for the evaluation of recreational freshwaters for fecal contamination (USEPA 1986a, b) since its presence suggests the presence of human pathogens such as harmful bacteria, protozoa, and viruses. In addition, *E. coli* bacteria are more significantly correlated with human gastrointestinal illness than are fecal coliforms (of which *E. coli* are a subset; USEPA 1986b). Due to these factors and in part due to the Beaches Environmental and Coastal Health (BEACH) Act of 2000, many state regulatory agencies have adopted *E. coli* as the primary indicator organism for assessing bacterial contamination (USEPA 2000). *E. coli* bacteria are good indicator organisms of fecal contamination for several reasons. First, they are found predominantly in humans and other warm blooded animals and generally live longer than pathogens. They are also found in greater numbers in the environment. Finally, they are generally less risky to collect (as many strains are not pathogenic) and easier and cheaper to culture than most pathogens.

Water quality monitoring for *E. coli* is important to ensure safe recreational opportunities. While many states have beach monitoring programs (i.e., BEACH Act; USEPA 1999, 2003) to assess fecal contamination at the state or local level, few states have the capacity or budget for bacteria monitoring in streams, even though streams are routinely used for fishing, swimming, kayaking, and canoeing.

Several economical *E. coli* monitoring kits that have been released and tested may be used to provide valuable data in such assessments. A chromogenic substrate method and an enzymatic method have been found to be comparable or significantly correlated with mTEC, the modified *E. coli* analysis approved by EPA and used by many certified state laboratories (Umble et al. 1999; Vail et al. 2003). Similarly, although Noble et al. (2003) noted that differences in analytical endpoints between methods may lead to varied results, the differences among the methods they tested (i.e., membrane filtration, multiple tube fermentation, and chromogenic substrate technology kits manufactured by IDEXX Laboratories, Inc.) were small in comparison to inherent measurement error. They also noted little variability among different laboratories using the same methods.

Even with such *E. coli* monitoring kits available, staff limitations can also be a problem for state agencies or local communities wishing to assess bacteria in surface waters over a broad area or time period. Several states have recently focused on training volunteers to assume a role in their states' monitoring efforts to expand data collection and resource assessment. Volunteer water quality monitoring programs operate throughout the US and many rely on trained volunteers to collect critical baseline data (Ely and Hamingson 1998). O'Leary et al. (2002) demonstrated that citizen volunteers can be trained in a relatively short time frame in the techniques required to collect and process *E. coli* samples. Such *E. coli* monitoring kits provide an opportunity for volunteers to assess the quality of local waterways in a cost-effective manner.

However, while a variety of *E. coli* test kits may be scientifically accurate when used by water resource professionals, few studies have comprehensively evaluated use of *E. coli* monitoring kits by volunteers. This paper reports on the evaluation of two *E. coli* test kits used by volunteers to determine how closely each matched results of EPA-approved laboratory analyses. Volunteer preferences were also considered.

Based on the findings of six methods tested in a scoping study (O'Brien 2006), two methods, Coliscan Easygel® (incubated) and 3M™ Petrifilm™, were recommended for this study. Coliscan Easygel® is EPA-approved in some states (e.g., EPA Region 4 for surface water monitoring by the Alabama Water Watch program) but not in others. 3M™ Petrifilm™, described for use in enumerating *E. coli* in food and dairy products, is not EPA approved for surface water testing but has shown favorable results in other studies

(Vail et al. 2003; Beloti et al. 2003). The study investigated the degree to which these methods compared with EPA-approved laboratory membrane filtration methods (mTec and modified mTec, USEPA 2002, 2006b) when split samples were enumerated by trained volunteers and sent to certified laboratories for analyses. Over 150 volunteers from six states (Indiana, Iowa, Michigan, Minnesota, Ohio, and Wisconsin) were trained during the study. Of those, 79 volunteers collected and analyzed samples and submitted data used in this research.

Methods

Seventy-seven percent of volunteers who participated in this 2005–2006 study had previous water quality monitoring experience but not necessarily in monitoring *E. coli*. They volunteered to participate in the study based on local advertising within each state's Land Grant University Extension System volunteer monitoring community. In addition, each volunteer participated in a 4-hour training workshop that included background on bacteria, potential health risks, monitoring techniques, and hands-on practice preparing and interpreting test plates. Quality assurance, chain-of-custody practices, and consistency were emphasized during the training workshops to ensure high-quality data. Volunteer confidence and preferences were assessed at the end of each monitoring season to evaluate the methods based on the volunteers' experiences.

Each volunteer collected a single grab sample in mid-stream after rinsing a sterile collection bottle three times with stream water and then split the sample. Samples were put on ice and either shipped to a state-certified laboratory, utilizing a professional shipping service, or taken home for analysis. The volunteers followed a chain of custody procedures throughout this process. Only samples that arrived at the laboratory within 24 hours were included in the study. Certified laboratories in five states used the USEPA-approved Modified m-TEC method, Method 1603 (USEPA 2002), while one state laboratory used the m-TEC method 1103.1 (USEPA 2006b).

For each sample, the same volunteer who collected and shipped the sample prepared Coliscan Easygel® and 3M™ Petrifilm™ test plates for incubation according to manufacturers' recommendations (3M 2008a; Micrology Laboratories 2008). Each volunteer then incubated (Hova-Bator (Model 1602N)) samples for 24 and 48 hours and recorded the number of bacteria colonies present at the end of each time period.

The results of the volunteers' test methods and those of certified laboratories were compared using analysis of covariance (ANCOVA). Data were natural log transformed to meet assumptions of ANCOVA. The ANCOVA assessed equivalence of slopes between the regression curves of the two volunteer methods and the laboratory results for both 24 and 48 hour incubation times. Estimates of the slopes and the standard errors of the slopes were determined from the ANCOVA.

Volunteer results were also compared with laboratory results assessing which exceeded and which fell below the EPA body contact standard for *E. coli* in recreational surface waters of 235 colony forming units (cfu)/100 mL. This analysis allowed identification of false positives and missed risk, sometimes referred to by statisticians as missed hits. Laboratory results were assumed to represent the true amount of *E. coli* in the water. For purposes of this study, a result was considered a "false positive" when volunteer test methods indicated *E. coli* levels in the sample exceeded the standard (235 cfu/100 mL), and laboratory results did not. "Missed risk" occurred when results of volunteer test methods indicated that *E. coli* levels in the water were below the standard and laboratory results exceeded the standard. An overall weighted accuracy rate was determined for each of the test methods by determining the fraction of times the kit and laboratory test both exceeded or both fell below the 235 cfu/100 mL standard.

The detection limit for the 3M™ Petrifilm™ is 100 cfu/100 mL since each plate holds only 1 mL. For Coliscan Easygel®, the detection limit varies, depending on the volume of sample used. Volunteers used between 1 and 5 mL; thus, the detection limit for a single plate was between 100 and 20 cfu/100 mL. Since volunteers ran tests in triplicate and averaged the results, detection

Table 1 ANCOVA estimates of slopes and regression results

Volunteer method		Slope						Regression results
Method	Incubation, hours	Parameter estimate	Standard error	Test slope not 0		Test slope not 1		r^2
				t	P value	t	P value	
Coliscan Easygel®	24	0.822	0.027	30.765	<0.001[a]	−6.640	<0.001[a]	0.499
Coliscan Easygel®	48	0.841	0.028	29.776	<0.001[a]	−5.639	<0.001[a]	0.490
3M™ Petrifilm™	24	0.953	0.030	31.908	<0.001[a]	−1.583	0.114	0.536
3M™ Petrifilm™	48	0.942	0.029	32.112	<0.001[a]	−1.973	0.049[a]	0.528

All t tests have 1,828 degrees of freedom

[a]Indicates significance at 0.05

limits were lower. For example, Petrifilm had a detection limit of 100 cfu/100 mL since only 1 mL of sample could be used. If one cfu was found on one plate and none on the other two, then the average of the three replicates was reported as 33 cfu/100 mL. Half detection limits were used in the statistical analyses for any data that were reported as "less than detection limit" as in Robertson et al. (2008). Half detection limit data, however, were censored from ANCOVA analyses to meet assumptions of ANCOVA.

Results

ANCOVA results indicated that 3M™ Petrifilm™ measurements had better agreement with laboratory results than Coliscan Easygel® measurements (Table 1). The slope of 3M Petrifilm results at 24 hours of incubation as compared to laboratory results was closest to 1, and results were not found to be significantly different from the laboratory results ((t1828 df) = −1.583, $P = 0.114$). Additionally, results from Coliscan Easygel® and 3M™ Petrifilm™ were statistically different from one another (Table 2).

Although there was a significant difference between the Coliscan Easygel® results and the laboratory results, there was some relationship between the two, since the slope did not equal 0 (Table 1). This enables the use of a correction factor (i.e., the parameter estimate) for Coliscan Easygel® results to equate them to laboratory results.

When assessing differences between 24- and 48-hour incubation times, ANCOVA showed no significant difference between the slopes or intercepts in the lines relating the volunteer measurements to the laboratory measurements for either method (Table 2). There was a difference in rate of false positives and missed risk between incubation times for each method (Table 3).

Overall accuracy rates were similar for the two volunteer methods when volunteer and laboratory

Table 2 ANCOVA estimates between kits

Volunteer methods				Statistical tests			
First method		Second method		Equal slopes		Equal intercepts	
Method	Incubation (h)	Method	Incubation (h)	t	P value	t	P value
Coliscan Easygel®	24	3M™ Petrifilm™	24	3.250	0.001[a]	4.612	<0.001[a]
Coliscan Easygel®	24	Coliscan Easygel®	48	−0.465	0.642	0.298	0.172
3M™ Petrifilm™	24	3M™ Petrifilm™	48	0.011	0.798	0.023	0.981
Coliscan Easygel®	48	3M™ Petrifilm™	48	−2.474	0.013[a]	3.166	0.002[a]

All t tests have 1,828 degrees of freedom

[a]Indicates significance at 0.05

Table 3 Comparison of false positives and missed risk above and below the EPA body contact standard for recreational surface water (235 cfu/100 ml) for 3M™ Petrifilm™ and Coliscan Easygel® ($n = 1, 171$)

Method and incubation time	Rate of false positives, %	Rate of missed risk, %	Overall accuracy rate, %
3M™ Petrifilm™, 24 h	14.92	19.62	83.18
3M™ Petrifilm™, 48 h	18.08	17.72	82.07
Coliscan Easygel®, 24 h	9.18	34.39	80.61
Coliscan Easygel®, 48 h	16.07	23.21	81.04

results were compared for agreement when they exceeded or fell below the 235 cfu/100 mL EPA standard (Table 3). Results from 3M™ Petrifilm™ correctly indicated the water condition 83.18% of the time at 24 hours of incubation and 82.07% of the time at 48 hours of incubation. Coliscan Easygel® correctly identified the water condition 80.61% of the time at 24 hours of incubation and 81.04% at 48 hours of incubation. However, the rate of false positives was considerably lower than the rate of missed risk for Coliscan Easygel®, especially at 24 hours of incubation. While the overall accuracy rate was reasonable, the rate of missed risk was over three times the rate of false positives. These error rates were more balanced for 3M™ Petrifilm™ (Table 3).

Missed risks and false positives were visually assessed when ANCOVA results were plotted alongside a 1:1 reference line. A solid cross-hair line was added to the graphs to indicate where the standard of 235 cfu/100 mL would occur (Figs. 1 and 2). The dotted line representing the relationship between Coliscan Easygel® results and laboratory results intersects the bolded reference line at 543 cfu/100 mL plotted on Fig. 1 as ln 6.3. It lies above the reference line for lower bacterial levels and below the reference line for higher bacterial levels. This demonstrates that unadjusted Coliscan Easygel® measurements after 24 hours of incubation underestimate bacterial levels, resulting in a high level of missed risk. The 3M™ Petrifilm™ line also crosses the reference line (Fig. 2), but it does so at a lower *E. coli* level than the 235 cfu/100 mL EPA body contact standard, presenting less of a problem in terms of potential risk.

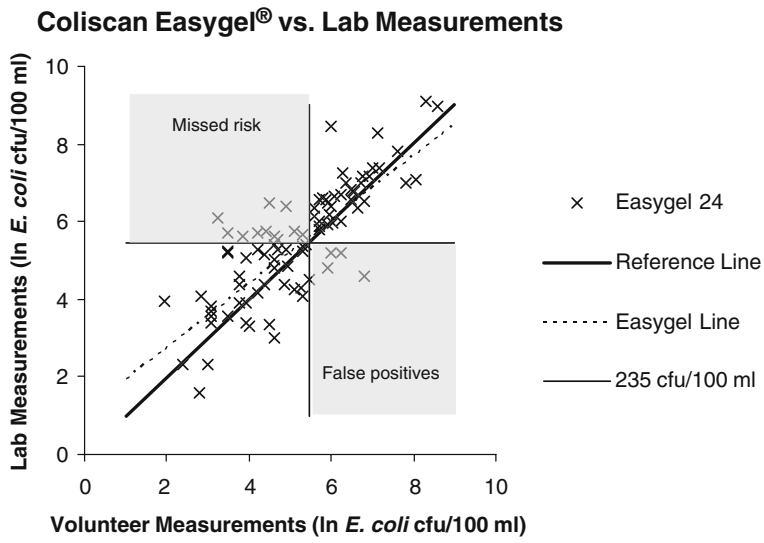

Fig. 1 Laboratory versus volunteer measurements for Coliscan Easygel® ($n = 1, 171$). A 1:1 reference line (*bold*), EPA's 235 cfu/100 mL standard (i.e., *the solid cross-hair line forming the edges of the missed risks and false positives areas*), and areas of false positives and missed risk are indicated. To avoid overwhelming the graph with the full data set, 10% of the data selected at random are displayed

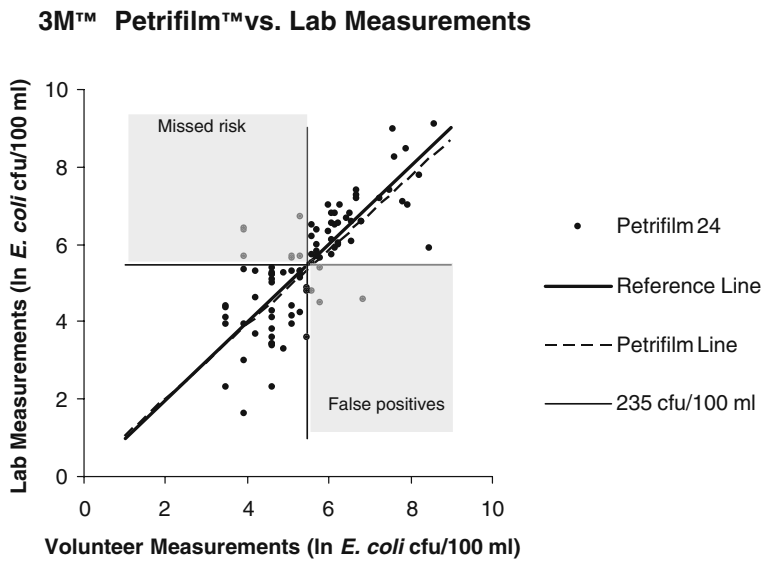

Fig. 2 Laboratory versus volunteer measurements for 3M™ Petrifilm™ ($n = 1,171$). A 1:1 reference line (*bold*), EPA's 235 cfu/100 mL standard (i.e., *the solid cross-hair line forming the edges of the missed risk and false positives areas*), and areas of false positives and missed risk are indicated. To avoid overwhelming the graph with the full data set, 10% of the data selected at random are displayed

Discussion

3M™ Petrifilm™ provided more similar results to laboratory analyses of *E. coli* bacteria in surface waters than Coliscan Easygel® in a direct comparison between laboratory and kit results. When comparing results that exceeded and fell below the 235 cfu/100 mL EPA body contact standard for recreation, both kits had nearly the same overall accuracy (81%–83%) and did a reliable job of assessing when water was safe for recreation. However, 3M™ Petrifilm™ had a reasonably low and more equal rate of false positives and missed risk. At the close of the study, two-thirds of the volunteers indicated they preferred the 3M™ Petrifilm™ method. It is important to note that 3M™ Petrifilm™ is designed for use of *E. coli* analysis in food; thus, the use of this method may be more appropriate for screening purposes. On the other hand, Coliscan Easygel® has an advantage in that it is approved for use in analysis of surface waters in Alabama, so a model exists for other states to accept the data obtained using this method (Deutsch and Busby 1999).

When plates were read by volunteers, Coliscan Easygel® consistently underestimated the true bacteria count measured by the laboratory. Thus, the parameter estimates obtained in ANCOVA were used to adjust the Coliscan Easygel® measurements. A second Coliscan Easygel® dataset ($n = 184$) consisting of volunteer-generated data from Minnesota from the years 2007–2008 was adjusted by applying the ANCOVA results from this study. The resulting adjusted Coliscan Easygel® scores were then compared to laboratory results using simple linear regression. The line representing the relationship between Coliscan Easygel® and the laboratory results showed that the two were not significantly different from one another ($m = 1.092$, $t(43) = 0.9293$, P value $= 0.358$).

In regard to using either method for screening purposes to assess if bacteria counts in waters exceed or fall below the 235 cfu/100 mL EPA standard, users should consider costs to a community related to missed risks and false positives. If waters are deemed safe for recreation when indeed they are not, individuals could be put at risk (i.e., missed risk). On the other hand, if a test falsely indicates that bacteria levels exceed the 235 cfu/100 mL standard (i.e., false positive), there is potential cost in lost recreation and associated revenue. An ideal statistical model would eliminate both false positives and missed risks, but this is highly unlikely in a natural system which is expected to be highly variable (Lear et al. 2008; Clark and Gamper 2003; Costerton et al. 1978).

In this study, after 24 hours of incubation, the Coliscan Easygel® method misidentified when water exceeded the EPA body contact standard 34% of the time. 3M™ Petrifilm™ misidentified

this nearly 20% of the time. At 48 hours of incubation, missed risks were reduced for both methods, though minimally for 3M™ Petrifilm™. Thus, there may be a benefit to incubate samples for 48 hours to minimize risk to community members, although the added delay in obtaining results would delay decision making. Alternatively, at 24 hours of incubation, Coliscan Easygel® resulted in false positives 9% of the time, while the 3M™ Petrifilm™ resulted in false positives 14% of the time. After 48 hours of incubation, both methods resulted in higher rates of false positives (16% and 18%, respectively). It is important to note that both methods when used by volunteers had overall accuracy rates above 80%, which should give local communities confidence in results of such monitoring. Utilizing a 24 hour incubation period would allow communities to make decisions in a more timely fashion and avoid loss of tourism revenue or diminished water-related recreational opportunities. Local community groups using either method should both be aware of these nuances and educate their constituents regarding results to minimize fear or lost revenue.

Additionally, local community groups wishing to conduct *E. coli* bacteria screening in surface waters must assess available funding, volunteer time, and desired data uses prior to determining the method they will use to assess bacterial contamination in surface waters. A benefit of both methods assessed in this study is that each is far less costly per test than laboratory analyses (i.e., approximately $3.00 versus costs from $15.00 to $35.00 per test plus overnight shipping costs for laboratory analyses). Thus, 3M™ Petrifilm™ or Coliscan Easygel® could be used for regular screening to develop an ongoing record of *E. coli* bacteria levels in surface waters or to characterize watershed health. Use of these methods could be supplemented by laboratory analyses when problems are found. 3M™ Petrifilm™ or Coliscan Easygel® could also be used by citizens to obtain general information about bacteria levels during runoff events when multiple laboratory analyses would be too expensive. Furthermore, the methods could be used to locate "hot spots" of pollution to help target more in-depth investigation. In this way, citizen efforts can effectively help to extend agency resources for monitoring bacteria.

In a study using professionals to read plates, Vail et al. (2003) found a much stronger relationship in regression analysis between 3M™ Petrifilm™ and three standard methods for monitoring *E. coli* bacteria than was found in this study. A possible reason for the weaker relationships found in this study may be related to the variability in streams, water chemistry, and *E. coli* across the Great Lakes region. Exploring that type of variability was beyond the scope of this study. Many streams monitored in this study had very low bacteria counts. Manufacturers of both Coliscan Easygel® and 3M™ Petrifilm™ indicate that their methods are most reliable at elevated *E. coli* counts (Micrology Labs 2008; 3M 2008a). This may further help explain the weaker relationships between test kit results with laboratory results.

Another possible reason for the weaker relationships between volunteer and laboratory methods may be variability in experience level between laboratory professionals and volunteers in preparing samples and interpreting bacteria colonies. In the study by Vail et al. (2003), consistent personnel prepared and interpreted plates for all types of methods. In this study, only 18 of 79 volunteers had prior experience monitoring *E. coli* bacteria. Four individuals had monitored *E. coli* bacteria professionally. Thus, 77% of the volunteers were newly trained to conduct this type of monitoring, and errors may have resulted due to their inexperience with the methods. Indeed, while some volunteer-generated data have been found to correlate well with professionally collected data (Obrecht et al. 1998; Canfield et al. 2002; Noble et al. 2003; Cohn 2008), others have found that certain types of data collection, such as macroinvertebrate identification, may present challenges for volunteers (Nerbonne and Vondracek 2003), and this may indeed be the case with bacteria monitoring as well. Furthermore, quality assurance and quality control issues often elicit concern about the quality of the data when collected by volunteers (Engel and Voshell 2002). The process volunteers followed to collect water samples, prepare plates, and count bacteria colonies may well have introduced a variety of errors.

One other reason for differences between volunteer and laboratory results could be the delay due to shipping. Volunteers began their analyses soon after the sample was collected while the laboratory began their incubation up to 24 hours later. If 3M™ Petrifilm™ and Coliscan Easygel® methods are similar to other methods that have been studied in this regard, the time difference in sample preparation should not have made a significant difference (Selvakumar et al. 2004; Pope et al. 2003). Vail et al. (2003) minimized such variation by preparing all samples for analysis within four hours following collection. Future studies could investigate if differences in holding time significantly affect bacteria colony counts using 3M™ Petrifilm™ and Coliscan Easygel®.

Conclusions

Statistically, 3M™ Petrifilm™ results were more similar to laboratory analyses of *E. coli* bacteria in surface waters than Coliscan Easygel®. However, the use of a correction factor more closely aligned the results of Coliscan Easygel® with laboratory results in the direct comparisons. Coliscan Easygel® performed nearly as well as the 3M™ Petrifilm™ in the analyses that were conducted comparing results that exceeded or fell below 235 cfu/100 mL. The kits' overall weighted accuracy rates, which exceed 80% for each method, suggest that these methods can be effectively used as screening tools to extend agency resources or to help characterize overall health of watersheds. Additional studies could consider whether differences in holding time significantly affect bacteria colony counts or the reliability of test kits for monitoring streams with very low bacteria.

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References

3M (2008a). 3M Petrifilm™ Coliform Count plate interpretation guide. http://multimedia.3m.com/mws/mediawebserver?66666UuZjcFSLXTt4XMa4XM_EVuQEcuZgVs6EVs6E666666–. Accessed 16 December 2008.

Beloti, V., de Souza, J. A., de Aguiar Ferreira Barros, M., Nero, L. A., de Mattos, M. R., Gusmão, V. V., et al. (2003). Evaluation of Petrifilm™ EC and HS for total coliforms and *Escherichia coli* enumeration in water. *Brazilian Journal of Microbiology, 34*(4), 301–304.

Canfield, D. E., Jr., Brown, C. D., Bachmann, R. W., & Hoyer, M. V. (2002). Volunteer lake monitoring: testing the reliability of data collected by the Florida LAKEWATCH program. *Lake and Reservoir Management, 18*(1), 1–9.

Clark, M. L., & Gamper, M. E. (2003). *A synoptic study of fecal-indicator bacteria in the Wind River, Bighorn River, and Goose Creek Basins, Wyoming, June to July 2000*. Water resources investigations report 03-4055. US Department of the Interior, US Geological Survey.

Cohn, J. P. (2008). Citizen science: Can volunteers do real research? *BioScience, 58*(3), 192–197.

Costerton, J. W., Geesey, G. G., & Cheng, K. J. (1978). How bacteria stick? *Scientific American, 238*(1), 86–95.

Deutsch, W. G., & Busby, A. L. (1999). *Alabama water watch quality assurance plan for bacteriological monitoring*. Alabama Water Watch, Auburn University, AL (63 pp). https://aww.auburn.edu/Docs/manuals/QABacManual99.pdf. Accessed February 25, 2010.

Ely, E., & Hamingson, E. (1998). *National directory of volunteer environmental monitoring programs* (5th Edn.). Washington, DC: United States Environmental Protection Agency.

Engel, S. R., & Voshell Jr., J. R. (2002). Volunteer biological monitoring: Can it accurately assess the ecological condition of streams? *American Entomologist, 48*(3), 164–177.

Lear, G., Anderson, M. J., Smith, J. P., Boxen, K., & Lewis, G. D. (2008). Spatial and temporal heterogeneity of the bacterial communities in stream epilithic biofilms. *FEMS Microbiology Ecology, 65*(3), 463–473.

Micrology Laboratories (2008). Detection of waterborne coliforms and *E. coli* with Coliscan Easygel. http://www.micrologylabs.com/Home/Our_Methods/Coliscan_Easygel/Coliscan_Easygel_Instructions. Accessed 16 December 2008.

Nerbonne, J. F., & Vondracek, B. C. (2003). Volunteer macroinvertebrate monitoring: Assessing training needs through examining error and bias in untrained volunteers. *Journal of the North American Benthological Society, 22*(1), 152–163.

Noble, R. T., Weisberg, S. B., Leecaster, M. K., McGee, C. D., Ritter, K., Walker, K. O., et al. (2003). Comparison of beach bacterial water quality indicator measurement methods. *Environmental Monitoring and Assessment, 81*, 301–312.

O'Brien, E. (2006). Volunteers conduct bacteria methods study. *The Volunteer Monitor, 18*(1), 3–6.

O'Leary, N., Wagenet, L., Pfeffer, M., & Vawter, T. (2002). *Linking science and the public: Monitoring water quality of streams*. American Water Resources Association Annual Conference. Philadelphia, PA. November 4–7, 2002.

Obrecht, D. V., Milanick, M., Perkins, B. D., Ready, D., & Jones, J. R. (1998). Evaluation of data generated from lake samples collected by volunteers. *Lake and Reservoir Management, 14*(1), 21–27.

Pope, M. L., Bussen, M., Feige, M. A., Shadix, L., Gonder, S., Rodgers, C., et al. (2003). Assessment of the effects of holding time and temperature on *Escherichia coli* densities in surface water samples. *Applied and Environmental Microbiology, 69*(10), 6201–6207.

Robertson, D. M., Weigel, B. M., & Graczyk, D. J. (2008). Nutrient concentrations and their relations to the biotic integrity of nonwadeable rivers in Wisconsin: US Geological Survey Professional Paper 1754, p. 81.

Selvakumar, A., Borst, M., Boner, M., & Mallon, P. (2004). Effects of sample holding time on concentrations of microorganisms in water samples. *Water Environment Research, 76*(1), 67–72.

Umble, A. K., Kolo, L. E., Newvine, L. A., Roth, G. N. & Roth, J. N. (1999). Coliform counts. *Water Environment & Technology, 11*(4), 57–59.

USEPA (1986a). *Quality criteria for water—1986*. EPA-440/5-86-001, Washington, DC.

USEPA (1986b). *Ambient water quality criteria for bacteria—1986*. US Environmental Protection Agency. EPA-440/5-84-002.

USEPA (1999). *Action plan for beaches and recreational waters: reducing exposures to waterborne pathogens*. EPA/600/R-98/079. Washington, DC: Office of Water, US Environmental Protection Agency.

USEPA (2000). Beaches Environmental Assessment and Coastal Health Act of 2000. Public law 106–284. http://www.epa.gov/waterscience/beaches/files/beachbill.pdf. Accessed March 29, 2010.

USEPA (2002). Method 1603: *Escherichia coli (E. coli) in water by membrane filtration using modified membrane-thermotolerant Escherichia coli agar (modified mTEC)*. EPA 821-R-02-023. Washington, DC: Office of Water, US Environmental Protection Agency.

USEPA (2003). Bacterial water quality standards for recreational waters (freshwater and marine waters) status report. http://www.epa.gov/waterscience/beaches/local/statrept.pdf. Accessed 1 September 2009.

USEPA (2006a). Overview of current total maximum daily load—TMDL—program and regulations. http://www.epa.gov/owow/tmdl/. Accessed 30 June 2008.

USEPA. (2006b). *Method 1103.1:* Escherichia coli *(*E. coli*) in water by membrane filtration using membrane-thermotolerant* Escherichia coli *Agar (mTEC)*. EPA-821-R-06-010. Washington, DC: Office of Water, US Environmental Protection Agency.

Vail, J. H., Morgan, R., Merino, C. R., Gonzales, F., Miller, R., & Ram, J. L. (2003). Enumeration of waterborne *Escherichia coli* with Petrifilm plates: comparison to standard methods. *Journal of Environmental Quality, 32*, 368–373.