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A Comparison Between Fecal Coliform, *E. coli*, and Enterococci as Bacterial Indicators in Southeast Texas Surface Waters

Field Operations Division

TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

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Region 12 - Houston

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ABSTRACT

In fiscal year 2001, the Texas Commission on Environmental Quality (TCEQ) Region 12-Houston office collected 445 surface water samples to compare different bacterial indicators. The traditional fecal coliform (FC) test was conducted on all samples using Standard Method 9222D Fecal Coliform Membrane Filter Procedure (MF). In freshwater, *Escherichia coli* (EC) was measured using the IDEXX Colilert® method. In estuarine waters, Enterococci (EN) was measured using the IDEXX Enterolert®method. The two newer bacterial indicators were used since Texas has adopted them into the Texas Surface Water Quality Standards (TSWQS). The United States Environmental Protection Agency (EPA) has shown EC and EN to be more reliable indicators of human health risk in surface waters compared to FC.

The objectives of this study were to (1) determine if FC counts (colony forming units/100ml) were significantly different from EC in freshwater, and EN in estuarine water; (2) determine potential correlations between the indicators; (3) compare the number of detections and exceedances of TSWQS between indicators; and (4) determine variability in testing methodologies via duplicate sampling.

All freshwater samples were treated as one group and the estuarine samples were divided into two groups. The first estuarine group (EN1) consisted of the tidally influenced segments of the tributaries to the bays and estuaries (Segments 801-1501). These segments typically receive direct inputs from wastewater discharges and non-point sources, and are more indicative of polluted waters. The second estuarine group (EN2) consisted of sites in the Bays and Estuaries Basin (Segments 2421-2501) which are typically less impaired.

1) Statistical analyses showed that FC and EC results were not significantly different in freshwater. FC and EN results were significantly different for both estuarine groups.

2) A positive correlation was observed between FC and EC, and between FC and both EN groups. A stronger correlation was observed in freshwater.

3) The number of detections and exceedances of TSWQS was greater for EC and EN, compared to FC. This indicates higher sensitivity with the newer indicators. A more noticeable difference between indicators was observed in estuarine water compared to freshwater.

4) All three testing methodologies were determined to be highly reproducible. Slightly less variability was observed with the FC test (MF) compared to the EC and EN tests (IDEXX).

The EPA is currently reviewing TCEQ's adoption of EC and EN as bacterial indicators. If approved, use of the new indicators will likely result in more 303(d) listed water bodies, especially in estuarine waters. This will ultimately affect all stakeholders as efforts are made to meet the new standards.

TABLE OF CONTENTS

ACKNOWLEDGMENTS iii

ABSTRACT v

INTRODUCTION 1

Membrane Filtration 2

E.coli and Enterococci as Bacterial Indicators 3

Texas Surface Water Quality Standards (TSWQS) 4

METHODS AND MATERIALS 7

Study Area 7

Sampling Procedures 7

Laboratory Analyses 8

Data Analyses 12

RESULTS AND DISCUSSION 13

Statistical Results 13

Quality Assurance 21

Factors Affecting Bacteriological Analyses 22

CONCLUSIONS 25

LITERATURE CITED 27

LIST OF TABLES

1. Basin and segment descriptions for the stations sampled for bacteriological analyses in the TCEQ Region 12-Houston area during fiscal year 2001 9

2. Descriptive statistics for the bacterial indicators analyzed in the TCEQ Region 12-Houston area during fiscal year 2001 14

3. Summary of detections and exceedances of the TSWQS for the three bacterial indicators analyzed in the TCEQ Region 12-Houston area during fiscal year 2001 15

4. Results of correlation analyses between the different bacterial indicators analyzed in the TCEQ Region 12-Houston area during fiscal year 2001 16

LIST OF FIGURES

1. *E. coli* and fecal coliform results for the freshwater stations sampled in TCEQ Region 12-Houston surface waters during fiscal year 2001 17

2. Enterococci and fecal coliform results for the tidal stream stations (EN1) sampled in TCEQ Region 12-Houston surface waters during fiscal year 2001 18

3. Enterococci and fecal coliform results for the Bays and Estuaries stations (EN2) sampled in TCEQ Region 12-Houston surface waters during fiscal year 2001. 19

4. Number of detections and exceedances of the TSWQS for the three bacterial indicators (fecal coliform, *E. coli*, and Enterococci) analyzed in TCEQ Region 12-Houston surface waters during fiscal year 2001. 20

INTRODUCTION

Bacterial indicators are measured instead of pathogenic organisms because the indicators are safer, and can be measured with faster, less expensive methods than the pathogens of concern (McGee et al., 1997). An ideal microbial indicator of fecal contamination in water should be present in feces of humans and warm-blooded animals; its potential for growth in the aquatic environment should be minimal, and should never surpass those of pathogens; it should be readily detectable by simple means, and produce unique and characteristic reactions to provide unambiguous identification of the group; it should always be present when pathogens are present; and it should show increased resistance to disinfectants compared to pathogens (Elmund et al. 1999).

Coliform bacteria comprise a heterogenous group of lactose positive bacteria belonging to the family Enterobacteriaceae. The most abundant and common coliform genera are *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella*, and may be either of environmental or fecal origin (Ostensvik, 2000). Many regulatory surface water monitoring programs, including that of the Texas Commission on Environmental Quality (TCEQ), have historically utilized fecal coliform (FC) as the primary bacterial indicator in their water quality standards. Developed in 1904, the FC test takes advantage of the fact that most *E. coli* (EC) will tolerate temperatures of 44.5ºC, whereas most total coliforms will not.

The literature, however, has identified a lack of both sensitivity and specificity on the part of the FC test (Edberg, 1994). Mummert and McGinis (1996) noted increased variability in surface water FC densities. Their counts were affected by a number of factors including storm water runoff, stream flow, sediment disturbance, and water chemistry. FC methods have also been faulted because several members of the group may have non-fecal origins (Covert et al., 1992). Thermo-tolerant *Klebsiella*, for example, has been found in pulp and paper mill effluents, textile processing plant effluents, cotton mill wastewater, and sugar beet wastes (Mates and Schaffer, 1988). Santiago-Mercado and Hazen (1987) found high densities of FC at numerous sites in Puerto Rico in the complete absence of any known sources of fecal contamination.

In response to problems associated with the FC test, research has focused on additional bacterial indicators and analysis techniques to better assess fecal contamination in surface waters. Most studies have shown EC and enterococci (EN) to be better indicators in freshwater and estuarine water, respectively. Subsequently, both indicators were recently adopted into the Texas Surface Water Quality Standards (TSWQS). The primary objective of this paper was to compare the newer indicators to FC. This assessment was conducted to determine the potential impact this changeover may have on surface water quality monitoring across the state.

Although EC and EN are the current indicators of choice, the EPA (1999) has acknowledged the need for rapid indicators of fecal pollution to identify risk before exposure takes place, as most microbial testing methods take 24 to 48 hours. They state that real-time or near-time analytical methods, such as simple "dipstick" color-change test for detecting human fecal contamination, must be developed to provide an immediate identification of potential problems. In addition, the EPA (1999) stated that indicators that distinguish between human and animal fecal contamination would allow health officials a potential means of tracking water pollutants to their sources. Several researchers, including Carson et al. (2001), have used ribotyping to effectively distinguish fecal EC of human origin from EC isolates of non-human origin, including cattle, pigs, horses, chickens, and dogs. They state this could assist with the formation of pollution reduction plans.

Membrane Filtration

Membrane filtration (MF) has historically been the method of choice for FC analysis. MF (Method 9222d) is the current method of choice for the TCEQ laboratory in Houston. One of the problems associated with this method includes the lack of suitability of some membranes in common use (Fricker and Fricker, 1996). MF tests are also labor and maintenance intensive, precise control of laboratory conditions is required, and a high degree of technical skill is necessary to perform and interpret results (Elmund et al., 1999). Budnick et al. (1996) noted that the MF method may provide inaccurate results due to the presence of toxic or growth promoting substances on the membrane, and obstruction of the agar surface by particulate matter. Growth-inhibiting chloro-organic complexes may also become trapped on membrane surfaces in samples with high levels of suspended solids. In addition, Budnick et al. stated that problems with the reaction may vary in intensity, especially when large numbers of variably sized colonies are present, resulting in counting errors. In some cases fecal coliform colonies may appear red or pink on the membrane, instead of the distinct blue color, giving inaccurate results (TNRCC, 1999).

Quality control is also more difficult with MF since the method requires filter and equipment maintenance, and plate agar batch testing. In addition, the agar plate media for MF is good for only two weeks (ampules of the media are available with longer shelf lives), which creates logistical concerns (Budnick et al., 1996). Newer bacterial procedures, such as the IDEXX methods, have better quality control due to their one time use, and longer (one year) reagent shelf life. Results are also available in 24 hours compared to the 48 hours required by MF for confirmation of bacterial strains (Budnick et al., 1996).

E.coli and Enterococci as Bacterial Indicators

From 1972 to the early 1980's, the EPA conducted studies to determine the relationship between indicator organisms and the incidence of intestinal illness or gastroenteritis. For marine waters, the highest correlation was found for EN, and in freshwater the highest correlation was found for EC. Correlations between the FC test and intestinal illness were relatively poor. Due to the extensive epidemiological study, the EPA recommended EC be used as the bacterial indicator species for surface water quality standards in freshwater, and EN in estuarine waters (EPA, 1986; EPA 2000; PBS&J, 2000). Dufour (1984) also demonstrated a direct relationship in freshwater between the rate of gastroenteritis among swimmers and the concentrations of EC, but not FC. Additionally, Covert et al. (1992) stated that EC is often preferred as an indicator because it indicates recent fecal contamination and the possibility of pathogens.

A review of the literature showed a positive correlation between FC and the new indicators, EC and EN. Eckner (1998) stated that the Colilert® and Enterolert® methods are statistically as good as, if not superior to the Swedish MF method for determining EC and EN in bathing beaches. Budnick et al. (1996) found no statistical difference between Enterolert® and MF (for the detection of EN) and lower incidence of false-positives and false-negatives with Enterolert®. Fricker and Fricker (1996) determined that Enterolert® gave more accurate results and had more detections than MF (for the detection of EN), while both methods had similar false-negative and false-positives. They also noted that counting colonies greater than 80 are difficult and subject to error using MF. The Enterolert® test range was noted to be much higher and better for samples heavily contaminated with non-target organisms. Furthermore, Chen (1996) evaluated 821 water samples and observed a good agreement between Enterolert® and MF for the detection of EN.

Edberg et al. (1989) found Colilert® to give a slightly higher response than MF, although not statistically different. McGee (1997) found no significant difference between Colilert® and MF, although they did note small differences between Enterolert® and MF. Lewis and Mak (1989) found a 97% correlation between MF and Colilert® Presence-Absence techniques (for the detection of EC) from 950 drinking water samples. Cowburn et al. (1994) determined that Colilert® recovered bacterial levels comparable to MF. Elmund et al. (1999) stated that Colilert® may be the method of choice to provide quantitative Most Probable Number (MPN) EC data on receiving streams. DeRoubin et al. (1997) compared Colilert® to the French MF technique and found the two methods to be equivalent.

Texas Surface Water Quality Standards (TSWQS)

Texas Administrative Code (TAC) Title 30, Chapter 307, Texas Surface Water Quality Standards (effective August 17, 2000) describes new criteria for site specific standards (contact and non-contact recreation) for both EC and EN, which are currently under review by the EPA (TNRCC, 2000). Prior to the proposed new criteria, criteria in Texas remained virtually identical to the 1968 recommendations by the National Technical Advisory Committee of the Department of the Interior which were endorsed by the EPA in 1976 (PBS&J, 2000). As of 1998, 44 states relied on FC as the primary bacterial indicator in their standards for contact recreation in freshwater, and 17 states relied on FC in marine and estuarine contact recreation waters (PBS&J, 2000). The EPA (2001) proposed a suite of MPN and MF test methods for enumerating EC and EN in surface waters in place of the total coliform and FC indicators in water quality-based monitoring programs. Two of the proposed test methods were the IDEXX Colilert® and IDEXX Enterolert® tests. Both methods are currently being used by the TCEQ SWQM program across the state.

None of the freshwater samples collected in this study were from non-contact recreational waters. Therefore, all EC results were compared to the single sample standard (TSWQS) of 394 colony forming units (cfu)/100 ml. All FC samples collected in freshwater were compared to the standard of 400 cfu/100 ml.

Two of the estuarine segments are classified as non-contact recreation or navigation/industrial supply; Segments 1006 and 1007 in the Houston Ship Channel. Both were compared to the single sample standard of 4000 cfu/100 ml and 500 cfu/100 ml for FC and EN, respectively. Contact recreation samples in estuarine waters were compared to the single sample standard of 400 cfu/100 ml and 89 cfu/100 ml for FC and EN, respectively. Oyster waters, which have different standards, were not evaluated in this report since FC is the only bacterial indicator group for this aquatic life use subcategory. Uses and criteria are as follows (excerpt from TSWQS)(TNRCC, 2000):

Chapter §307.7(b)(1)

(A) Freshwater.

(i) Contact recreation. The geometric mean of *E. coli* should not exceed 126 per 100 ml. In addition, single samples of *E. coli* should not exceed 394 per 100 ml. Contact recreation applies to all bodies of freshwater except where specifically designated otherwise in §307.10 of this title.

(ii) Non-contact recreation. The geometric mean of *E. coli* should not exceed 605 per 100 ml.

(B) Saltwater.

(i) Contact recreation. The geometric mean of Enterococci should not exceed 35 per 100 ml. In addition, single samples of Enterococci should not exceed 89 per 100 ml. Contact recreation applies to all bodies of saltwater, except where specifically designated otherwise in §307.10 of this title.

(ii) Non-contact recreation. The geometric mean of Enterococci should not exceed 168 per 100 ml. (note - single sample limits are found in §307.10)

(C) Fecal coliform bacteria. Fecal coliform bacteria can be used as an alternative instream indicator of recreational suitability until sufficient data are available for *E.coli* or Enterococci. For segments designated as oyster waters in §307.10 of this title, fecal coliform can continue to be used as an indicator of recreational suitability because fecal coliform is used as the indicator for suitability of oyster water use as described in paragraph (3)(B) of this subsection. Fecal coliform can also continue to be used as a surrogate indicator in effluent limits for wastewater discharges. Fecal coliform criteria are the same for both freshwater and saltwater, as follows.

(i) Contact recreation. The geometric mean of fecal coliform should not exceed 200 per 100 ml. In addition, single samples of fecal coliform should not exceed 400 per 100 ml.

(ii) Non-contact recreation. Fecal coliform shall not exceed 2,000 per 100 ml as a geometric mean. In addition, single samples of fecal coliform should not exceed 4,000 per 100 ml.

METHODS AND MATERIALS

Study Area

A total of 445 bacteriological samples were collected between November 14, 2000 and August 22, 2001. Samples were collected as part of the 2001 fiscal year workplan for the TCEQ Region 12-Houston office. One-hundred sixteen different stations, representing 49 segments (Table 1) were sampled on a quarterly basis. Of the 445 samples, 115 were collected at freshwater stations, and 330 were collected at bay/estuarine or tidal stations. All stations were within the Region 12-Houston area which encompasses Harris/Galveston counties and their 11 surrounding counties. The estuarine stations were part of the Galveston/Trinity Bay and Matagorda Bay systems.

Sampling Procedures

Field measurements were made in accordance with the procedures outlined in the SWQM Program Procedures Manual (TNRCC, 1999). Temperature, pH, dissolved oxygen, specific conductance (conductivity) and salinity were measured with a Hydrolab or YSI multiprobe instrument. Water transparency (turbidity) was measured in centimeters using a standard Secchi disk.

Each water sample for bacteriological analysis was collected in a sterilized Nasco® Whirl-Pak Thio-Bag. Each bag contained a 10 mg non-nutritive sodium thiosulfate pill to eliminate any potential chlorine in the sample. Two samples were taken at each location; one for FC analysis, and one for either EC or EN analysis dependent upon segment classification (freshwater or estuarine). The FC test was continued by the TCEQ to allow comparative data to be collected during this transitional period of moving to the new indicators. For quality assurance purposes, field duplicate samples were collected every tenth sample. FC samples were transported on ice to the TCEQ laboratory in Houston within six hours of collection time. Samples must be in the incubator within eight hours according to the method. EC and EN samples were transported to the TCEQ Region 12-Houston office, setup, and placed in incubators within eight hours as well.

Laboratory Analyses

FC analysis was conducted on all samples at the TCEQ laboratory in Houston using Standard Method 9222 D Fecal Coliform Membrane Filter Procedure (APHA, 1995). Samples collected for the new indicators were analyzed at the TCEQ Region 12-Houston office. The new indicators were analyzed using the IDEXX Colilert® (freshwater) or Enterolert® (estuarine) tests combined with the IDEXX Quanti-Tray® enumeration system (IDEXX, 1996; IDEXX 2000).

Various dilutions were performed by the TCEQ Houston laboratory to obtain FC counts. Most IDEXX samples were diluted to 10% with Type II deionized water, although some samples were diluted to 20% or 1% based upon expected results from historical samples. The presence of *Bacillus* spp. can interfere with the testing of marine waters and a dilution of 1:10 is recommended to eliminate interference (ASTM, 1999). The diluted IDEXX samples were mixed with the appropriate powdered reagent in a sterile 100 ml plastic jar, shaken to dissolve, and poured into Quanti-Trays®. The trays were then sealed in an IDEXX Quanti-Tray® sealer and placed directly into a Thermoclyne Type 142300 Incubator. A blank sample of Type II deionized water was analyzed with each batch of samples for quality assurance/control purposes. IDEXX samples were incubated between 24 and 28 hours at 35 $\pm$0.5ºC for Colilert®, and 41 $\pm$0.5ºC for Enterolert®.

After incubation, trays were counted within one foot of a 365 nm UV lamp that was contained in a dark box. Tray cells were counted positive if they turned yellow (indicating presence of total coliforms), and exhibited blue fluorescence under the lamp (indicating presence of EC or EN). Samples demonstrating borderline fluorescence were left in the incubator for additional time (<28 hours total incubation) and compared to a comparator tray provided by IDEXX, for positive growth verification. IDEXX Quanti-Tray/2000® MPN Table (cfu per 100ml) and 51-Well Quanti-Tray® MPN Table's were used to enumerate sample results.

Table 1. Basin and segment descriptions for the stations sampled for bacteriological analyses in the TCEQ Region 12-Houston area during fiscal year 2001

FRESHWATER SEGMENTS (EC)		
SEGMENT	**BASIN**	**NAME**
802	TRINITY RIVER	Trinity River Below Lake Livingston
902	TRINITY-SAN JACINTO COASTAL	Cedar Bayou Above Tidal
1000	SAN JACINTO RIVER	Unclassified Waters
1002	SAN JACINTO RIVER	Lake Houston
1009	SAN JACINTO RIVER	Cypress Creek
1014	SAN JACINTO RIVER	Buffalo Bayou Above Tidal
1016	SAN JACINTO RIVER	Greens Bayou Above Tidal
1017	SAN JACINTO RIVER	White Oak Bayou Above Tidal
1102	SAN JACINTO-BRAZOS COASTAL	Clear Creek Above Tidal
1108	SAN JACINTO-BRAZOS COASTAL	Chocolate Bayou Above Tidal
1110	SAN JACINTO-BRAZOS COASTAL	Oyster Creek Above Tidal
1202	BRAZOS RIVER	Brazos River Below Navasota River
1245	BRAZOS RIVER	Upper Oyster Creek
1302	BRAZOS-COLORADO COASTAL	San Bernard River Above Tidal
1305	BRAZOS-COLORADO COASTAL	Caney Creek Above Tidal
1402	COLORADO RIVER	Colorado River Below La Grange
1502	COLORADO-LAVACA COASTAL	Tres Palacios Creek Above Tidal

Table 1 (Continued). Basin and segment descriptions for the stations sampled for bacteriological analyses in the TCEQ Region 12-Houston area during fiscal year 2001

TIDAL STREAM SEGMENTS (EN1)		
SEGMENT	**BASIN**	**NAME**
801	TRINITY RIVER	Trinity River Tidal
901	TRINITY-SAN JACINTO COASTAL	Cedar Bayou Tidal
1001	SAN JACINTO RIVER	San Jacinto River Tidal
1005	SAN JACINTO RIVER BASIN	Houston Ship Channel/San Jacinto River Tidal
1006	SAN JACINTO RIVER BASIN	Houston Ship Channel Tidal
1007	SAN JACINTO RIVER BASIN	Houston Ship Channel/Buffalo Bayou Tidal
1013	SAN JACINTO RIVER BASIN	Buffalo Bayou Tidal
1101	SAN JACINTO-BRAZOS COASTAL	Clear Creek Tidal
1105	SAN JACINTO-BRAZOS COASTAL	Bastrop Bayou Tidal
1107	SAN JACINTO-BRAZOS COASTAL	Chocolate Bayou Tidal
1109	SAN JACINTO-BRAZOS COASTAL	Oyster Creek Tidal
1111	SAN JACINTO-BRAZOS COASTAL	Old Brazos River Channel Tidal
1113	SAN JACINTO-BRAZOS COASTAL	Armand Bayou Tidal
1201	BRAZOS RIVER BASIN	Brazos River Tidal
1301	BRAZOS-COLORADO COASTAL	San Bernard River Tidal
1304	BRAZOS-COLORADO COASTAL	Caney Creek Tidal
1501	COLORADO-LAVACA COASTAL	Tres Palacios Creek Tidal

Table 1 (Continued). Basin and segment descriptions for the stations sampled for bacteriological analyses in the TCEQ Region 12-Houston area during fiscal year 2001

BAY AND ESTUARINE SEGMENTS (EN2)		
SEGMENT	**BASIN**	**NAME**
2421	BAYS AND ESTUARIES	Upper Galveston Bay
2422	BAYS AND ESTUARIES	Trinity Bay
2423	BAYS AND ESTUARIES	East Bay
2424	BAYS AND ESTUARIES	West Bay
2425	BAYS AND ESTUARIES	Clear Lake
2431	BAYS AND ESTUARIES	Moses Lake
2432	BAYS AND ESTUARIES	Chocolate Bay
2434	BAYS AND ESTUARIES	Christmas Bay
2437	BAYS AND ESTUARIES	Texas City Ship Channel
2438	BAYS AND ESTUARIES	Bayport Channel
2439	BAYS AND ESTUARIES	Lower Galveston Bay
2441	BAYS AND ESTUARIES	East Matagorda Bay
2451	BAYS AND ESTUARIES	Matagorda Bay/Powderhorn Lake
2452	BAYS AND ESTUARIES	Tres Palacios Bay/Turtle Bay
2501	GULF OF MEXICO	Gulf of Mexico

Both IDEXX tests are described as ‘defined substrate technology' which Fricker and Fricker (1996) stated is a patented process where the major carbon and energy sources for bacterial growth serve as indicators of growth. The Enterolert® test is a MPN statistical method for determining bacterial density based on the Poisson distribution (ASTM, 1999). It is a rapid 24-hour test that uses 4-methylumbelliferyl-B-D-glucoside as the defined substrate nutrient-indicator. This compound is metabolized/hydrolyzed by EN B-glucosidase (produced by bacteria such as *E. faecium and E. faecilis*) which releases 4-methlyumbelliferone and glucose. The glucose is further metabolized by EN to promote their growth, while the 4-methlyumbelliferone compound exhibits blue fluorescence under a UV 365nm lamp. Most non-target microbes do not typically possess the necessary enzyme, therefore, cannot digest the indicator nutrient. Bacteria that do possess the enzyme are selectively suppressed by antimicrobial agents (IDEXX, 2000).

Colilert® uses two nutrient indicators, ONPG (o-nitrophenyl and B-D-galactopyranoside) and MUG (4-methyl-umbelliferyl and B-D-glucuronide), as the two major sources of carbon and energy. These are metabolized by the coliform enzyme B-galactosidase and the EC enzyme B-glucoronidase, respectively. When these compounds are metabolized, the indicator portion is cleaved and changes color allowing presence/absence confirmation (IDEXX, 1996). Killan and Bullow (1976) showed that the production of B-glucurodinase (MUG from Colilert®) is specific for EC in 97% of the strains tested by them. The only Enterobacteriaceae known to produce this enzyme are some strains of *Salmonella* spp. and *Shigella* spp. (Feng and Hartman, 1982).

Data Analyses

Data were analyzed using SPSS® Base 7.5 for Windows. Distribution of the data sets were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk normality tests, box plots, and histograms. Non-parametric statistics were conducted on the data sets that did not meet the assumptions for parametric testing (normal distribution, similar variance, and skewness/kurtosis limits).

All bacterial results were (ln) transformed and non-detectable samples (<10 cfu/100 ml) were assigned a value of 5 cfu/100 ml for statistical purposes. The data were analyzed in three separate groups according to type (freshwater or marine) and location (tidal stream or open bay). Freshwater samples were represented by the EC group. The EN1 group included tidal stream stations (Segments 801-1501) which are usually closer to point and non-point source discharges, and more indicative of polluted waters. The EN2 group (Segments 2421-2501) included stations in the bays (Bays and Estuaries Basin) which are typically less polluted. FC was analyzed at all stations.

A Wilcoxon signed rank test was conducted to determined if the FC results were significantly different from the respective EC or EN values. A paired sample t-test was conducted on the duplicate samples to compare the reliability of the test methods and for quality assurance purposes. A Spearman rank correlation test or Pearson correlation test was conducted to test for correlations between the data sets. Statistical significance was assessed at the 95% or 99% confidence level depending on the specific tests. Scatter plots were created to illustrate the degree of linear correlation between the bacterial indicators.

RESULTS AND DISCUSSION

Statistical Results

In freshwater, FC results averaged 826 cfu/100ml, and EC results averaged 1,009 cfu/100 ml (Table 2). Mean bacterial densities for FC and EC were not significantly different using the Wilcoxon signed rank test (p value 0.066). The two indicators were strongly correlated (r value 0.684) using the Spearman rank correlation test. The number of freshwater detections (results greater than or equal to 10 cfu/100 ml) was slightly more for EC (111) compared to FC (103). Both methods were in agreement and positive in 88.7% of the samples (Table 3 and Figure 4). The number of samples that exceeded the applicable single sample standard (TSWQS) was greater for EC (56) compared to FC (48), indicating that the EC method may be more protective of human health.

EN and FC results were significantly different for both estuarine groups using the Wilcoxon signed rank test (p value 0.01 for EN1; p value 0.000 for EN2). Both EN groups significantly correlated with FC using the Spearman rank correlation test (r value 0.442 for EN1; r value 0.350 for EN2)(Table 4). Scatter plots of the results for all samples are represented in Figures 1, 2, and 3.

In the tidal streams (group EN1), mean EN densities (178 cfu/100 ml) were much lower than the corresponding FC densities (759 cfu/100 ml). The number of detections, however, was more for EN (127) compared to FC (106), indicating that EN is only a portion of the bacterial community assessed by the FC test. There was a greater number of exceedances of the TSWQS (single samples) for EN (41) compared to FC (21). This also indicates that EN may be more protective of the contact recreation use than FC.

In the open bay (group EN2), mean results for FC and EN were 8 and 15 cfu/100 ml, respectively. Neither FC nor EN was detected at 60 percent of the stations probably due to the remoteness from point and non-point sources. The number of detections for FC and EN was 29 and 78, respectively, while the number of exceedances was 2 and 7, respectively.

Overall, the magnitude of difference between FC (MF) counts and EC/EN (IDEXX) was least in freshwater. This suggests that most of the FC at these stations are EC, or that the membrane filtration method underestimates FC counts. According to Smith (1994), EC represents 90% of fecal coliforms.

Table 2. Descriptive statistics for the bacterial indicators analyzed in the TCEQ Region12-Houston area during fiscal year 2001

	FRESHWATER (cfu/100ml)		TIDAL STREAMS (cfu/100ml)		BAYS AND ESTUARIES BASIN (cfu/100ml)	
	FC	EC	FC	EN1	FC	EN2
Mean *	826	1009	759	178	8	15
Standard Deviation	3054	3271	8338	1405	94	103
Median	240	368	153	63	5	5
Minimum	5	5	5	5	5	5
Maximum	21000	24192	60000	10462	1000	782
Number of samples	115	115	137	137	193	193
Mean difference between indicators **	170		-577		6.9	
Standard Deviation of the difference	1209		8132		138	
Median difference	5.0		-62		0	
Minimum difference	-4108		-59010		-938	
Maximum difference	4114		8642		777	

* The 5% trimmed mean was calculated by ordering the values within each group from largest to smallest. The top and bottom 5% were then deleted and the mean was computed from the observations that remained (SPSS Base 7.5 Applications Guide).

** Differences were obtained by subtracting FC from EC/EN (i.e. a negative difference indicates higher FC results compared to EC/EN).
EN1 - Enterococci samples collected from tidal streams
EN2 - Enterococci samples collected from the bay
EC - *E. coli* samples collected from freshwater

Table 3. Summary of detections and exceedances of the TSWQS for the three bacterial indicators analyzed in the TCEQ Region 12-Houston area during fiscal year 2001

	FRESHWATER		TIDAL STREAMS		BAYS AND ESTUARIES	
	FC	EC	FC	EN1	FC	EN2
Number of samples	115	115	137	137	193	193
Number of detects (≥10 cfu/100ml)	103 (89.6)	111 (96.5)	106 (76.3)	127 (91.4)	29 (15.2)	78 (40.1)
Number of exceedances compared to the standard*	48 (41.7)	56 (48.7)	21 (15.1)	41 (29.9)	2 (1.0)	7 (3.7)
FC > IDEXX	53 (46.1)		80 (58.4)		17 (8.8)	
FC = IDEXX	2 (1.7)		4 (2.9)		108 (56.0)	
FC < IDEXX	60 (52.2)		53 (38.7)		68 (35.2)	
Both FC and IDEXX detected	102 (88.7)		100 (73.0)		22 (11.4)	
Both FC and IDEXX non-detected (<10 cfu/100ml)	2 (1.7)		4 (2.9)		108 (60.0)	
FC detected and IDEXX non-detected	1 (0.9)		6 (4.4)		7 (3.6)	
IDEXX detected and FC non-detected	10 (8.7)		27 (19.7)		56 (29.0)	
Both FC and IDEXX exceeded the standard	39 (33.9)		12 (8.8)		0 (0)	
FC exceeded the standard and IDEXX did not	9 (7.8)		9 (6.6)		2 (1.0)	
IDEXX exceeded the standard and FC did not	17 (14.8)		29 (21.2)		7 (3.6)	

* Exceedances of the TSWQS are based on the appropriate limits for a single sample. Numbers in parentheses represent percentages.

Table 4. Results of correlation analyses between the different bacterial indicators analyzed in the TCEQ Region 12-Houston area during fiscal year 2001

	Number of Samples	Wilcoxon signed-rank test (p-value)	Paired sample *t* test (p-value)	Pearson correlation test (r-value)	Spearman rank correlation test (r-value)
FC (MF) versus EC (IDEXX)	115	*0.066			**0.684
FC (MF) versus EN (IDEXX) Group 1	137	0.010			**0.442
FC (MF) versus EN (IDEXX) Group 2	193	0.000			**0.350
EC and EN (IDEXX) versus Duplicates	32		*0.392	**0.908	
FC (MF) versus Duplicates	32		*0.323	**0.964	

* Significant at the 0.05 level (2-tailed) (Groups are not significantly different)
** Significant at the 0.01 level (2-tailed) (Groups are correlated)

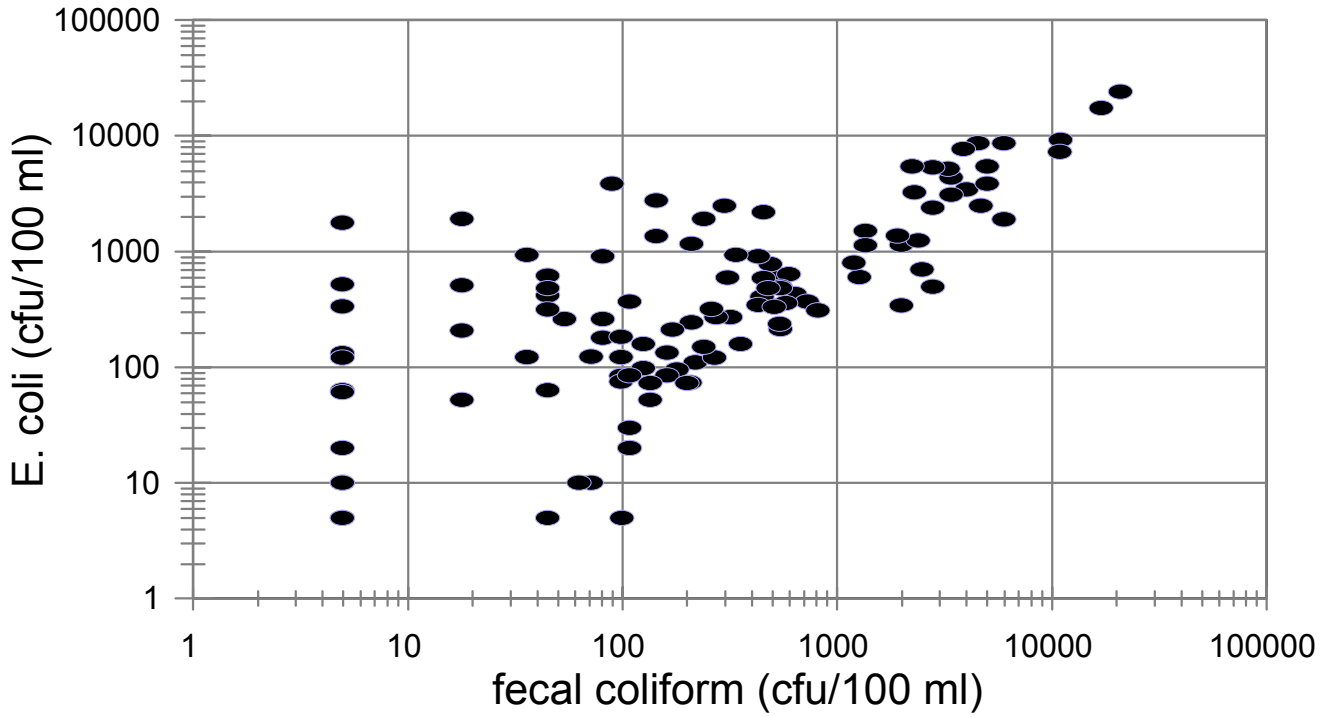

Figure 1. *E. coli* and fecal coliform results for the freshwater stations sampled in TCEQ Region 12-Houston surface waters during fiscal year 2001. Samples with the same results are represented by one point.

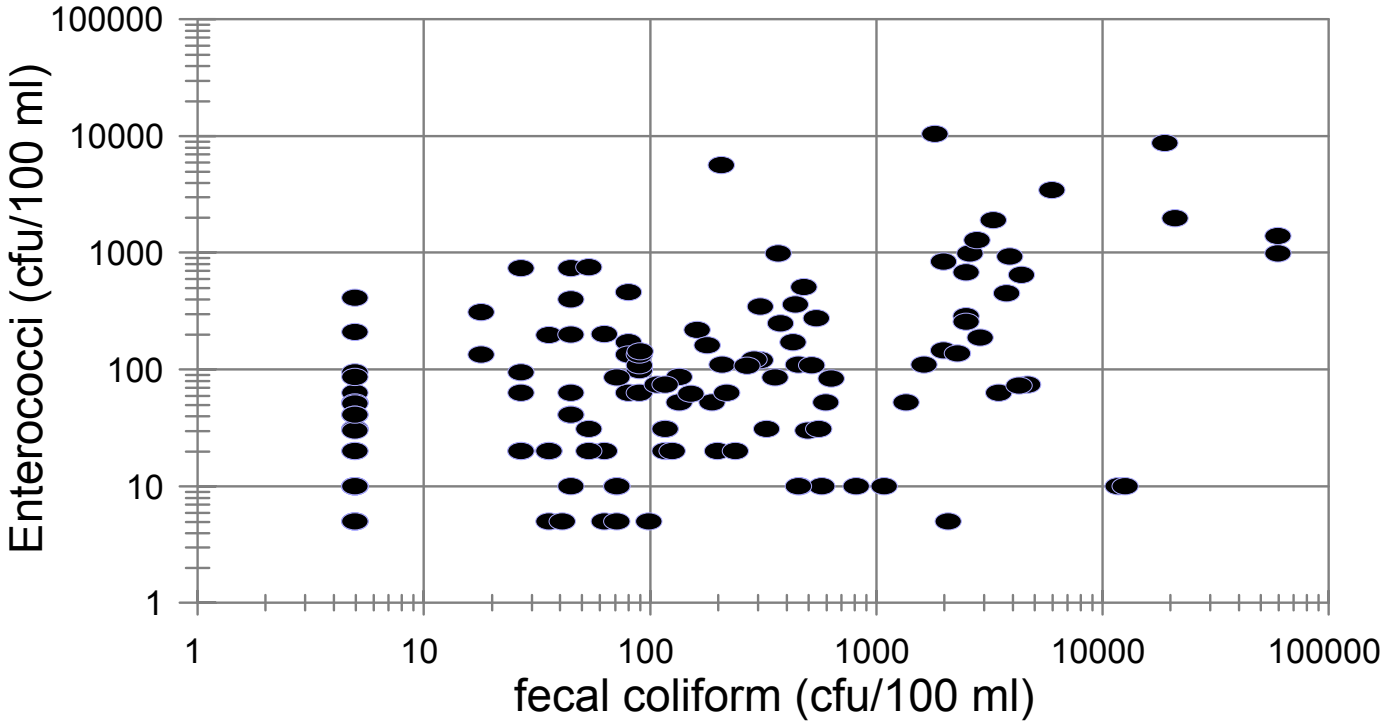

Figure 2.

Enterococci and fecal coliform results for the tidal stream stations (EN1) sampled in TCEQ Region 12-Houston surface waters during fiscal year 2001. Samples with the same results are represented by one point.

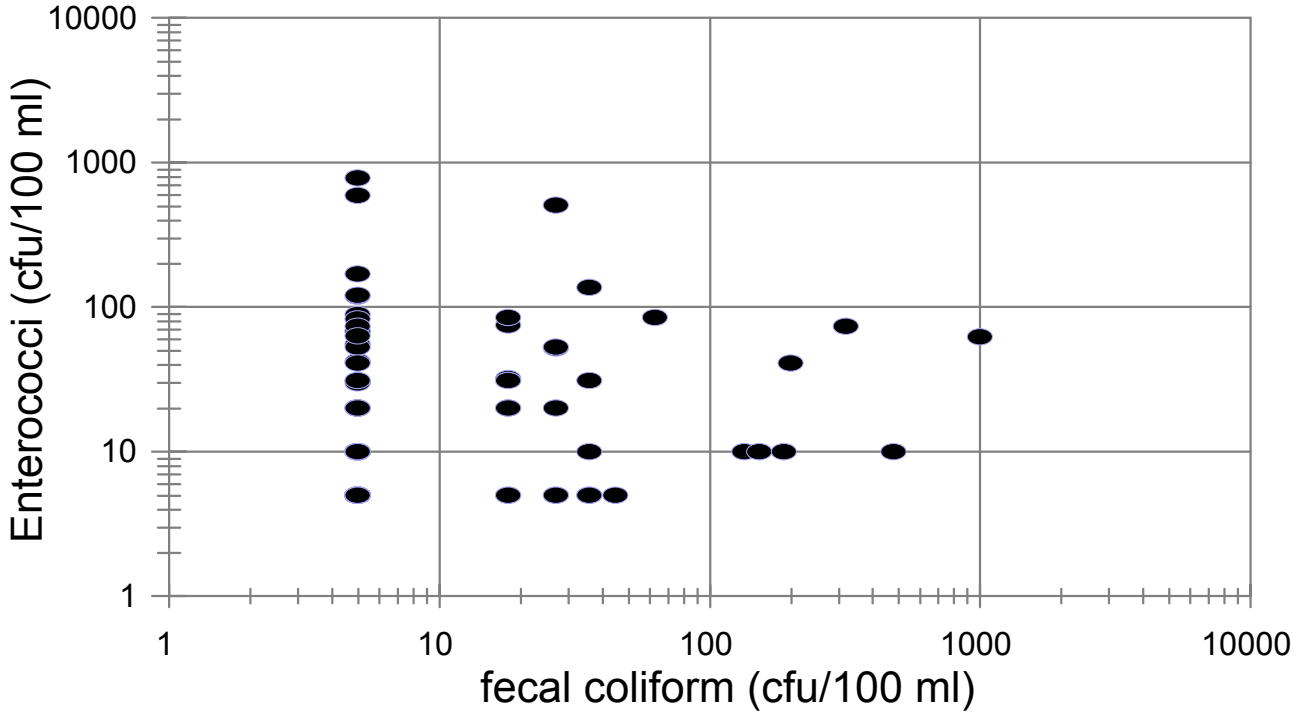

Figure 3. Enterococci and fecal coliform results for the Bays and Estuaries stations (EN2) sampled in TCEQ Region 12-Houston surface waters during fiscal year 2001. Samples with the same results are represented by one point. There were 108 samples where both fecal coliform and Enterococci were non-detectable (5 cfu/100 ml).

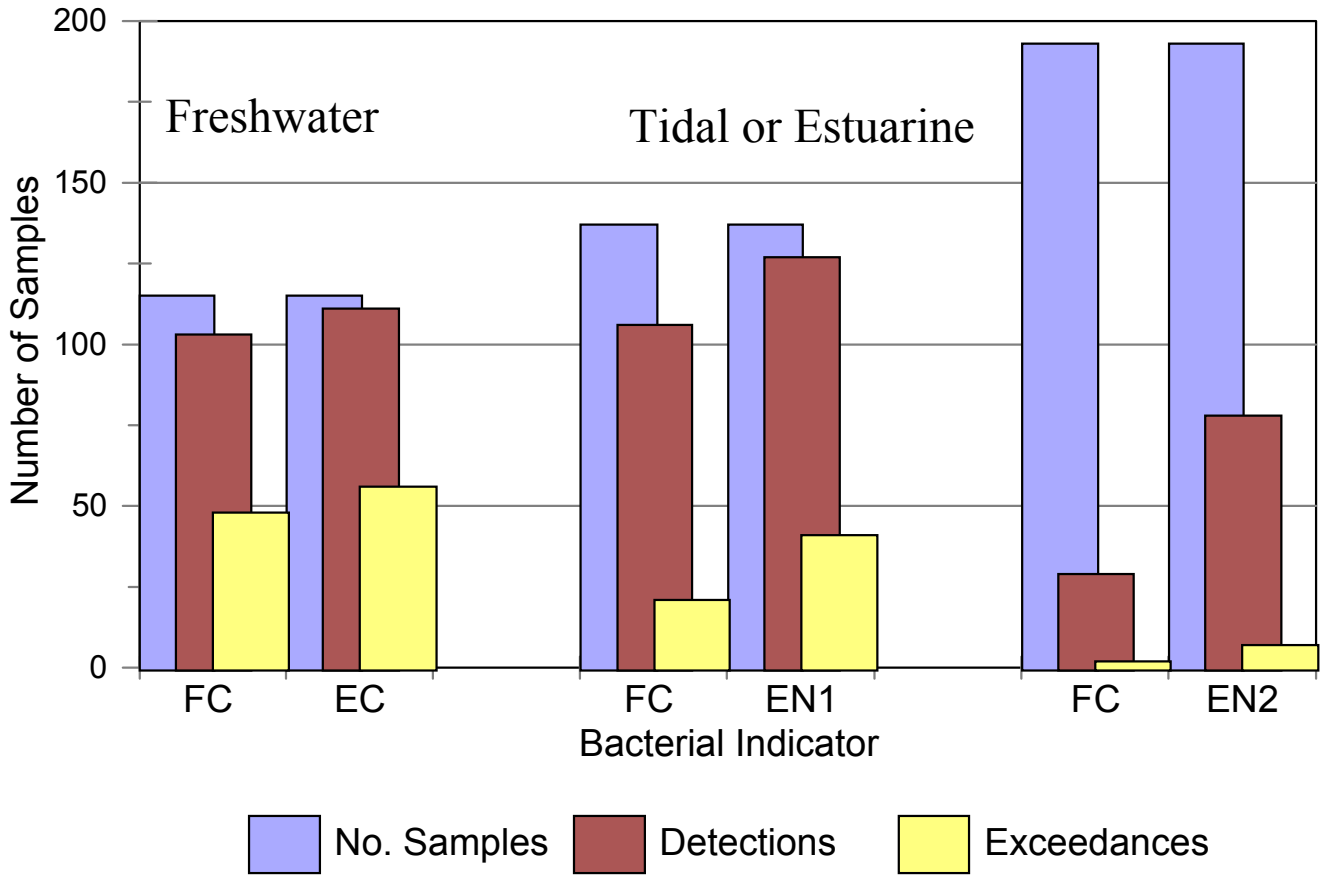

Figure 4. Number of detections and exceedances of the TSWQS for the three bacterial indicators (fecal coliform, *E. coli*, and Enterococci) analyzed in TCEQ Region 12-Houston surface waters during fiscal year 2001. EN1 represents the tidal stream sites and EN2 represents sites in the Bays and Estuaries Basin.

FC results were greater than EN results more often at the tidal stream stations (58.4% of the time) compared to the bay stations (8.8%). It is unknown whether this difference was in response to the higher bacterial counts at the tidal sites, differences in bacterial composition, salinity differences, or some other factor. FC results were higher at the freshwater and tidal stream sites compared to the bay stations. This was expected due to their proximity to sources of pollution.

There were numerous samples where EN was detected when FC was non-detected (<10 cfu/100ml). This occurred 19.7% of the time at the tidal stream sites, and 29.0% of the time in the open bay. In 8.7% of the freshwater samples, EC was detected when FC was not. This was not expected since EC and EN are subgroups of FC. When they were detected, FC should have been detected as well. Conversely, there were only a few samples (<5%) where FC was detected when EN and EC were non-detected. It was unknown whether differences in samples identified false results (positive or negative) for EC/EN or for FC, as the colonies were not further cultured for verification.

Quality Assurance

Thirty-two duplicate samples were collected in this study for data validation and quality assurance purposes. The FC duplicate samples and the IDEXX duplicate samples (EC and EN combined) were determined to have no significant difference using paired sample t-tests (p value 0.323 for FC; p value 0.392 for EC/EN)(Table 4). The FC duplicates demonstrated a statistically significant positive correlation using the Pearson correlation test (r value 0.964). EC and EN duplicate samples (combined) also demonstrated a positive correlation (r value 0.908)(Table 4). These results indicate that all three test methodologies are equally reproducible. The MF method was slightly more precise than the IDEXX methods.

Every day that EC or EN samples were analyzed at the TCEQ Region 12-Houston office, a lab blank of Type II distilled water was also analyzed. A positive sample was found in only one tray (result of 9.9 cfu/100 ml) out of 106 blank samples, indicating low incidence of laboratory contamination for the IDEXX methods.

When time permitted, IDEXX samples that were positive for EC or EN growth were left in the incubator for additional time after the initial reading, but not exceeding the 28 hours total allowed by the test methods. Out of 131 samples rechecked, 49 increased in cfu/100 ml by an average of 24.6%. This demonstrated that the longer the samples incubated, the greater the bacterial counts were. This is important from a regulatory standpoint since permitted discharge limits are often governed by surface water assessments.

Factors Affecting Bacteriological Analyses

A number of environmental factors injure or kill coliform bacteria in water including sunlight, temperature, bacteriophages, predators, sedimentation, toxic substances, and lack of nutrients (Barcina et al., 1989; Olson 1978; Fiskal 1994). Davies et al. (1994) also stated that several plant extracts and algae could significantly interfere with the detection of coliform bacteria and EC with the use of rapid assays, including Colilert®, on the basis of the production of the same enzymes. They suggest the need for masking agents to reduce contribution of enzymes from plant and algal mass.

Bacteria also often adhere to solid particles in the water column. Differences in testing methods, therefore, probably affected the bacterial counts in this study. The IDEXX methods do not require filtration, while MF does. Interactions between bacteria and sediment have also been widely documented. This is important since sediments may be re-suspended by a variety of factors including dredging activities, run-off, wind and wave action, swimmers, and boating activities. Francy and Darner (1998) showed the importance of physical disturbance on the resuspension of sediment-stored bacteria in lake bottom sediments, and their importance in degradation of recreational waters. They found longer survival of EC in sediment than in water due to the higher content of organic matter in the sediment. Laboratory experiments by Sherer (1992) found that the half-lives of FC ranged from 11 to 30 days in fine to coarse sediments, while the half-life of the bacteria in the overlying water was only 2.8 days. Davies et al. (1995) determined that FC can survive in freshwater much longer, even up to 60 days. Marino and Gannon (1991) stated that storm-drain sediments act as reservoirs of fecal indicators during warm, dry weather periods for up to six days.

An important aspect of the different testing methodologies is the incidence of false-positives and false-negatives. False-positive results are usually due to contamination, or the growth of non-target organisms. False-negative results are due to inadequate detection of the target organism or group. Most of the literature reviewed showed a much higher false-positive rate for FC than for either EC or EN. This indicates problems affecting recovery and retention of cells common to procedures using membrane filters (Budnick et al., 1996). DeRoubin et al. (1997) found a low false-positive rate of 2.4% for EC, and a false-negative rate of 3.9% for EC using Colilert ®. Chen et al. (1996) found a low false-negative rate of 1% for EN, and a false-positive rate of 5% for EN, using Enterolert®.

Budnick et al. (1996) noted one sample in their study where FC <10 cfu/100 ml, and EN >2005 cfu/100 ml, which they relate to Enterolert's® ability to recover injured EN. The sample was taken near the effluent of a chlorinated wastewater treatment plant. Fricker and Fricker (1996) also noted that EN are generally more resistant to chlorination and other environmental stressors.

Davies et al. (1995) demonstrated the potential for marine bacteria of the genus *Vibrio* to cause false-positive reactions in coliform assays based on B-D-galactosidase activity. They stated that marine *Vibrio* spp. have a competitive advantage over coliforms in seawater since it is their natural habitat. They are also more likely to be a problem in warmer months when their numbers are higher.

Niemi et al. (1997) stated that there are characteristic differences in the composition of total coliform flora isolated from different point sources. Their FC tests mainly detected EC in natural or pristine waters, but was less reliable in domestic waters where EC and *Klebsiella* spp. dominate. Since approximately 15% of *Klebsiella* spp. (non-fecal origin) are thermo-tolerant, many fecal coliform positive isolates are due to the presence of those species, or related coliforms, and not EC. This diminishes the ability of a fecal coliform test to signal a true fecal contamination event (Edberg and Smith,1994). Edberg (1991) also noted that between 10% and 15% of EC are not thermo-tolerant and would be missed under the elevated temperature conditions (44.5ºC) of the FC test. Elmund et al. (1999) compared FC to EC using Colilert®. He found that *Klebsiella pneumoniae* interfered with the recovery of fecal coliforms using the MF technique, while it did not interfere with the enumeration of EC. Edberg et al. (1990) showed the Colilert® system to be resistant to noncoliform heterotrophic bacteria whereas MF was not. They also found that the Colilert® system does not support the growth of *Aeromonas* spp. and similar lactose-fermenting non-coliforms, minimizing false-positives.

This study identified three issues with the IDEXX methods that potentially affected EC and EN bacterial counts. Temperatures often dropped one or two degrees Celsius as the incubator door was opened to place the Quanti-Trays® inside. The time required to reach the optimum temperature for each test was unknown, but may have taken a couple of hours and may have affected test results. Cowburn et al. (1994) noted that the time taken for samples to reach 35ºC (using Colilert®) could be up to 8 hours, especially when a large number of samples were placed in a relatively small incubator. Secondly, enumeration is subjective and we noticed a slight degree of difference among individuals reading the Quanti-Tray® (determining fluorescence), even with the use of a comparator tray supplied by the manufacturer. Most of the time the differences were negligible. Thirdly, and most importantly, we documented a discernible difference in bacterial counts dependent upon incubation time. Readings may be made

anytime between 24 and 28 hours, however, counts varied within this time frame. From a regulatory standpoint, consistency must be implemented across the state. These issues are currently being evaluated by the TCEQ SWQM team.

Differences between indicators in this study were based on only three or four individual samples which were compared to the TSWQS for single samples. Comparing the results to the TSWQS for multiple samples (lower limit than single samples) would have been more statistically valid, however, a minimum of 10 samples is required.

CONCLUSIONS

The new indicators, EC in freshwater, and EN in estuarine waters (using IDEXX methodologies), correlated positively with FC (using traditional MF). Both were also more sensitive than FC based on the greater number of detections and exceedances of TSWQS. The bacterial densities for both EN groups were significantly different from FC, while EC was not. This suggests a larger degree of difference between bacterial indicators in estuarine waters than in freshwater. This is probably because EC is a larger proportion of the FC group than EN is. All three methodologies (MF and the two IDEXX methods) were reliable given that duplicate samples correlated well with each other.

These results should give the TCEQ greater confidence in the changeover to the newer indicators. Exclusive use of EC/EN will likely result in more 303(d) listed water bodies (especially in estuarine waters), each which may require a Total Maximum Daily Load (TMDL). This may have a profound effect on industrial and municipal wastewater permit holders since permit limits may become more stringent in an effort to meet the new bacterial TSWQS. Similar conclusions have been made in other studies. Francy et al. (1993) stated the EC criteria recommended by the EPA will be more difficult to meet than FC standards. Nuzzi and Burhans (1997) concluded that switching to a EN standard from the FC standard will result in more beach closures.

Analysis of FC will continue until at least 10 comparative samples are collected at each station. A state-wide evaluation should then be conducted to more adequately assess the impact of the switch to the new bacterial indicators. The TCEQ must also stay abreast of technological advances, such as ribotyping, to ensure adequate assessment of the surface waters in the state.

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