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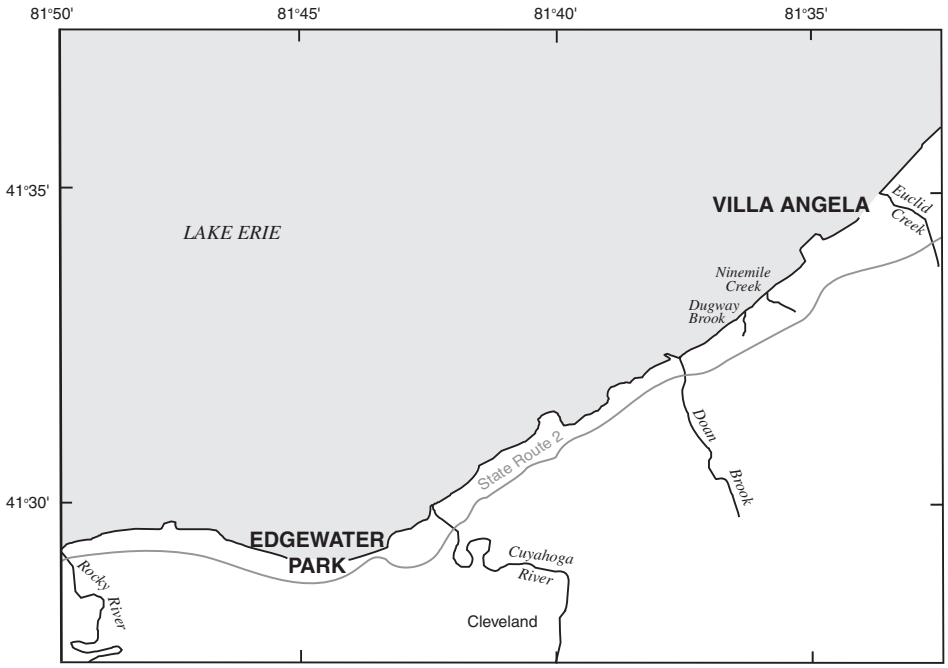
Evaluation of Real-Time Quantitative Polymerase Chain Reaction (qPCR) to Determine *Escherichia coli* Concentrations at Two Lake Erie Beaches



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Conversion Factors

Multiply	By	To obtain
	Length	
meter (m)	3.281	foot (ft)
	Volume	
milliliter (mL)	0.03381	ounce, fluid (fl. oz)
microliter (µL)	0.00003381	ounce, fluid (fl. oz)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$°F=(1.8\times°C)+32$

Concentrations of reagents are given in millimolar (mM) or micromolar (µM).

Concentrations of bacteria are given in colony-forming units per 100 milliliters (CFU/100mL).

Evaluation of Real-Time Quantitative Polymerase Chain Reaction (qPCR) To Determine *Escherichia coli* Concentrations at Two Lake Erie Beaches

By Christopher M. Kephart and Rebecca N. Bushon

Abstract

During the recreational seasons of 2006 and 2007, the quantitative polymerase chain reaction (qPCR) method was used to determine *Escherichia coli* (*E. coli)* concentrations in samples from two Lake Erie beaches. Results from the qPCR method were compared to those obtained by traditional culturing on modified mTEC agar. Regression analysis showed strong, statistically significant correlations between results from the two methods for both years. Correlation coefficients at Edgewater and Villa Angela Beaches were 0.626 and 0.789 for 2006 and 0.667 and 0.829 for 2007, respectively. Linear regression analyses were done to determine how well *E. coli* concentrations could have been predicted from qPCR results. These hypothetical predictions were compared to the current practice of determining recreational water quality from *E. coli* concentrations determined for samples collected on the previous day. The qPCR method resulted in a greater percentage of correct predictions of water-quality exceedances than the current method for both beaches and both years. However, because regression equations differed somewhat between both sites and both years, the study did not result in any single relation reliable enough to use for actual real-time prediction of water-quality exceedances for either beach; therefore, a posterior analysis of data was done. Additional years of data may be needed to develop such a relation. Results from this study support the continued development and testing of a qPCR method for providing rapid and accurate estimates of *E. coli* concentrations for monitoring recreational water quality.

Introduction

Recreational water-quality impairment due to fecal pollution can adversely impact the health of the public. Levels of impairment from fecal pollution are assessed on the basis of concentrations of bacterial indicator organisms. Traditional culture-based methods to determine fecal-indicator bacteria concentrations typically require 18–24 hours to obtain results. Studies have demonstrated that fecal-indicator bacteria concentrations can fluctuate rapidly (Leecaster and Weisberg, 2001; Boehm and others, 2002; Boehm, 2007). The delay in obtaining results when using the culture-based method combined with the temporal variability of bacterial concentrations can result in incorrect assessments of the current quality of public waters. At beaches, posting of water-quality hazard advisories when the exposure risk is low can result in loss of revenue and recreational use (Rabinovici and others, 2004). In contrast, not posting advisories when the risk is high can result in the public coming into contact with water that contains unsafe levels of pathogenic microorganisms. One study has estimated that, for one frequently used coastal beach, as many as 40 percent of the advisories based on traditional methods for water-quality assessment were erroneous (Kim and Grant, 2004).

One way to improve the accuracy and efficiency of recreational water-quality monitoring programs is the use of rapid methods to reduce the time required to determine bacteria concentrations. Recent advancements in molecular and immunological techniques have led to development of various methods capable of yielding results in less than 4 hours from the time of sample collection, a short enough time to permit managers to make public health decisions based on current-day results (Griffith and Weisberg, 2006). Many of these rapid methods are based on enumeration of cellular components, such as genetic material or cell-surface structures. These methods typically are very sensitive so they can be vulnerable to interference from complex water-sample matrices. Consequently, substantial testing is required to determine whether they are capable of replacing traditional culture-based methods. Recent studies evaluating rapid-detection methods have found several to be promising, yet there is a need for further evaluation and epidemiological studies to establish direct relations of method results to public health risk (Griffith and others, 2004; Noble and Weisberg, 2005).

One rapid method uses real-time quantitative polymerase chain reaction (qPCR) to quantify fecal-indicator bacteria. This method, which detects and enumerates unique genetic sequences within target bacteria, can be done in less than 3 hours. The enumeration relies on the accumulation of a fluorescent signal that is produced during PCR cycling.

A fluorescent dye is released during replication of the target DNA sequence; accumulation of the dye is proportional to the amount of target DNA present. Quantification of bacteria in a sample is done by means of a standard curve. The standard curve is developed from qPCR results for a series of samples with known concentrations of the target organism.

The qPCR method for quantifying fecal-indicator bacteria has been tested for enterococci, a U.S. Environmental Protection Agency (USEPA)-recommended indicator for marine water and freshwater (Haugland and others, 2005; Frahm and Obst, 2003; He and Jiang, 2005) and to a lesser extent, *Escherichia coli (E. coli)*, a USEPA-recommended indicator for freshwater (Foulds and others, 2002; Frahm and Obst, 2003). Additionally, an epidemiological study was done relating results from the qPCR rapid method for enterococci to swimming-associated health effects (Wade and others, 2006). Although the qPCR method for *E. coli* quantification has been evaluated in recent studies, there is a need for better understanding of method performance in environmental waters, especially samples collected at beaches.

The U.S. Geological Survey (USGS), in cooperation with the Northeast Ohio Regional Sewer District (NEORSD), evaluated a qPCR method for determining *E. coli* concentrations at two Lake Erie beaches, Edgewater and Villa Angela, in 2006 and 2007 (fig. 1). Both beaches are sand beaches; Villa Angela Beach is 900 feet in length and Edgewater Beach is 1,500 feet in length. In 2006, a total of 617,000 people visited both beaches combined and in 2007, a total of 616,649 people visited both beaches combined. According to an estimate by Cleveland Lakefront State Park (managers of Edgewater and Villa Angela Beaches), at least 90% of the visitors during both years were to Edgewater Beach (oral communication, March 2, 2009).

This report describes the performance of the qPCR method as compared to traditional culturing on a modified mTEC medium. Performance of the qPCR method was also evaluated for accuracy in predicting exceedances of water-quality standards. An effective rapid method for determining *E. coli* concentrations would enable local water-resource managers to make more informed decisions and could enhance public safety during recreational water use.

Methods of Study

Sample Collection

During the recreational seasons (May–September) of 2006 and 2007, the NEORSD conducted a routine monitoring program at Edgewater and Villa Angela Beaches. That program included collection of two water-quality samples at each beach, 5 days per week. The samples were individually analyzed for *E. coli* concentrations by use of the traditional

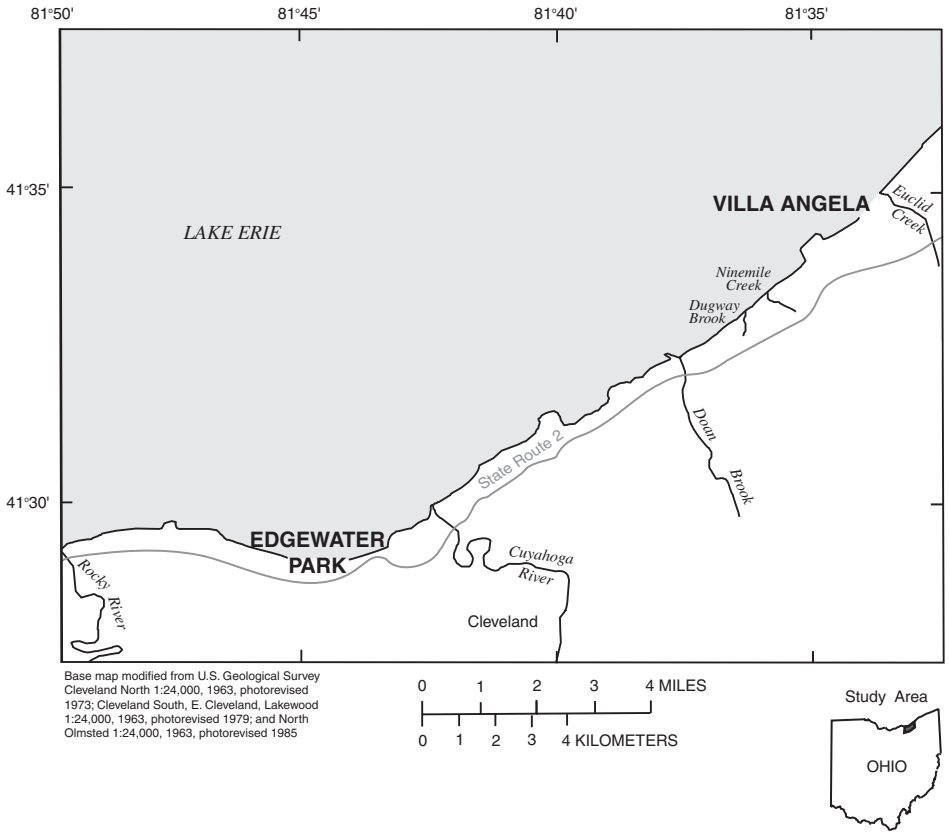

Figure 1. Location of Edgewater and Villa Angela Beaches, Cleveland, Ohio.

culture-based method. Additionally, the two samples from each beach were composited (Bertke, 2007) 3 days per week for 12 consecutive weeks, during both years. The composite samples were analyzed for *E. coli* concentrations by both the traditional and qPCR methods. The NEORSD collected all samples between 9:30 and 11:30 a.m where the water was 1 m deep in an area of the beach used for swimming. All samples were collected into presterilized bottles approximately 0.3 m below the water surface by means of a grab-sampling technique (Myers and others, 2007). Samples were placed on ice until analysis.

Membrane Filtration and DNA Extraction/Purification

Composited water samples were analyzed for concentrations of *E. coli* within 6 hours of collection at the NEORSD Laboratory by use of the modified mTEC membrane-filtration method 1603 of the USEPA (2006). Also at the NEORSD Laboratory, samples were processed for subsequent qPCR analysis by filtering 100 mL of the sample (or the maximum volume capable of passing through the filter) through a 0.4-µm polycarbonate filter (Whatman, Florham Park, N.J.). The filtration apparatus was rinsed copiously with phosphate-buffered saline (PBS). The filter was then aseptically folded and placed, with the perimeter of the filter first, inside a 2.0-mL screw-cap vial containing 0.3 g of acid-washed glass beads (Sigma, St. Louis, Mo.) and 550 µL of bead solution from the MO BIO UltraClean Fecal DNA Isolation Kit (MO BIO Laboratories, Carlsbad, Calif.). These vials were mixed briefly by vortexing and then stored at -70°C until further analysis by the USGS Ohio Water Microbiology Laboratory (OWML).

Once the water-sample filters were received at the OWML, DNA was extracted from the filters and purified by use of a modified protocol for the MO BIO UltraClean Fecal DNA Isolation Kit. The initial steps of the DNA extraction-kit protocol were modified to accommodate the variation in starting material (filter-preserved DNA), and various wash steps were modified to enhance quantitative recovery. Briefly, the modifications included the following:

- Samples were processed in an eight-position mini bead beater (Biospec Corp., Bartlesville, Okla.) for 2 minutes at maximum rate.
- After centrifugation, entire supernatants were removed without disturbing the pellets, and subsequent reagent volumes were adjusted to the larger volumes of supernatant, proportionally.
- Samples were bound to the spin filters by use of a vacuum manifold (Qiagen, Valencia, Calif.) amended with vacuum adapters (Promega Corp., Madison, Wis.).

The final extract volume for each sample was 50 µL. All DNA extracts were stored at 4°C until qPCR analysis, which was done within 24 hours of DNA extraction.

qPCR Analysis

Analysis by qPCR was accomplished by use of lyophilized bead-based reagents. The qPCR master mix was prepared by combining OmniMix HS beads (Cepheid, Sunnyvale, Calif.) and Scorpion *E. coli* primer and probe beads (Cepheid) in a microcentrifuge tube and reconstituting with molecular-grade water. Each OmniMix HS bead was adequate for two 25-µL reactions containing 1.5 units of TaKaRa hot start Taq polymerase, 200 µM deoxyribonucleotides, 4 mM magnesium chloride, and 25 mM HEPES buffer. Each Scorpion bead was adequate for four 25-µL reactions containing 0.25 µM of a proprietary primer-probe complex targeting *E. coli* species. qPCR reactions were processed in optical 96-well reaction plates (Applied Biosystems, Foster City, Calif.) as 25-µL volumes consisting of 20 µL of the qPCR master mix and 5 µL of DNA extract template. The reactions took place in an Applied Biosystems 7500 Real-Time PCR System. Thermal cycling conditions were as follows: 2 minutes at 95°C, followed by 45 cycles of 5 seconds at 95°C and 43 seconds at 62°C.

A standard curve was developed to quantify environmental samples. The standards were created from a pure culture of *E. coli,* American Type Culture Collection (ATCC) 25922, grown at 36°C in tryptic soy broth for 18 hours with shaking. The pure culture was diluted 1:10 in PBS. Initial attempts at extracting DNA from the undiluted culture were unsuccessful (data not shown); therefore, a 1:10 dilution was necessary prior to extraction. The pure culture was diluted further to determine concentrations of *E. coli* by use of USEPA method 1603 (2006). The 1:10 dilution of pure culture was prepared for DNA extraction by filtering in duplicate through a polycarbonate filter and storing briefly at -70°C, in the same manner as water samples. Filters were then processed through DNA extraction and purification in the same manner as above. Resulting DNA extracts were each serially diluted 1:10 to a final dilution (including the 1:10 dilution done before DNA extraction) of 10^{-9}. The final five dilutions from each of the duplicate DNA extracts (10^{-5}–10^{-9}) were analyzed by qPCR in triplicate in the same manner as above. A compiled standard curve that included three standard curve runs on different dates was used for final data analysis.

Quality-Assurance and Quality-Control Procedures

General laboratory quality-assurance (QA) procedures followed those of Francy and others (2007). Quality-control samples included whole-method, DNA extraction, and qPCR controls.

Two whole-method control samples were run, consisting of field blanks (one per recreational season) and split replicate samples (one per week). Field blanks were processed by pouring approximately 500 mL of sterile PBS into a sterile collection bottle in the field and then subjecting them to the

same conditions and processes as the beach samples. Split replicate samples were processed by filtering a separate aliquot of water from the same sample bottle.

DNA extraction controls consisted of a negative and positive extraction control, which were included with each DNA extraction run. The negative extraction control was processed by filtering approximately 100 mL of PBS and preserving the filter, as described for beach samples. The positive extraction control was processed by adding 1 µL of DNA extract from one of the standards for the standard curve to 4 µL of negative control DNA extract.

Quality-control samples for qPCR included no-template controls (NTCs), matrix spikes, and qPCR positive controls. The NTC was processed once per qPCR run by using sterile molecular-grade water as the DNA template. The effect of the matrix on qPCR was assessed by processing matrix spikes. In 2006, matrix spikes were analyzed by qPCR for every sample that was processed. In 2007, matrix spikes were analyzed for only a portion of the sample set, approximately one for every eight samples. Matrix spikes were processed by adding 1 µL of DNA extract from one of the standards for the standard curve to 4 µL of sample DNA extract to serve as the DNA template for qPCR. The qPCR positive control was processed by adding 1 µL of DNA extract from the same standard as was used for the matrix spike to 4 µL of sterile, molecular-grade water.

Data Analysis

After completion of the qPCR analyses, various settings in the Sequence Detection Software (SDS) (Applied Biosystems) were optimized to analyze the data. The optimized settings were established on the basis of the standard-curve output, and these settings were applied to each run of environmental samples. Output from the analysis is displayed in an amplification plot, where the increase in reporter dye fluorescence (Delta Rn) is plotted against PCR cycle number, as illustrated in figure 2. First, the baseline (initial cycles of PCR in which little change in the fluorescent signal is seen) was defined manually. A threshold value was then set manually as a level of increasing fluorescence that was above the baseline and within the exponential phase of the amplification curve. Cycle threshold (Ct) values were calculated by the SDS software for each reaction. A Ct value is the cycle at which the sample fluorescence passes the threshold; higher concentrations of the target sequence result in a lower Ct value because the fluorescent signal meets the threshold earlier in the PCR amplification.

Conversion of Ct values to *E. coli* concentrations was accomplished by plotting the Ct values from each standard against $\log_{10}$-transformed *E. coli* concentrations that were determined by the traditional culture-based method. A line

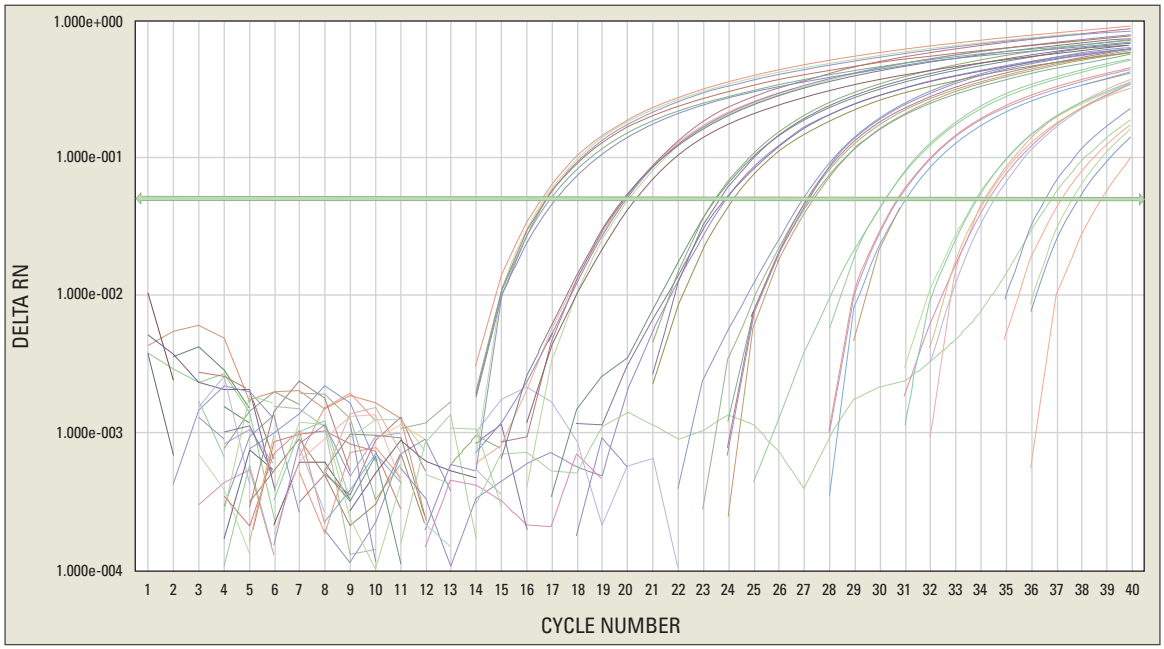

Figure 2. Example qPCR amplification plot showing the accumulation of fluorescence (Delta Rn) plotted against PCR cycle number.

of best fit was applied to this plot (fig. 3), and the resulting equation used to describe this line was used to calculate sample *E. coli* concentrations based on their Ct values.

Correlations between the qPCR and the traditional culture-based method results were determined by computing Pearson's correlation coefficient (r). In this study, the r-values were considered statistically significant if the p-values were less than 0.05. For data analysis, all nondetects (Ct values greater than 40) were set to an upper limit of 40 prior to determining CFU/100 mL from the standard curve.

Concentrations calculated from the qPCR standard curve were commonly higher than concentrations obtained from the traditional culture-based method. Consequently, to compare the qPCR results to the recreational bathing-water standard, simple linear regression models were developed for each year at each beach to "predict" (in retrospect) cultivable bacterial concentrations as a function of the estimated concentration as determined by qPCR. Pearson's correlation coefficients and simple linear regression equations were both computed from $\log_{10}$-transformed data.

Relations Between Results from qPCR and Culture-Based Methods

E. coli concentrations determined by the traditional culture-based method generally were higher during the 2007 recreational season than in 2006. *E. coli* concentrations from composited samples collected at Edgewater Beach ranged from 13 to 760 CFU/100 mL with a median value of 75 CFU/100 mL in 2006 and ranged from 10 to 1,800 CFU/100 mL with a median value of 120 CFU/100 mL in 2007 (Appendix 1–1). *E. coli* concentrations from composited samples taken at Villa Angela Beach ranged from 4 to 4,100 CFU/100 mL with a median value of 230 CFU/100 mL in 2006 and ranged from 10 to 24,000 CFU/100 mL with a median value of 400 CFU/100 mL in 2007 (Appendix 1–2).

The standard curve for *E. coli* that was used for interpretation of qPCR data was determined from composited data that was collected on three separate dates (fig.3). The standard curve had an R^2 value of 0.9497, with an amplification efficiency of 91.1 percent and a dynamic range of 14–23,000 CFU/100 mL. The amplification efficiency was calculated by use of the equation $10^{(-1/slope)} - 1$. The dynamic range of the standard curve is the lowest and highest concentrations of the standards that were used in the regression analysis. The following equation for the standard curve was used to compute concentrations based on Ct values:

Predicted log CFU/100 mL = (Ct value – 44.835)/ – 3.5551

Statistically significant linear correlations were found between results from the qPCR method and the traditional culture-based method for *E. coli* (fig. 4). At Edgewater Beach, 35 samples in 2006 and 45 samples in 2007 were analyzed by the two methods. Of the 45 samples analyzed in 2007, 17 did not have qPCR results as a result of a quality-control failure (duplicate qPCR reactions did not produce similar Ct values); these samples were not included in further statistical analyses. Outside of these 17 samples, this phenomenon has not been observed. Precision issues were resolved in other datasets, and

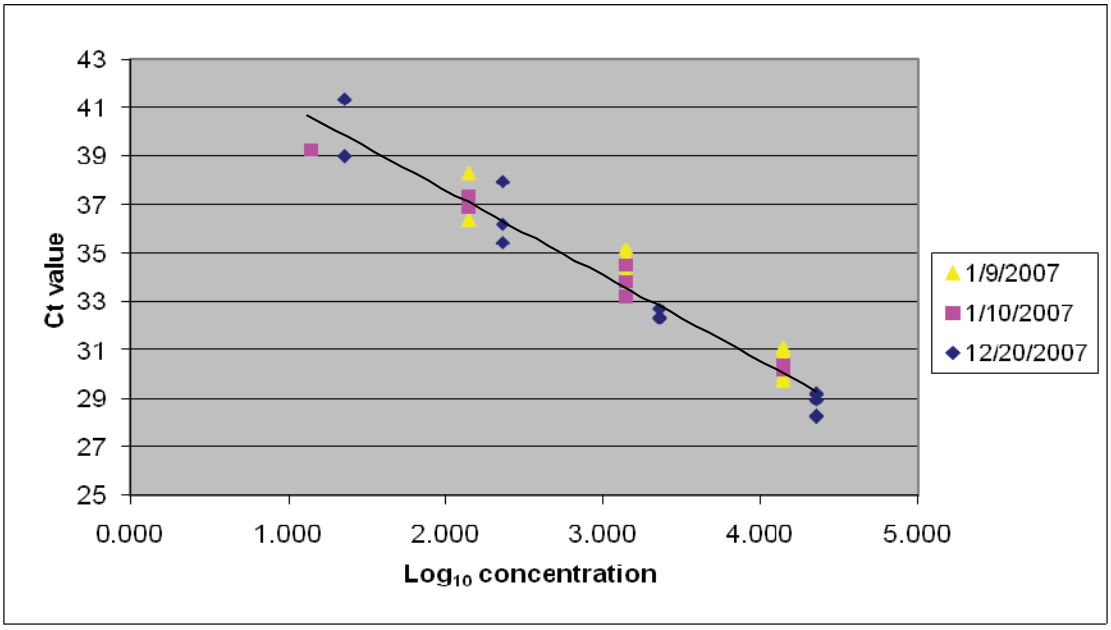

Figure 3. Compiled standard curve for *Escherichia coli*. $R^2 = 0.9497$

no other data were discarded. The Pearson's correlation coefficients relating qPCR method results and culture-based method results were 0.626 ($p<0.0001$) in 2006 and 0.667 ($p<0.0001$) in 2007. At Villa Angela Beach, 36 samples were analyzed in 2006 and 38 samples in 2007. The Pearson's correlation coefficients were 0.789 ($p<0.0001$) in 2006 and 0.829 ($p<0.0001$) in 2007.

As shown in figure 4, the relation between the qPCR estimations of *E. coli* concentrations and the culture-based *E. coli* concentrations appeared to be different for each year. To determine whether the two years of data could be combined for each beach, the slopes of the regression equations were compared to determine whether they were statistically different (University of California, Los Angeles, [2008]). A p-value of less than 0.05 would indicate that the slopes were statistically different and that the data could not be combined. For both beaches, using a students t-test, the slopes were found to be statistically different ($t = -2.13$, $p = 0.0369$ for Edgewater; $t = -2.84$, $p = 0.0059$ for Villa Angela); therefore, a single relation could not be used to describe the relation for both years at a given beach.

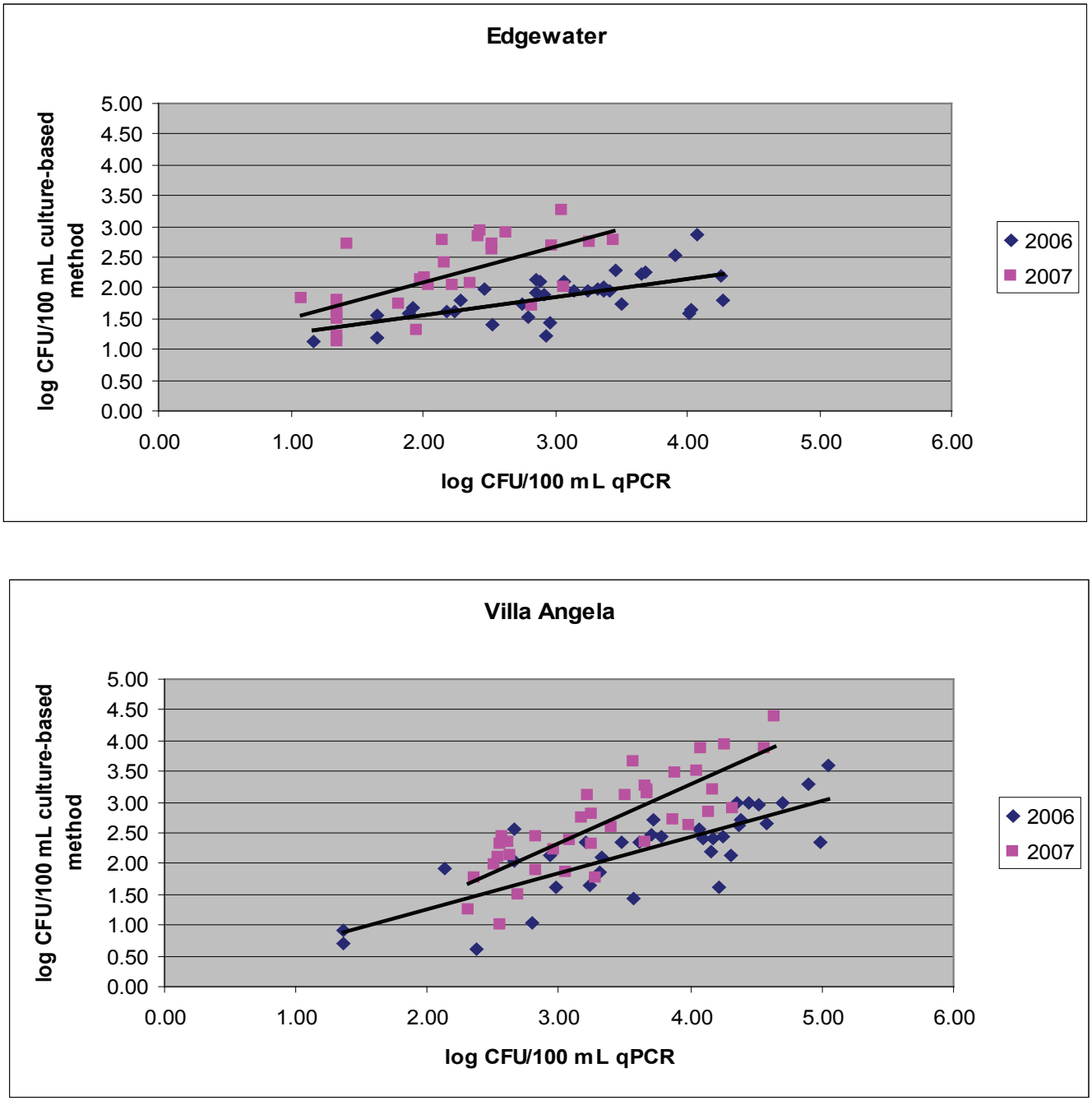

Figure 4. *Escherichia coli* concentrations as measured by qPCR compared to results obtained from the traditional culture-based method at Edgewater and Villa Angela beaches in 2006 and 2007.

As seen in the data (Appendix 1) and in the literature (He and Jiang, 2005; Haugland and others, 2005), the estimated bacterial concentrations, as determined by qPCR, can be significantly higher than concentrations determined by the traditional method. These findings highlight the need for developing new standards based on epidemiological studies using qPCR. Overestimation by the qPCR method can occur because qPCR detects target DNA regardless of cell viability. Consequently, simple linear regression analysis was done to predict *E. coli* concentrations from qPCR results, by site, for each year. These predictions, as well as the current procedure for determining recreational water quality (using the previous day's *E. coli* concentrations), were compared.

The percentage of correct classifications, false positives, and false negatives of the qPCR method with respect to exceedances of the recreational bathing-water standard of 235 CFU/100 mL are listed in table 1. These percentages are a proportion of the total number of samples collected. False positive results occurred when the *E. coli* concentration was below the standard as measured by the traditional culture-based method, but qPCR results predicted exceedance of the standard. False negative results, which are important in that they result in a greater risk to human health, occurred when the *E. coli* concentration exceeded the standard as measured by the traditional culture-based method, but qPCR results predicted the concentration was below the standard.

Table 1 also shows the statistical sensitivity and specificity results for the traditional culture-based and qPCR methods. Sensitivity is defined as the percentage of time a method result indicated an exceedance of the standard when the standard was actually exceeded according to culture-based analysis of samples collected that day. Specificity is defined as the percentage of time a method result indicated nonexceedance of the standard when culture-based analysis of samples collected that day indicated the standard was not exceeded.

Split replicate sample results for qPCR were combined for both beaches and years for a total of 20 replicate pairs. On the basis of a paired t-test on the $\log_{10}$-transformed data, the difference between replicate sample results was not statistically different from zero ($t = 0.1702$, $p = 0.8666$). This indicates that there is no statistical difference between replicate sample results.

Summary and Conclusions

Traditional culture-based methods for determining fecal indicator bacteria concentrations in recreational waters require about 18–24 hours to produce results, often leading to inaccuracies in public health reporting systems such as beach water-quality advisories. Recent emergence of rapid methods such as qPCR for determining fecal indicator concentrations may help solve the latency issue. Some advantages of using qPCR are that the method is both sensitive and specific (Mackay, 2004). An additional benefit of using qPCR is that the method is versatile and can be amended to detect different targets. The qPCR method can be applied for other purposes such as the direct quantification of pathogens (Guy and others, 2003; He and Jiang, 2005) and quantification of source-specific molecular markers (Layton and others, 2006; Noble and others, 2006). These applications can help answer other public-health research questions and provide an increased understanding of the microbial water quality at affected sites.

In this study, *E. coli* concentrations were determined by use of qPCR for samples collected during the recreational seasons of 2006 and 2007 from two Lake Erie beaches, Edgewater and Villa Angela. Results were compared to concentrations determined by means of the traditional method involving membrane filtration on modified mTEC agar.

Table 1. Method performance statistics related to the *Escherichia coli* bathing-water standard of 235 colony-forming units per 100 milliliters

Year	Predictive tool	Sample size	Correct responses[a]	False positives[a]	False negatives[a]	Sensitivity[b]	Specificity[b]
			Edgewater Beach				
2006	qPCR	35	94% (33/35)	0% (0/35)	5.7% (2/35)	0% (0/2)	100% (33/33)
	Previous day's result	24	83% (20/24)	8.3% (2/24)	8.3% (2/24)	0% (0/2)	91% (20/22)
2007	qPCR	28	75% (21/28)	7.1% (2/28)	18% (5/28)	58% (7/12)	88% (14/16)
	Previous day's result	25	64% (16/25)	12% (3/25)	24% (6/25)	40% (4/10)	80% (12/15)
			Villa Angela Beach				
2006	qPCR	36	78% (28/36)	11% (4/36)	11% (4/36)	76% (13/17)	79% (15/19)
	Previous day's result	25	52% (13/25)	24% (6/25)	24% (6/25)	50% (6/12)	54% (7/13)
2007	qPCR	38	87% (33/38)	7.9% (3/38)	5.3% (2/38)	91% (21/23)	80% (12/15)
	Previous day's result	33	73% (24/33)	9.1% (3/33)	18% (6/33)	70% (14/20)	77% (10/13)

[a] In parentheses, number of responses divided by the total number of samples analyzed.

[b] In parentheses, number of responses divided by the number of exceedances (sensitivity) or nonexceedances (specificity).

Statistically significant correlations between results from the qPCR method and results from the culture-based method were found for both beaches, during both years of data collection, with stronger correlations observed at Villa Angela Beach. The magnitude of correlation determined by Haugland and others (2005) between the qPCR method and the culture-based method for quantification of enterococci, another fecal-indicator microorganism, was similar to those reported in this study (r = 0.68). Griffith and Weisberg (2006) reported correlation coefficients ranging from 0.44 to 0.96 for enterococci and 0.44 to 0.79 for *E. coli* in an evaluation study of multiple rapid-method technologies.

At each beach, data from the two sampling years were not combined because of a statistical difference in the slopes of the regression equations relating qPCR-based estimates of *E. coli* concentrations to culture-based concentrations. One possible reason for the different regressions for the two years may be from using two different lot numbers of DNA extraction kits. In a separate study, anecdotal data suggest that DNA-extraction efficiencies can vary significantly between lot numbers of a commercially available extraction kit.

Estimated *E. coli* concentrations determined from the qPCR standard curve were frequently higher than concentrations determined by the traditional culture-based method. This may result from detection of nonculturable organisms or free genetic material in the water by the qPCR method. Although this may be viewed as a disadvantage in comparison to the traditional methods, these inputs still provide information on levels of fecal contamination. Additionally, culture-based methods may be underestimating contamination levels by not quantifying viable but nonculturable cells (Tamanai-Shacoori and others, 1996).

For the purposes of this study, estimated concentrations from qPCR results were transformed by use of single linear regression models so that results could be compared with the current practice of predicting exceedances of the water-quality standard using the previous day's *E. coli* concentrations. Comparing the methods in this way is unrealistic given that the transformed results were based upon a regression developed using data collected during an entire recreational season. At both beaches, during both years, results obtained from qPCR provided more accurate predictions compared to results obtained from the previous day's *E. coli* concentrations (table 1); however, because of the posterior data analysis, results from this study could not have been provided rapidly.

The qPCR method had a lower percentage of false positive and false negative results than use of the previous day's culture-based results (the traditional method), and it had greater sensitivity and specificity (with the exception of Edgewater Beach in 2006, where both methods were unable to predict two exceedances and had a sensitivity of 0 percent). In the cases where water-quality standards were exceeded and the water was considered unsafe for recreation, the results from the qPCR method would have resulted in fewer opportunities for public contact with levels of fecal contamination that exceeded the standard. For example, at Villa Angela Beach during the 2007 recreational season, the qPCR method would have resulted in posted advisories for 21 of the 23 exceedances, whereas the traditional method correctly predicted only 14 out of 20 exceedances.

Findings from a separate study support the use of qPCR at Villa Angela Beach for assessing recreational water quality (Francy and Darner, 2008). Previous studies at this beach (2004–7) showed that predictive modeling relying on environmental factors alone for estimating *E. coli* concentrations did not result in a more accurate forecast than the traditional method. Results from this study suggest that forecast improvements may be gained from other approaches, such as rapid methods.

Based on this study, use of qPCR to determine *E. coli* concentrations at recreational beaches may be a viable option for obtaining rapid results. Although this study demonstrates the promise of this method, adoption of qPCR as the standard method for determining fecal indicator concentrations for regulatory purposes is not yet practical. Future work should include development of a consensus methodology, a simplification of methods—including laboratory techniques and data analysis—and development of standard quality-control measures. Ultimately, epidemiological studies would be needed to relate qPCR results to the incidence of waterborne illness so that new standards can be developed.

Acknowledgments

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Appendix 1.

Appendix 1–1. Concentrations of *Escherichia coli (E. coli)* in colony-forming units per 100 milliliters (CFU/100 mL) in composite water samples from Edgewater Beach for the traditional culture-based method, quantitative polymerase chain reaction (qPCR) method, and predictions from the simple linear regression (SLR) model, 2006 and 2007.

[--, not determined]

Date	*E. coli* – culture-based method, CFU/100 mL	*E. coli* – qPCR		*E. coli* – SLR predicted CFU/100 mL
		Average Ct value	Estimated CFU/100 mL	
6/7/2006	13	40.00	23	23
6/8/2006	36	38.94	46	28
6/12/2006	97	36.09	290	49
6/14/2006	17	34.43	840	67
6/19/2006	61	36.71	190	43
6/20/2006	92	32.68	2,600	93
6/21/2006	62	30.03	19,000	170
6/26/2006	40	37.24	180	42
6/27/2006	42	38.13	150	40
6/28/2006	88	34.37	1,800	84
7/6/2006	88	33.92	2,400	91
7/10/2006	170	31.86	4,500	110
7/11/2006	130	34.60	760	65
7/12/2006	100	32.89	2,300	90
7/17/2006	39	38.09	79	33
7/18/2006	330	32.00	8,200	130
7/19/2006	140	34.66	730	64
7/24/2006	15	38.98	44	28
7/25/2006	92	33.66	1,400	78
7/26/2006	75	34.49	810	66
7/31/2006	54	32.36	3,200	99
8/1/2006	39	30.55	10,000	140
8/2/2006	45	30.50	11,000	140
8/7/2006	160	29.72	18,000	170
8/8/2006	130	33.93	1,200	74
8/9/2006	26	35.84	340	51
8/14/2006	97	33.03	2,100	88
8/15/2006	760	30.35	12,000	150
8/16/2006	28	34.33	900	68
8/21/2006	82	34.70	710	63
8/22/2006	34	34.88	630	61
8/23/2006	56	35.03	570	59
9/5/2006	46	37.99	84	34
9/6/2006	190	32.55	2,900	97
9/7/2006	180	31.73	4,900	110
6/12/2007	130	37.78	96	120
6/13/2007	800	35.46	430	290
6/14/2007	21	37.86	92	120
6/19/2007	32	40.00	23	52
6/20/2007	590	32.59	1,800	670

Appendix 1–1. Concentrations of *Escherichia coli (E. coli)* in colony-forming units per 100 milliliters (CFU/100 mL) in composite water samples from Edgewater Beach for the traditional culture-based method, quantitative polymerase chain reaction (qPCR) method, and predictions from the simple linear regression (SLR) model, 2006 and 2007 —Continued

[--, not determined]

Date	*E. coli* – culture-based method, CFU/100 mL	*E. coli* – qPCR		*E. coli* – SLR predicted CFU/100 mL
		Average Ct value	Estimated CFU/100 mL	
6/21/2007	470	34.23	960	460
6/26/2007	68	40.00	23	52
6/27/2007	42	40.00	23	52
6/28/2007	700	37.29	260	220
7/3/2007	10	--[a]	--	--
7/5/2007	53	38.36	66	97
7/6/2007	410	36.01	330	250
7/9/2007	26	--[a]	--	--
7/10/2007	33	--[a]	--	--
7/11/2007	100	33.94	1,200	530
7/12/2007	370	--a	--	--
7/13/2007	120	--a	--	--
7/17/2007	65	40.00	23	52
7/18/2007	47	--[a]	--	--
7/19/2007	1,500	--[a]	--	--
7/24/2007	55	--[a]	--	--
7/25/2007	110	37.54	110	130
7/26/2007	160	--[a]	--	--
7/27/2007	250	37.12	150	160
7/31/2007	13	40.00	23	52
8/1/2007	36	40.00	23	52
8/2/2007	17	40.00	23	52
8/6/2007	500	35.88	330	250
8/8/2007	1,800	33.98	1,100	500
8/9/2007	840	36.16	280	220
8/10/2007	600	37.18	140	150
8/14/2007	54	--[a]	--	--
8/15/2007	120	36.46	230	200
8/16/2007	82	--[a]	--	--
8/17/2007	390	--[a]	--	--
8/20/2007	300	--[a]	--	--
8/21/2007	140	37.67	100	120
8/22/2007	80	--[a]	--	--
8/23/2007	580	--[a]	--	--
8/24/2007	510	39.74	27	57
8/27/2007	44	--[a]	--	--
8/28/2007	51	34.78	670	370
8/29/2007	120	--[a]	--	--
8/30/2007	550	33.25	1,800	670
8/31/2007	110	36.92	170	170

[a] Ct value was not obtained because of a quality-control failure.

Appendix 1–2. Concentrations of *Escherichia coli (E. coli)* in colony-forming units per 100 milliliters (CFU/100 mL) in composite water samples from Villa Angela Beach for the traditional culture-based method, quantitative polymerase chain reaction (qPCR) method, and predictions from the simple linear regression (SLR) model, 2006 and 2007.

Date	*E. coli* – culture-based method, CFU/100 mL	*E. coli* – qPCR		*E. coli* – SLR predicted CFU/100 mL
		Average Ct value	Estimated CFU/100 mL	
6/7/2006	370	35.36	460	45
6/8/2006	230	33.46	1,600	92
6/12/2006	27	32.13	3,700	150
6/14/2006	84	37.26	140	22
6/19/2006	220	32.00	4,100	160
6/20/2006	920	28.79	33,000	540
6/21/2006	270	31.04	18,000	380
6/26/2006	280	31.40	6,000	200
6/27/2006	130	33.03	2,100	110
6/28/2006	520	29.28	24,000	450
7/6/2006	160	31.15	14,000	330
7/10/2006	960	29.04	28,000	490
7/11/2006	370	30.36	12,000	300
7/12/2006	4,100	26.89	110,000	1,100
7/17/2006	300	31.67	5,100	180
7/18/2006	980	29.35	23,000	440
7/19/2006	110	35.37	460	45
7/24/2006	1,000	28.11	51,000	690
7/25/2006	430	29.32	23,000	440
7/26/2006	510	31.64	5,100	180
7/31/2006	44	33.35	1,700	96
8/1/2006	260	30.02	15,000	340
8/2/2006	230	27.08	99,000	1,000
8/3/2006	250	30.27	12,000	300
8/7/2006	460	28.56	38,000	580
8/8/2006	140	29.54	20,000	400
8/9/2006	4	36.41	230	30
8/14/2006	70	33.07	2,000	100
8/15/2006	2,000	27.45	78,000	890
8/16/2006	40	29.85	16,000	350
8/21/2006	41	34.26	950	68
8/22/2006	140	34.42	850	64
8/23/2006	11	34.89	630	54
9/5/2006	5	40.00	23	8
9/6/2006	230	32.50	3,000	130
9/7/2006	8	40.00	23	8

Appendix 1–2. Concentrations of *Escherichia coli (E. coli)* in colony-forming units per 100 milliliters (CFU/100 mL) in composite water samples from Villa Angela Beach for the traditional culture-based method, quantitative polymerase chain reaction (qPCR) method, and predictions from the simple linear regression (SLR) model, 2006 and 2007 —Continued

Date	*E. coli* – culture-based method, CFU/100 mL	*E. coli* – qPCR		*E. coli* – SLR predicted CFU/100 mL
		Average Ct value	Estimated CFU/100 mL	
6/12/2007	59	36.72	230	47
6/13/2007	1,300	32.39	3,200	620
6/14/2007	3,100	30.43	11,000	2,100
6/19/2007	1,400	31.80	4,600	890
6/20/2007	7,600	30.34	12,000	2,300
6/21/2007	1,800	31.85	4,500	870
6/22/2007	380	32.72	2,600	510
6/26/2007	130	35.50	420	84
6/27/2007	790	29.46	21,000	3,900
6/28/2007	3,000	31.01	7,700	1,500
7/3/2007	4,600	32.18	3,600	700
7/5/2007	100	35.77	360	73
7/6/2007	620	33.26	1,800	350
7/9/2007	1,600	30.02	15,000	2,800
7/10/2007	510	31.08	7,400	1,400
7/17/2007	24,000	28.32	44,000	8,200
7/18/2007	540	33.56	1,500	300
7/19/2007	8,600	30.75	18,000	3,400
7/24/2007	80	34.77	680	140
7/25/2007	93	35.89	330	67
7/26/2007	230	35.53	410	83
7/27/2007	1,600	31.80	4,600	890
8/1/2007	18	36.60	210	43
8/2/2007	170	34.28	930	180
8/6/2007	1,300	33.38	1,700	330
8/10/2007	280	34.81	660	130
8/14/2007	130	35.83	340	69
8/15/2007	220	31.84	4,500	870
8/17/2007	410	30.66	9,100	1,700
8/20/2007	240	33.90	1,200	240
8/21/2007	270	35.72	370	75
8/22/2007	60	33.20	1,900	370
8/23/2007	7,500	28.63	36,000	6,700
8/24/2007	680	30.10	14,000	2,600
8/27/2007	10	35.78	350	71
8/28/2007	32	35.28	490	98
8/30/2007	100	33.27	1,800	350
8/31/2007	74	34.00	1,100	220