APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2008, p. 3319–3320 Vol. 74, No. 10
0099-2240/08/$08.00+0 doi:10.1128/AEM.02623-07

Comparison of Transcription-Mediated Amplification and Growth-Based Methods for the Quantitation of *Enterococcus* Bacteria in Environmental Waters[▽]

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Received 20 November 2007/Accepted 23 March 2008

An assay based on transcription-mediated amplification (TMA) technology was used to quantitate *Enterococcus* fecal indicator bacteria in environmental water samples. The results generated by this and two growth-based methods relative to the 104 most-probable-number or CFU-per-100-ml threshold show that the three methods are in good qualitative agreement when tested against a range of water samples taken from different locations. The results demonstrate sensitive and rapid detection (approximately 4 h from sample collection to result) and quantitation of *Enterococcus* bacteria compared to the results with the growth-based methods.

Enterococcus is the recommended fecal indicator bacterium for monitoring the presence of fecal contamination in recreational waters (5). Standards for the maximum acceptable level of *Enterococcus* bacteria in recreational waters have been established by the U.S. EPA (U.S. Environmental Protection Agency). The most common single-sample standard used for managing recreational waters is 104 most probable number (MPN) or CFU per 100 ml. Presently, most regulatory agencies worldwide use traditional growth-based tests, such as membrane filtration and multiple-tube fermentation, for the detection and quantitation of fecal indicator bacteria in water samples. These tests typically require 18 to 24 h to yield results.

This report describes a rapid test for the quantitation of *Enterococcus* rRNA that yields a result within 4 h. The method involves filtration of the water sample, lysis of the target organism, and purification of rRNA using magnetic-particle target capture, followed by amplification of the purified rRNA using transcription-mediated amplification (TMA) technology. Homogeneous, real-time detection of amplicons is achieved by using a fluorescent oligonucleotide probe.

TMA technology is both isothermal and rapid. This assay allows highly sensitive and specific quantitation using a standard curve to derive the equivalent of CFU-per-100-ml values. The assay does not detect nonenterococcal organisms that are known to cause false-positive results in some of the U.S. EPA-approved growth-based methods (2) or *Enterococcus* species that are underrepresented in human feces (3).

The optimization and development of the TMA method for the detection of *Enterococcus* deliberately spiked into seawater have been described previously (3), and the present study expands on this research to apply the method to a wide range of ambient environmental water samples. The goal of this study was to compare the results obtained with the TMA assay to results obtained with two of the most commonly used reference methods for quantitation of *Enterococcus* bacteria in water samples, Enterolert (Idexx Laboratories, Inc., Westbrook, ME) and U.S. EPA method 1600 (6), in estuarine, brackish, and storm water samples.

Different types of water samples were collected to obtain samples with various *Enterococcus* concentrations, environmental parameters, ionic characteristics, and high-molecular-weight organic matter levels. In total, 77 water samples were collected from a variety of sites in North Carolina, the United States, during different seasons. Water samples were collected from several geographic locations and depths on the Neuse River Estuary, along with water samples from nearby creeks and storm drains ($n = 55$). Storm water samples ($n = 22$) were included to have samples with expected higher *Enterococcus* concentrations.

Samples were collected and filtered as prescribed by U.S. EPA method 1600 (6). For the TMA assay, each water sample (100 ml) was filtered and extracted in triplicate and each extract was subsequently amplified in triplicate, yielding nine results per original water sample (3). The Enterolert assay (results reported in MPN per 100 ml) and U.S. EPA method 1600 (reported in CFU per 100 ml) were performed according to the manufacturer's instructions (4) and according to U.S. EPA guidelines (6), respectively. The sensitivities and specificities of the TMA assay and the Enterolert assay were estimated by comparing the test results to the U.S. EPA method 1600 results. In this study, the U.S. EPA-recommended threshold of 104 MPN or CFU per 100 ml of water (1) for a single sample was used to define a sample as positive or negative, though the method is applicable to any threshold determined by a given authority.

A series of solutions of purified *Enterococcus faecalis* rRNA (1 to 1,000,000 fg) were assayed in triplicate with the TMA assay with every experiment to create a standard curve. Inter-

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▽ Published ahead of print on 31 March 2008.

TABLE 1. Interassay regression parameters and precision data for 12 TMA assay standard curves[a]

Standard curve (fg)	Mean emergence time above threshold	Standard deviation	CV (%)
0	65.9	6.9	10.4
1	62.1	8.3	13.4
10	53.3	6.8	12.7
100	45.8	6.0	13.1
1,000	40.5	4.6	11.5
10,000	34.7	3.4	9.7
100,000	28.4	3.4	12.1
1,000,000	23.4	3.6	15.3

[a] Each rRNA concentration was assessed in triplicate with every experiment, and the mean of the results of 12 experiments was calculated. CV, coefficient of variation. r^2, 0.98; x, −0.141; intercept, 9.89.

assay regression parameters and precision data for 12 curves are shown in Table 1. The standard curve for each experiment was used to estimate the amount of rRNA per amplification reaction. In the first instance, environmental samples were collected from Neuse creek ($n = 8$) and measured using TMA, Enterolert, and U.S. EPA method 1600. Where Enterolert and the U.S. EPA method gave the same qualitative results (positive or negative against the 104 CFU threshold), the data were used to calculate a conversion factor. One CFU per 100 ml of *Enterococcus* bacteria corresponded to 5 fg of rRNA per reaction, which was in reasonable agreement with the conversion factor previously generated from laboratory-generated samples (1 CFU per 100 ml corresponded to 3 fg rRNA per reaction) (3). This factor was subsequently used to convert femtograms to CFU per 100 ml. TMA and U.S. EPA method 1600 results of <10 CFU were assigned a value of 10 to be consistent with the stated lower limit of quantitation of the Enterolert assay (when using the manufacturer-recommended 1:10 dilution).

In the 55 water samples from estuarine and brackish waters, the *Enterococcus* concentrations ranged from 10 to 2,867 CFU per 100 ml with a mean value of 92 CFU per 100 ml (as determined by U.S. EPA method 1600). In the 22 storm water samples, the *Enterococcus* concentrations ranged from 10 to 57,000 CFU per 100 ml with a mean value of 12,659 CFU per 100 ml.

The U.S. EPA method 1600, TMA assay, and Enterolert assay qualitative results are summarized in Table 2. The sensitivity and specificity of the TMA assay were 91.7% (22/24) and 96.2% (51/53), respectively, giving an overall accuracy of 94.8% (73/77) (relative to the results of U.S. EPA method 1600). Enterolert demonstrated a sensitivity of 83.3% (20/24) and a specificity of 96.2% (51/53), giving an overall accuracy of 92.2% (71/77).

The data were tested for normality using the D'Agostino and Pearson omnibus test and found to be nonparametric. Direct quantitative comparison of the TMA assay results to the Enterolert assay and U.S. EPA method 1600 data yielded Spearman's rank correlation coefficient (r) values of 0.82 and 0.88, respectively. The comparison of the Enterolert assay and U.S. EPA method 1600 data gave an r value of 0.92 (Prism version 4.03).

TABLE 2. Summary of results[a]

Assay	Total no. of samples	No. of samples with result			
		Positive	Negative	False negative (%)	False positive (%)
U.S. EPA method 1600	77	24	53	NA	NA
TMA	77	24	53	2 (2.6)	2 (2.6)
Enterolert	77	22	55	4 (5.2)	2 (2.6)

[a] The U.S. EPA-recommended threshold of 104 MPN or CFU per 100 ml of water for a single sample (1) was used to define a sample as positive (≥104 CFU) or negative (<104 CFU). NA, not applicable.

Conclusion. Comparison of the results generated by the three methods relative to the 104 MPN or CFU-per-100-ml threshold shows that the three methods are in good qualitative agreement when tested against a range of environmental water sample types varying in salinity, depth, organic-matter constituents, and total suspended solids.

The data generated in this study were compared to the U.S. EPA method 1600 as the current gold-standard method; however, this method is reported to have between 6 and 26% false-positive results (2, 6), with *Streptococceae* species, such as *S. bovis*, and *Aerococcus* species appearing identical to *Enterococcus* on membrane-*Enterococcus* indoxyl-D-glucoside agar (6). The TMA assay has been reported previously to have a good analytical specificity and does not cross-react with the species that are known to cause false positives in the U.S. EPA method. The main advantages of the TMA assay are the rapid time to result (approximately 4 h) and the high level of analytical specificity (3). The data presented here show that TMA-based methods hold promise for use in the future to accurately quantify fecal indicator bacteria in recreational waters.

This research was funded by a grant from Molecular Light Technology Research Limited, Cardiff, Wales, United Kingdom. MLT Research Ltd. is a subsidiary of Gen-Probe Incorporated.

All probes and primers were generously donated by Gen-Probe Incorporated, San Diego, CA.

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