RAPID *E. coli* TEST EVALUATION

AT

EIGHT BATHING BEACHES

By

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

BEACH STUDY 1996

Introduction

Summers in Ontario are greatly appreciated and the availability of recreational waters for swimming, jet skiing, water skiing and windsurfing is an important factor for the tourist industry, which contributes significantly to the economy of the area. People expect that beach waters are going to be accessible for weekend and holiday recreational activities.

The most common reason beaches on the Great Lakes are posted *("WARNING These waters are unsafe for swimming due to recent bacterial pollution.")* by a Medical Officer of Health is the development of elevated levels of *Escherichia coli.* A geometric mean concentration of 100 *Escherichia coli* cells per 100 mL of sample, based on a minimum of five samples, is the standard for the Province. The standard indicates that when *E. coli* levels exceed 100 *E. coli* per 100 mL of sample, the likelihood of contracting gastroenteritis or eye, ear, nose and throat infections is high. When the *E. coli* levels are below the standard, the chance of developing these illnesses falls within an acceptable range.

Because of the lack of funding for recreational water testing, public beaches are tested once or twice per week, or not at all. In fact, some beaches are posted in the month of June and remain posted for the entire summer. Other beaches are tested weekly on Mondays from May to September. If the test results, which are available on Wednesdays, are below the standard, the beach water is not retested until the following Monday. If, on the other hand, the results of the Monday testing show an elevated *E. coli* level, the beach is re-sampled on the next Wednesday. These results are available on Fridays and, if they are below the standard, the beach is left unposted for the weekend. If the results are adverse, however, the beach is posted until Wednesday of the following week.

The consequences of this limited testing, which takes from 28 to 36 hours for the results to be available, is that often beaches are posted when they could be open to the public and not posted when they should be. This testing/posting protocol is considered unacceptable by both the public and the tourist industry. There is an obvious need to improve the testing system in order to provide an accurate indication of the *Escherichia coli* concentrations in the water in a timely and cost-effective manner. The method of testing recreational waters has remained the same for the past twenty-five years and it is time for new methods to be evaluated, which is the reason that this project was initiated.

Figure 1 displays how rapidly the bacterial water quality of this Lake Huron beach can fluctuate. The samples were taken at a beach that was monitored five days a week, from Thursday through Monday. It is evident from Figures 1 through 8 that one day the *E. coli* levels, calculated as the geometric mean of five samples, are below the 100 standard, however, on the very next day the levels can be in excess of the standard. Based on these fluctuating concentrations of *E. coli,* it can be understood why managing the beach to protect the public's health is such a challenge.

The project began in the summer of 1995, when two of the five beaches being sampled were tested five days a week using the standard laboratory method for detecting *E. coli* as well as a new technology, invented by Charm Sciences, Malden, Massachusetts, that provided results (indicating whether the levels were above or below the 100 *E. coli* per 100 mL standard) within a six hour period. This comparison confirmed that *E. coli* detection in less than six hours was possible, however, further test refinements were required (Palmateer et al., 1995). During the months between the 1995 and 1996 recreational seasons, this testing technology was fine-tuned by the research and development staff of Charm Sciences and by the authors to improve its accuracy.

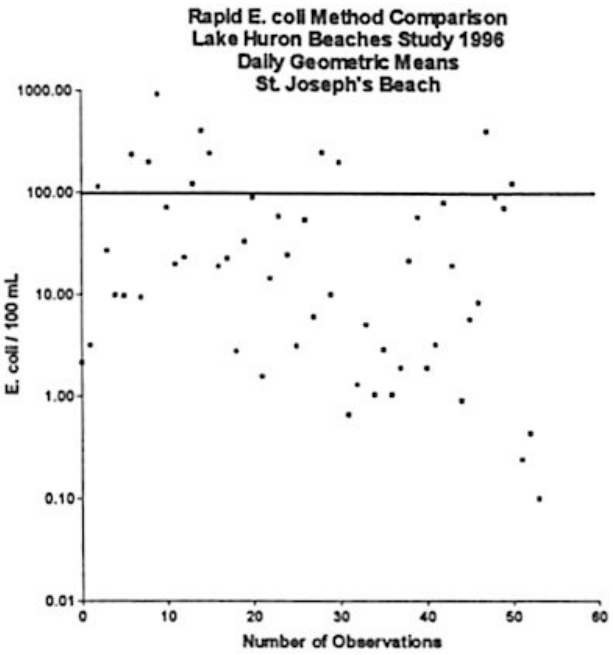


Figure 1: Daily Geometric Mean levels of E col for the summer months at St Joseph's Beach.

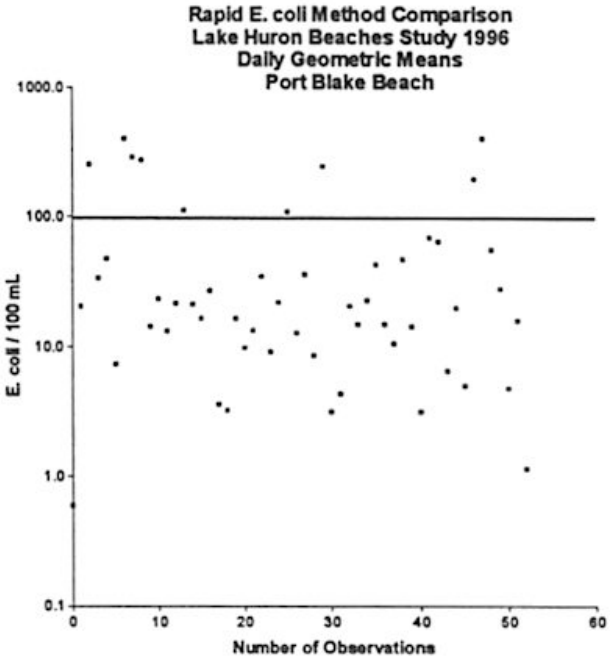


Figure 2: Daily Geometric Mean levels of E. coli for the summer months at Port Blake Beach.

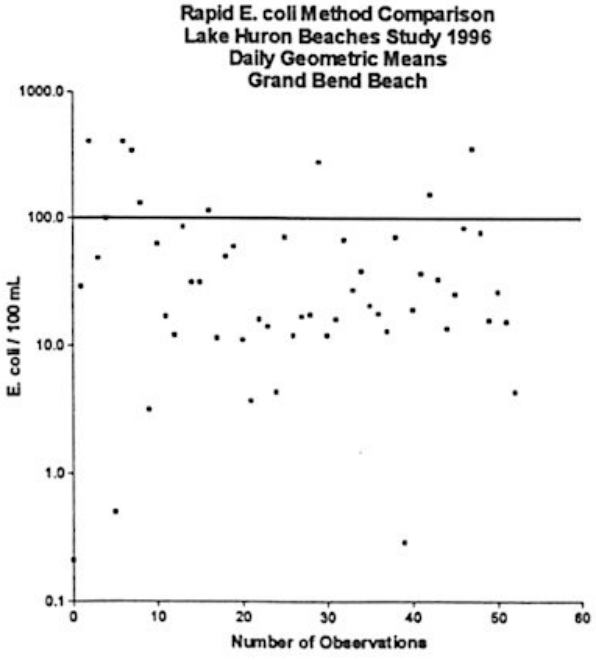


Figure 3: Daily Geometric Mean levels of E. coli for the summer months at Grand Bend Beach.

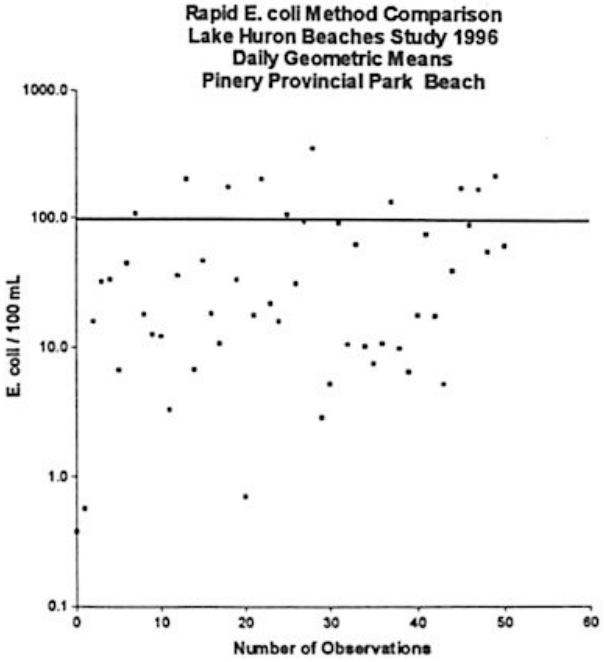


Figure 4: Daily Geometric Mean levels of E. coli for the summer months at Pinery Provincial Park Beech.

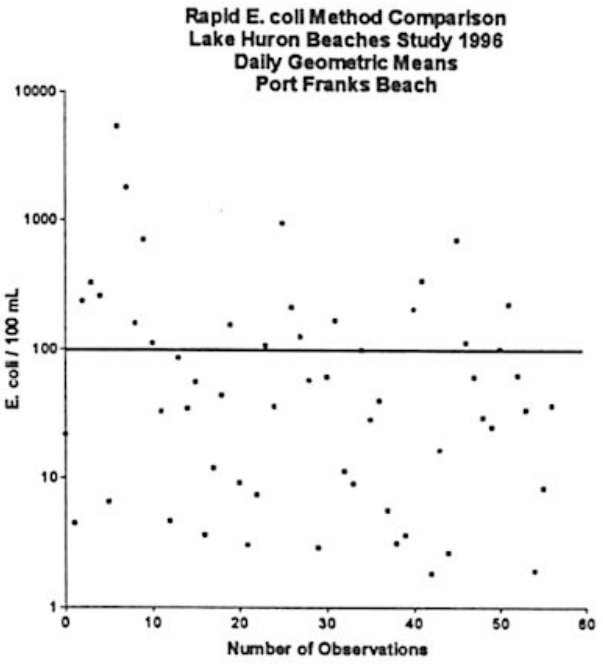


Figure 5: Daily Geometric Mean levels of *E. coli* for the summer months at Port Franks Beach.

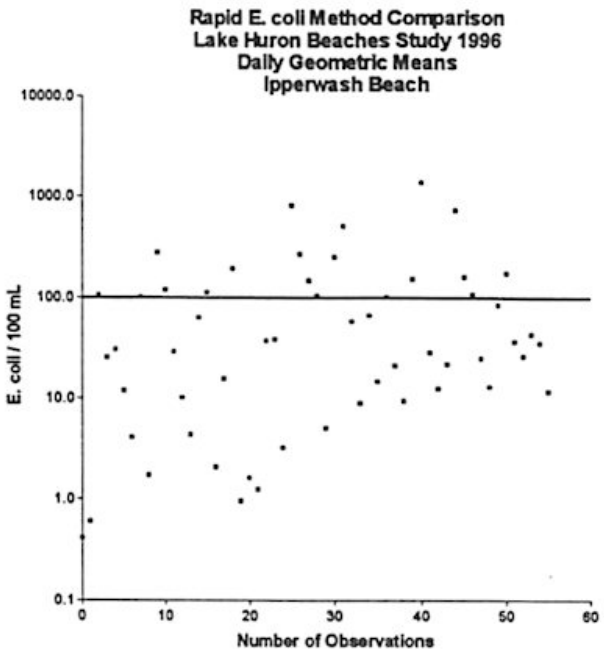


Figure 6: Daily Geometric Mean levels of *E. coli* for the *summer* months at Ipperwash Beach.

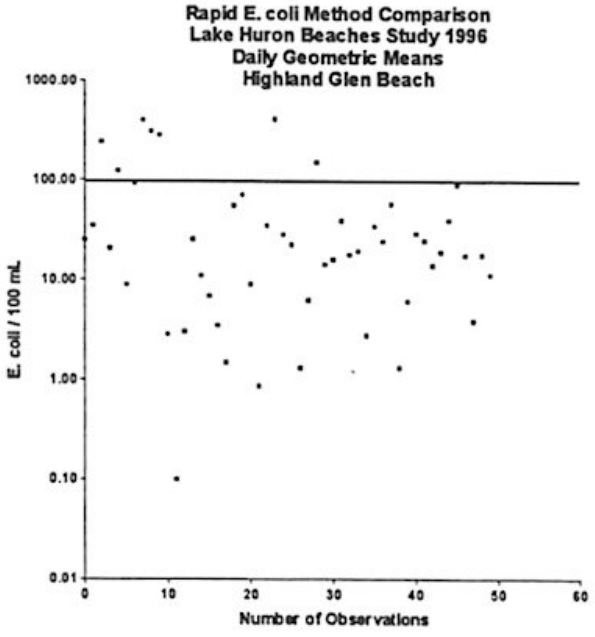


Figure 7: Daily Geometric Mean levels of *E. coli* for are summer months at Highland Glen Beach.

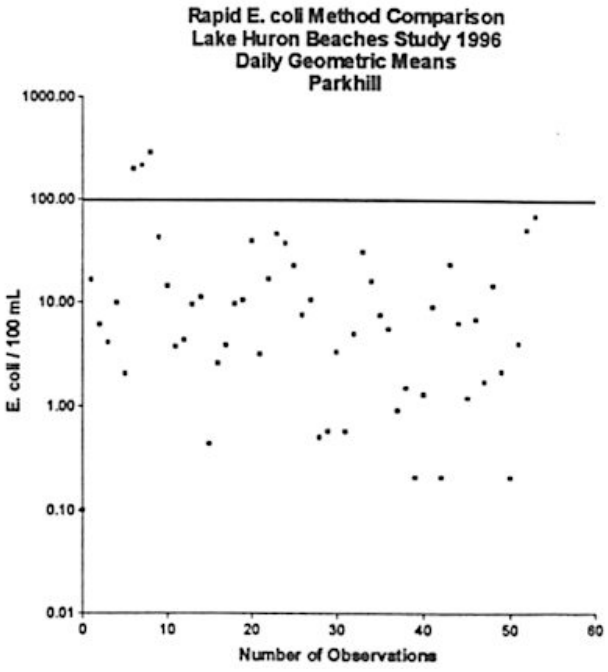


Figure 8: Day Geometric Mean levels of *E. coli* for the summer malls at Parkhill Beech.

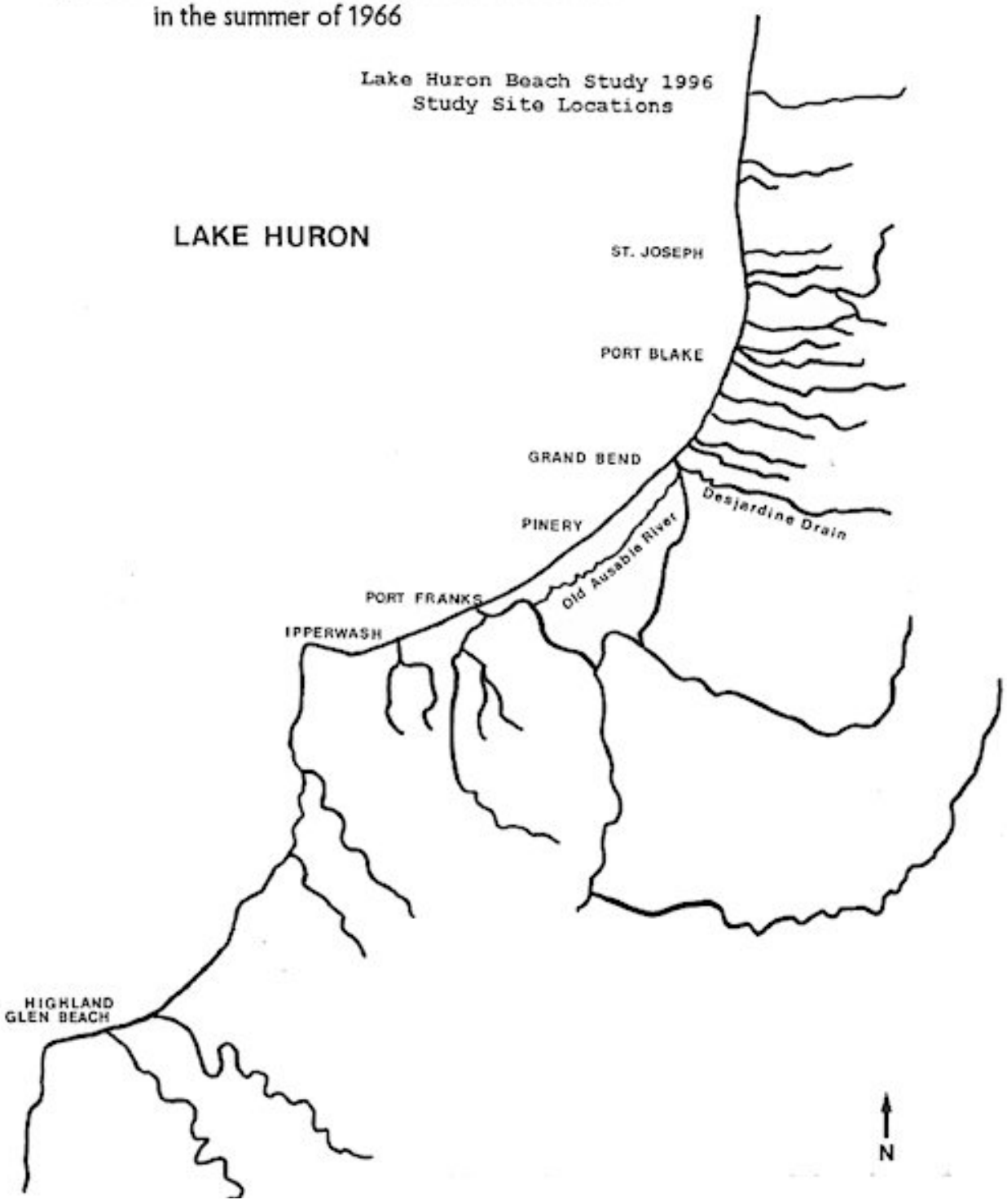
A number of reports have demonstrated that detection of *E. coli* in food and water can be facilitated using chromogenic substrates for the enzyme, ß-D-glucuronidase. The ß-D-glucuronidase enzyme (GUD) is present in most (greater than 94 percent) *E. coli,* however, the enterohemorrhagic strain, *E. coli* 0157,H7, is negative, although this strain has the gene that codes for the ß-D-glucuronidase enzyme. Other bacteria in the Enterbacteriaceae group that have ß-D-glucuronidase activity are as follows: 40 to 67 percent of the *Shigella* strains, 17 to 29 percent of the *Salmonella sp.,* a few strains of *Yersinia sp.* and occasional strains of *Citrobacter, Enterobacter, Edwardia* and *Hafmia sp.* However, Feng and Hartman, 1982, cite that the low incidence of ß-D-glucuronidase of non-E. *coli* pathogens in food and water samples is not considered to compromise significantly either the sensitivity or the purpose of the GUD assay.

The number of beaches chosen for the 1996 study was expanded from five to eight, using the standard testing protocol for *E. coli* on all eight, while the Rapid *E. coli* procedure was employed on five beaches on a rotational basis. The beaches sampled in the study are shown in Table 1 in conjunction with the sampling times for each beach. Grand Bend was sampled twice (once at 6:15 a.m. and again at 12:00 p.m.) to assess the potential for change in bacterial water quality during the approximately six hour period. In addition, one inland beach, Parkhill Reservoir, was chosen to assess a body of water other than Lake Huron. The map in Figure 9 indicates the locations of the Lake Huron beaches.

TABLE 1: Beaches sampled in 1996 and the sampling times.

BEACH LOCATION	TIME OF SAMPLING
St. Joseph's	8:45 a.m.
Port Blake	8:00 a.m.
Grand Bend - a.m.	6:15 a.m.
Pinery Provincial Park	6:50 a.m.
Port Franks	7:30 a.m.
Ipperwash	8:10 a.m.
Highland Glen	8:50 a.m.
Parkhill	8:15 a.m.
Grand Bend - p.m.	12:00 p.m.

Figure 9. Map showing Lake Huron beaches studied in the summer of 1966



It was understood that sampling in the morning was necessary in order to take advantage of the six hour test. If a sample was taken at 7:00 a.m., for example, the test result would be available by 1:30 p.m., which was just in advance of the peak swimming period between 2:00 to 4:00 p.m.

Sampling Protocol and Frequency

The beaches were sampled using the Ministry of Health protocol, where five samples were taken in waist-deep water (approximately 1.5 metres in depth) at a point 30 centimetres below the surface.

The five sites were chosen by dividing the bathing area of the beach into five equal sections of the shoreline.

The samples were taken in 500 mL sterile plastic bottles and, immediately following the sampling, were placed in coolers containing ice.

The water and air temperatures were taken at the time the samples were collected. The numbers of swimmers, seagulls and pets, as well as the wave height (which was shown to correlate with *E. coli* levels in the 1995 study) were recorded daily for each beach. Rainfall, wind speed and wind direction were also recorded daily throughout the entire study.

The study was expanded to determine the bacterial water quality of eight sites that were suspected of contributing *E. coli to* the beach water. These sites included creeks, agriculture drains and rivers. Table 2 lists the locations of these eight sites.

TABLE 2: Creeks and drains sampled during the summer of 1996.

RIVER / CREEK LOCATIONS
St. Joseph's Creek
Grand Bend Harbour
Stephen "B" Line Creek
Mud Creek
Jericho Creek
Highland Glen River
Ausable River
Parkhill Creek
Tri-County Bridge

In past studies, the water quality of specific beaches on Lake Huron has been shown to vary somewhat according to the slope of the bottom of the bathing area (i.e., approximately between 0 and 30 metres from shore). As a result, the slope of each beach, including the inland beach, was measured and recorded as indicated in Table 3.

TABLE 3: Slope of each beach in the 1996 study.

BEACH	SLOPE
St. Joseph's	0.07
Port Blake	0.08
Grand Bench	0.10
Pinery Provincial Park	0.05
Port Franks	0.04
Ipperwash	0.02
Highland Glen	0.08
Parkhill	0.07

Test Protocol

As indicated previously, the test protocol used for this study was modified from the 1995 study.

The water samples were first processed by drawing 100 mL through a 60 mL syringe using two aliquots of 60 mL and 40 mL. Each of the twenty-five syringes, the number required for twenty-five samples of the five beaches in the study, were connected to a vacuum manifold, which was, in turn, attached to a vacuum source.

Following the filtration of the sample through a 25 mm membrane filter with a 0.45 µm pore size, which was Luer-locked to the 60 mL syringe, the syringe was removed from the filter and replaced with a 3 mL syringe. The syringe plunger was removed and saved. A growth medium tablet containing the desired nutrients, including ß-D-galactoside and ß-D-glucuronide, was placed into the 3 mL syringe aseptically.

Two mL of Millipore Super Q water were dispensed into the 3 mL syringe containing the growth medium tablet. The tablet was left to dissolve for five to ten minutes, after which the syringe was shaken gently and 1 mL of liquid medium was discharged to waste. A cap was placed on the syringe tip, which then was incubated at 44.5°C for six hours. The procedure was repeated for the remaining samples.

The incubator was humidified through the passive technique of placing a tray containing water in the bottom of the incubator. This technique prevented excessive drying of the membrane filters during incubation.

After the six hour incubation period, the syringes and filters were prepared for the enzyme extraction with lysozyme, a LE reagent. The plunger of the syringe was aseptically removed and the LE reagent was dispensed into the syringe. With the plunger replaced, 1 mL of reagent solution was discharged onto the filter and was incubated for another five minutes at 44.5°C. Following this incubation period, the remaining LE reagent was dispensed into a test tube.

Next, 0.2 mL of gal-substrate or gluc-substrate were added to a microtube. Then 0.2 mL of the sample were added to the microtube, which was then placed in the block incubator at 44.5°C for ten minutes.

Following the ten-minute incubation period, a stop tablet was aseptically placed into the microtube, which was then placed in the luminometer. The luminometer was activated and a readout in Relative Light Units (RLU) was generated for either the galactosidase or glucuronidase enzymes. The resulting RLU values were then compared to the standard membrane filtration data conducted on the same samples.

The standard development of the relationships between the RLU values generated from the galactosidase and glucuronidase assays and the membrane filtration results are displayed in Figures 10 and 11. As the galactosidase substrate was utilized for a month before the glucuronidase substrate was available, there are more data comparisons for the galactosidase assays than for the glucuronidase assays.

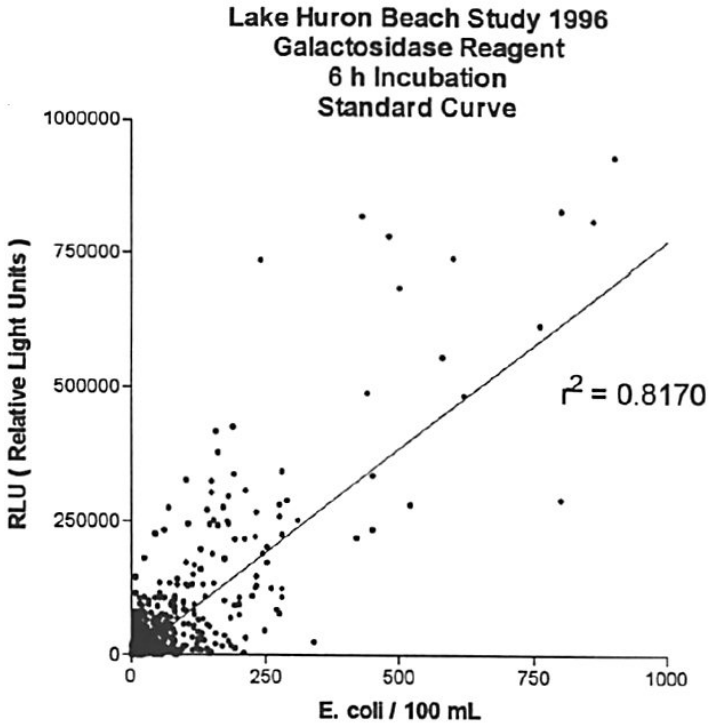


Figure 10: Relative Light Units using the Galactosidase assay compared to the membrane filtration test for *E. coli*.

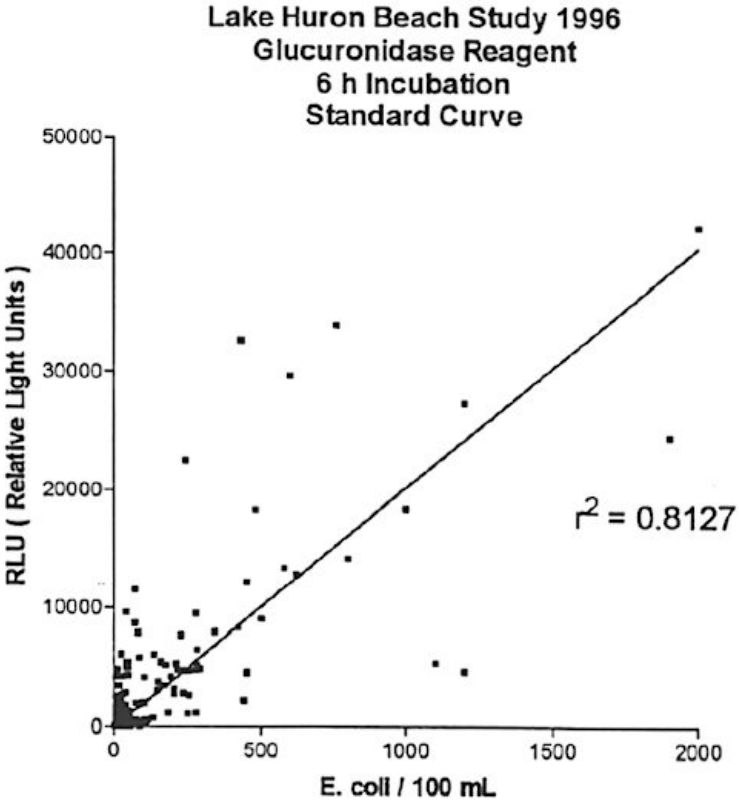


Figure 11: Relative Light Units using the Glucuronidase assay compared to the membrane filtration test for E. coli.

The standard curves produced initially were based on beach data. As the rivers and/or creeks were sampled, the resulting data comparisons (each Rapid *E. coli* test was accompanied by a standard membrane filtration test) were incorporated into the standard curves for the respective enzyme assays.

The geometric mean of the *E. coli* concentration, along with maximum and minimum data, are shown in Table 4 for each location. The *E. coli* levels determined by the Rapid Method were few in number. As a result, they were compared to the standard method using the paired t-test to check for a significant difference.

TABLE 4: Maxima, minima, and geometric mean data of *E. coli* levels at the nine creek and drain sites as determined by membrane filtration.

SITE	Maximum Conc. (*E. coli* / 100 ml)	Minimum Conc. (*E. coli* / *100* ml)	Geometric Mean Conc.
Ausable River	2,600	0.1	70.17
Drainage Ditch	4,100	500	1,175
Grand Bend Harbour	1,000	0.1	52.17
Highland Glen	3,000	0.1	121.4
Mud Creek	3,400	0.1	140.4
Jericho Creek	1,500	0.1	95.91
Stephen "B" Line	10,000	50	381.3
St. Joseph's	3,300	20	313.5
Tri-County Bridge	1,000	30	169.5

Zero, One and Four Hour Analyses

To evaluate the capability of the Rapid *E. coli* test to be reduced to a shorter incubation period, the six hour incubation period was altered to either zero, one or four hours, after which the standard six hour protocol was followed. The zero time analysis, a direct analytical protocol termed the Mini-Test, was conducted as described below. The one and four hour tests were conducted using the same protocol as the six hour test, only the incubation periods were shortened to one and four hours respectively.

The Mini-Test, or the zero time incubation period, was conducted by placing 0.2 mL of LE reagent into a microtube followed by the addition of 0.2 mL of the water sample. The microtube was mixed gently and incubated for five minutes at 44.5°C. Then, 0.2 mL of dioxetane-ß-D-galactosidase were added to the microtube, mixed and incubated for ten minutes at 44.5°C. After incubation, one stop tablet was added to the microtube and the tube was placed into the luminometer. The LUM assay was performed.

The RLU values did not correlate well with the membrane filtration data, as indicated by an R^2 value of 0.15 (see Figure 12). It is speculated that the number of *E. coli* in the 0.2 mL sample aliquot was too low to provide sufficient enzymes to produce enough light to be detected accurately. Perhaps a longer incubation period of twenty to thirty minutes was necessary, however, further testing at this time was not possible.

It has also been demonstrated by Davies *et al.*, 1994, that plant and algal interference occurs with both ß-D-galactosidase and ß-D-glucuronidase assays in surface waters. It is possible that, with the 0.2 mL of sample volume, the amount of enzymes from plant and algal sources, although low in concentration, may have been enough to affect the result with respect to the bacterial enzyme levels.

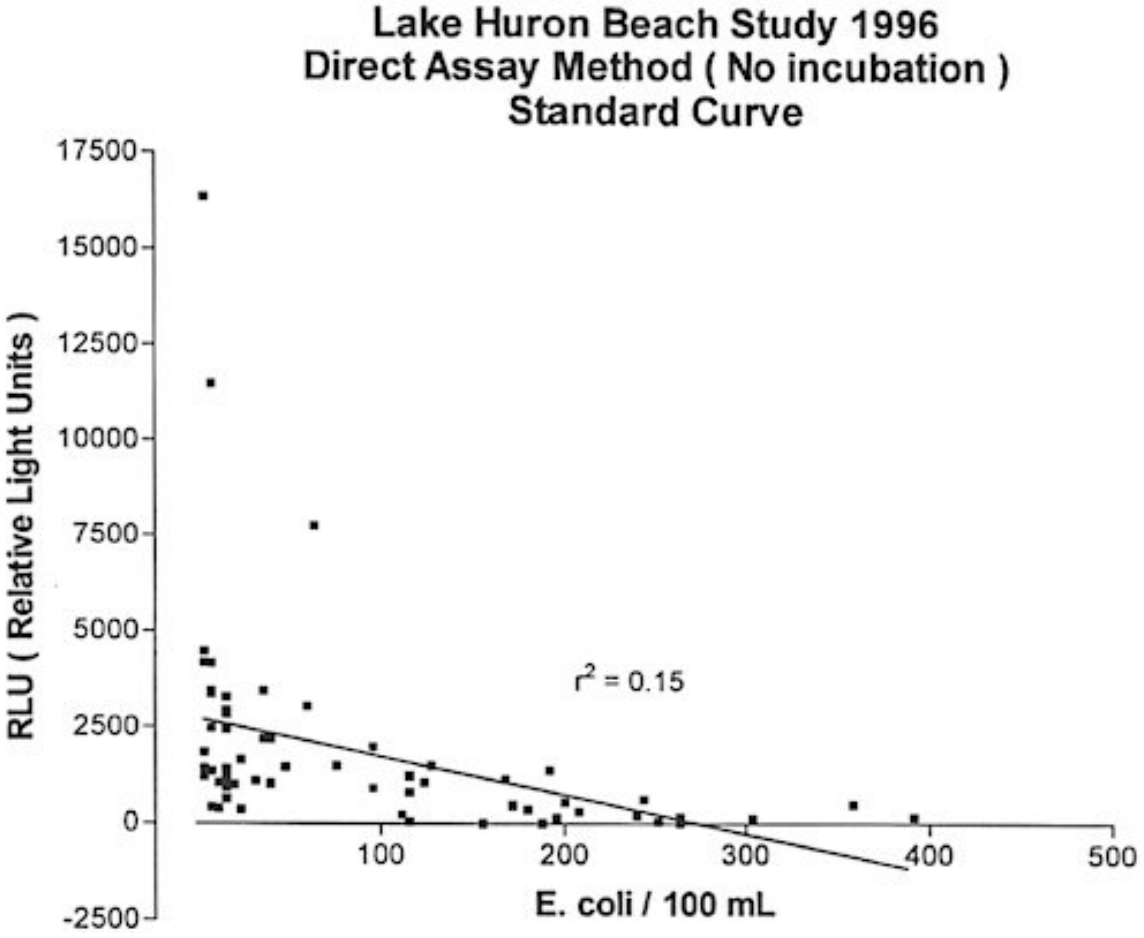


Figure 12: Relative Light Units using the Galactosidase assay with no incubation period compared to the membrane filtration test for *E. coli*.

The one hour assay for galactosidase produced an R^2 value of 0.6 while the one hour assay for glucuronidase produced an R^2 value of 0.72. (See Figures 13 and 14.) The chemo-luminescence visible at one hour was enough to demonstrate that a relationship existed. However, based on the protocol used, it was insufficient to ensue accurate predictions.

In contrast, the results of the four hour incubation for both the galactosidase and the glucoronidase assays were more encouraging. As can be seen in Figures 15 and 16, for the two enzyme assays of the Rapid Method, the regression of the four-hour Rapid Method data for the galactosidase assay against the corresponding Membrane Filtration data produced an R^2 value of 0.98, while the R^2 value for the glucuronidase assay was 0.99. Although the data comparisons were limited, the four hour incubation period exhibited comparative data that suggested the test may be reduced from six to four hours.

The results of the Rapid *E. coli* analyses using the galactosidase assay were evaluated initially by assessing the number of times the Rapid Method and the Standard Method produced results above the 100 *E. coli* per 100 mL standard and the percentage of agreement between both tests. Similarly, the number of times each method was below the *100 E. coli* per 100 mL standard and the number of times agreement occurred were evaluated. The results of the data comparisons are shown in the following tables (Tables 5 through 12) on an individual beach basis.

The comparison of the data from Grand Bend beach resulted in 47 occasions of 265 membrane filtrations where the data was above the 100 *E. coli* standard. Of the 55 Rapid Method tests using the galactosidase assay, nineteen were also above the 100 *E. coli* standard. Both tests agreed in 92 percent of the parallel testing, as shown in Table *7.*

In these 265 membrane filter tests, 218 were below the 100 *E. coli* standard. Of 155 Rapid Method tests, 136 were below the 100 *E. coli* standard. Both tests agreed in 98 percent of the parallel testing.

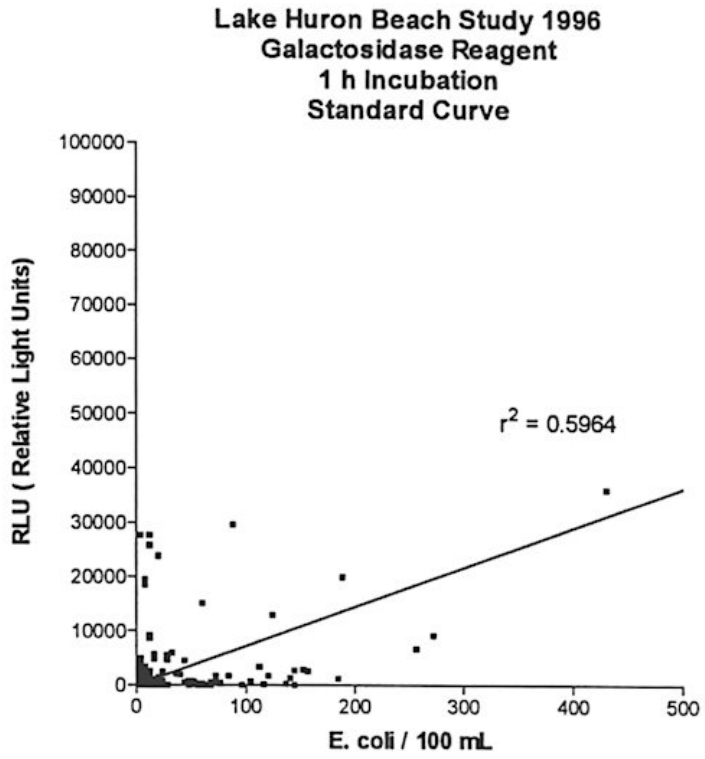


Figure 13: Relative Light Units using the Galactosidase assay with 1 hour incubation compared to the membrane filtration test for *E. coli*.

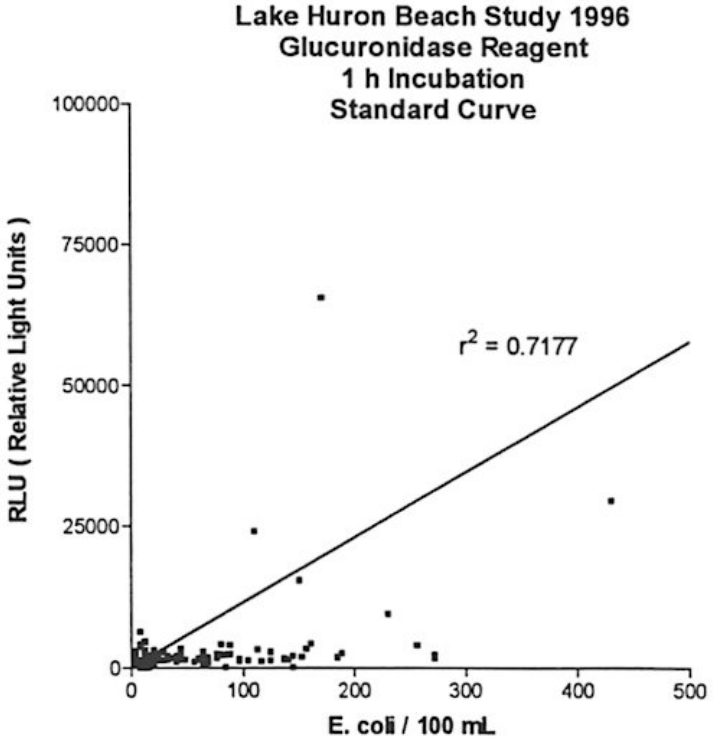


Figure 14: Relative Light Units using the Glucuronidase assay with 1 hour incubation compared to the membrane filtration test for *E. coli.*

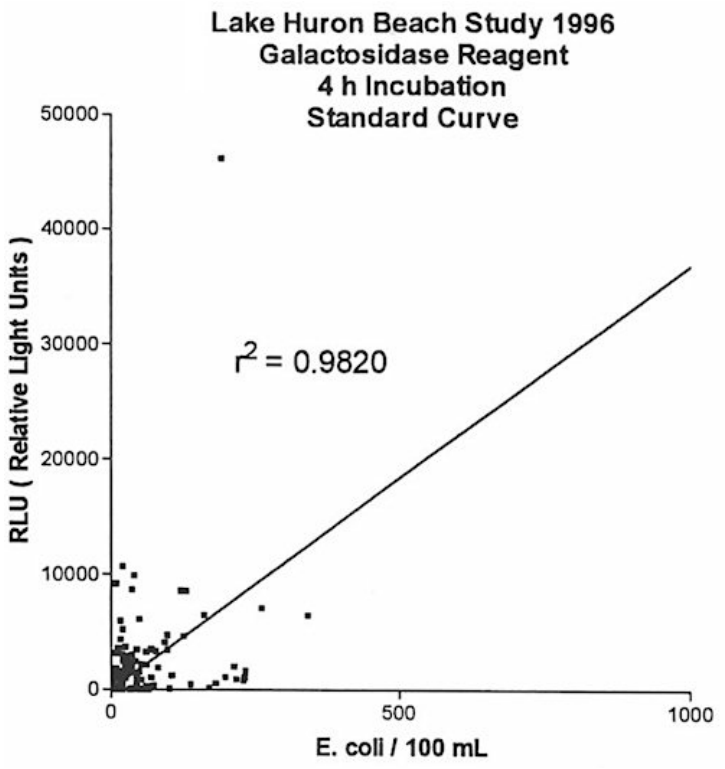


Figure 15: Relative Light Units using the Galactosidase assay with 4 hour incubation compared to the membrane filtration test for *E. coli*.

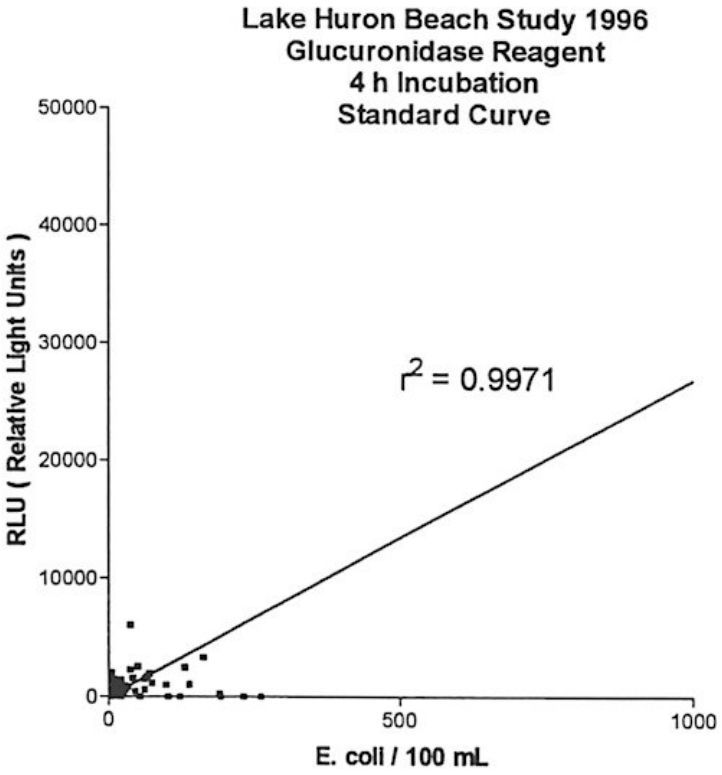


Figure 16: Relative Light Units using the Glucuronidase assay with 4 hour incubation compared to the membrane filtration test for *E. coli*.

Table 5. Statistical comparison of results above and below 100 *E. colt* per 100 mL for both the Rapid *E. coli* method and Membrane Filtration at St. Joseph's Beach.

Greater than 100 EC/ 100 mL			Less than 100 EC/ 100 mL		
MF	LUM	Both tests in agreement	MF	LUM	Both tests in agreement
54/270 20%	12/140 9%	5/20 25%	216/270 80%	128/140 91%	99/105 94%

Table 6. Statistical comparison of results above and below 100 *E. colt* per 100 mL for both the Rapid *E. coli* method and Membrane Filtration at Port Blake Beach.

Greater than 100 EC/ 100 mL			*Less* than 100 EC/ 100 mL		
MF	LUM	Both tests in agreement	MF	LUM	Both tests in agreement
44/264 17%	9/130 7%	1/7 14%	220/264 83%	121/130 93%	101/108 94%

Table 7. Statistical comparison of results above and below 100 *E. coli* per 100 mL for both the Rapid *E. coli* method and Membrane Filtration at Grand Bend Beach.

Greater than 100 EC/ 100 mL			Less than 100 EC/ 100 mL		
MF	LUM	Both tests in agreement	MF	LUM	Both tests in agreement
47/265 18%	19/155 12%	11/12 92%	218/265 82%	136/155 88%	121/123 98%

Table 8. Statistical comparison of results above and below 100 *E. colt* per 100 mL for both the Rapid *E. coli* method and Membrane Filtration at Pinery Provincial Park Beach.

Greater than 100 EC/ 100 mL			Less than 100 EC/ 100 mL		
MF	LUM	Both tests in agreement	MF	LUM	Both tests in agreement
52/255 20%	13/85 15%	8/14 57%	203/255 80%	72/85 85%	56/61 92%

Table 9. Statistical comparison of results above and below 100 *E. coli* per 100 mL for both the Rapid *E. coli* method and Membrane Filtration at Port Franks Beach.

Greater than 100 EC/ 100 mL			Less than 100 EC/ 100 mL		
MF	LUM	Both tests in agreement	MF	LUM	Both tests in agreement
97/285	32/100	23/38	188/285	68/100	47/51
34%	32%	61%	66%	68%	92%

Table 10. Statistical comparison of results above and below 100 *E. coli* per 100 mL for both the Rapid *E. call* method and Membrane Filtration at Ipperwash Beach.

Greater than 100 EC/ 100 mL			Less than 100 EC/ 100 mL		
MF	LUM	Both tests in agreement	MF	LUM	Both tests in agreement
88/280	26/85	24/35	192/280	59/85	35/35
31%	31%	69%	69%	69%	100%

Table 11. Statistical comparison of results above and below 100 *E. coli* per 100 mL for both the Rapid *E. coli* method and Membrane Filtration at Highland Glen Beach.

Greater than 100 EC/ 100 mL			Less than 100 EC/ 100 mL		
MF	LUM	Both tests in agreement	MF	LUM	Both tests in agreement
39/250	5/65	5/11	211/250	60/65	49/49
16%	8%	45%	84%	92%	100%

Table 12. Statistical comparison of results above and below 100 *E. coli* per 100 mL for both the Rapid *E. coli* method and Membrane Filtration at Parkhill Beach.

Greater than 100 EC/ 100 mL			Less than 100 EC/ 100 mL		
MF	LUM	Both tests in agreement	MF	LUM	Both tests in agreement
16/180	6/95	1/1	264/280	89/95	64/69
6%	6%	100%	94 %	94%	93 %

The comparisons for each method for each beach varied as can be observed in reviewing the data for the individual beach. The data from the inland beach, Parkhill Reservoir, was interesting, as it compares well with the Lake Huron beaches. When comparing the data above or below the 100 *E. coli* standard, the 100 and 93 percent agreements respectively using the galactosidase assay were impressive.

The comparison of the rapid method using the galactosidase assay to the membrane filtration method for all beaches, with results above the 100 *E.* coli per 100 mL standard, was 70 percent, whereas, the percent agreement for all beaches below the 100 *E. coli* per 100 mL standard was 92. In comparing the data from all beaches using the glucuronidase assay, the percent agreement for results above 100 *E. coli* per 100 mL was 83. For the data below 100 *E. coli* per 100 mL, the percent agreement between the rapid method using the glucuronidase assay and the membrane filtration method was 87.

The water at the Grand Bend beach was sampled at 6:15 a.m. and again at 12:00 p.m., which produced data that was very important to the study. It is well known that the bacterial water quality can change significantly from day to day. (Palmateer and Huber, 1984). If the Rapid *E. coli* test is to be effective, a beach must be sampled in the early morning, so that the Rapid *E. coli* Method can provide *E. coli* data by the peak swimming period of 2:00 to 4:00 p.m. If the water quality changed between early morning and noon, the value of the Rapid Test would be questionable.

The data from the Grand Bend beach was analyzed using a t-test to observe whether the *E. coli* data produced by the membrane filtration procedure was different when the 6:15 a.m. data was compared to the 12:00 p.m. data, based on the variance of the two data sets. Based on fifty-four data comparisons, the results of the t-test showed no significant difference.

It can be concluded, because the data generated from the 6:00 a.m. sampling was not different from the 12:00 p.m. sampling, that the bacterial water quality remained the same.

Consequently, the Rapid *E. coli* testing conducted on the early morning samples produced data relevant to the bacterial water quality at 12:00 p.m.

It can be observed that, from the comparison data above the 100 *E. coli* standard for the galactosidase assay, the rapid method was different to the membrane filtration method.

For both of the rapid method assays, the agreement between the rapid method and the membrane filtration procedure was lower for the comparison above the 100 *E. coli* standard than was the agreement between the methods for data below the 100 *E. coli* standard. It was presumed that with turbid water, which consistently has higher *E. coli* concentrations (above the 100 *E. coli* standard), there was an interference in the enzyme assay. It may have resulted from the chemo-luminescent substrate not being able to interact with the two enzymes of *E. coli.* It is possible that the lysozyme was not able to lyse the cell wall due to the physical interference of the particulates surrounding the *E. coli* cells on the membrane surface. This observation was consistently observed on the individual beach data, except for data from the Parkhill Reservoir beach.

It is interesting to compare the relative closeness of the data from the galactosidase and glucuronidase assays. From previous studies (Palmateer and Huber, 1984 and 1985), the levels of fecal coliform bacteria and the levels of *E. coli* in the recreational waters of Lake Huron were very similar. In this study, the *E. coli* concentrations were typically comprised of 92 to 97 percent of the fecal coliform concentrations in the same waters. Consequently, both the galactosidase and glucuronidase assays produced similar data.

The reproducibility of the Rapid Six Hour Method was compared to the Standard Method by analyzing the same sample ten times. Figure 17 shows the data comparison. Although the geometric means of 337 and 472 *E. coli* per 100 mL of the Standard Method and the Rapid Method respectively were significantly different based on a t-test, the bacteriological significance of the difference in the two results was

similar. The probability of disease-causing bacteria being present in the water or the risk of illness from swimming in the water with either geometric mean would be similar (Dufour *et al.,* 1982).

The creeks and rivers that flow into Lake Huron were tested at specific sites, as previously mentioned, using the Standard Method. The maximum, minimum and geometric means for the testing periods are shown in Table 4.

The Rapid Method was used to test the creeks and river sites and, on a few occasions, to test the Rapid Method in waters with heavy bacterial loadings. The t-test, comparing geometric means, showed no significant differences between the geometric means produced by either method, although only thirty-two comparisons were made.

The creeks and rivers investigated were found to vary dramatically in concentrations of *E. coli.* For example, the geometric mean of *E.* coli levels per 100 mL at the Stephen "B" Line site was 381, which is well above the 100 *E. coli* per 100 mL beach standard. The maximum and minimum value of the 28 tests was 10,000 and 50 respectively. Other creek and river sites with the potential to impact the beach showed similar results in that the geometric means were well above the beach standard and, at times, based on maxima data, show that major loadings of *E. coli* are reaching the beaches. See Table 4.

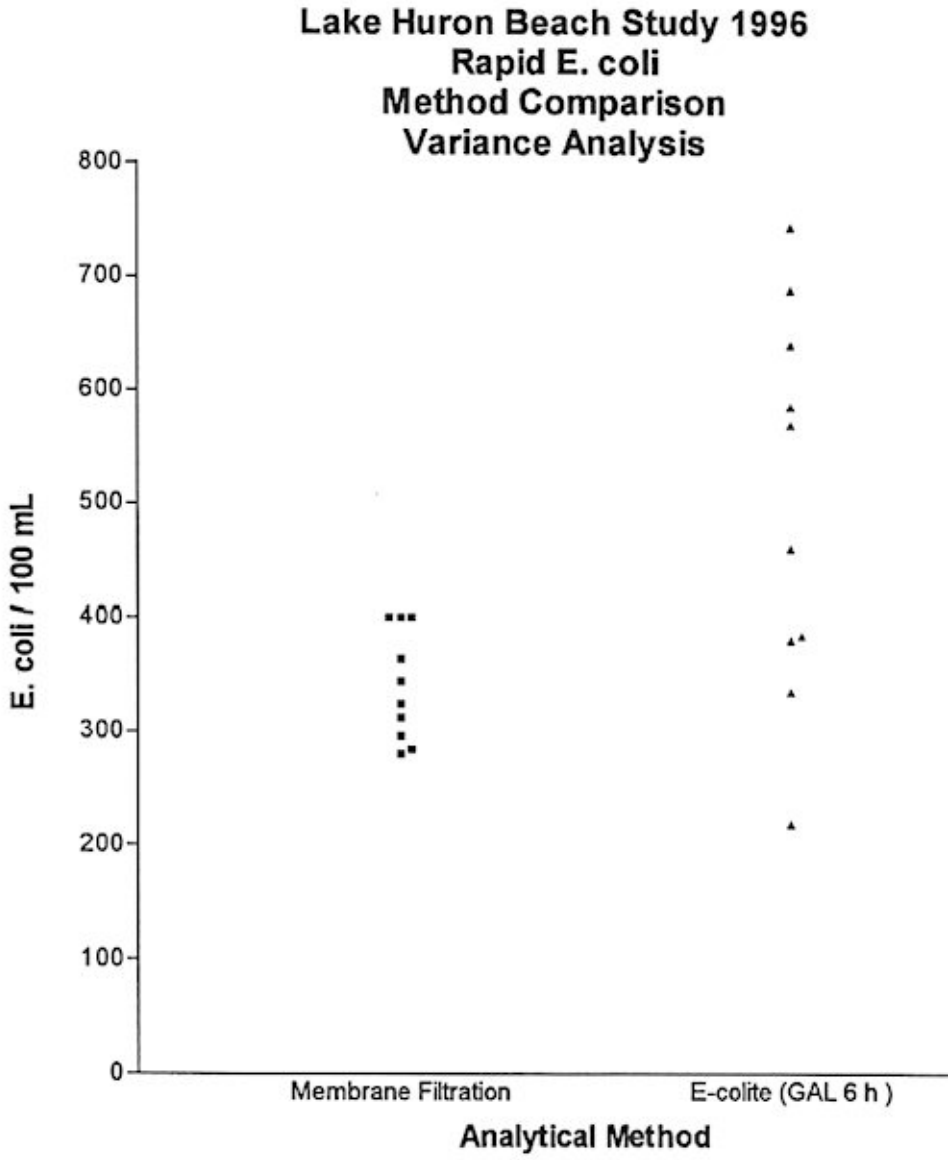


Figure 17: *Comparison of results with 10 replicates of the Rapid E.* coli test compared to results of 10 replicates using the membrane filtration test

The beaches studied showed a consistent pattern in the variability of the daily geometric means of *E. coli* concentrations. Table 13 displays the percentage of occurrences when the daily means were above the 100 *E. coli* standard.

TABLE 13: Geometric means for the entire season and frequency of means above the Provincial Standard

Beach	Geometric Mean	Percentage Above Standard
St. Joseph's	75.8	20
Port Blake	59.6	17
Grand Bend	66.2	15.1
Pinery	57.4	19.6
Ipperwash	117.8	32.1
Port Franks	233.7	33.3
Highland Glen	57.6	14
Parkhill Reservoir	24.1	5.6
Grand Bend	86.9	19.6

Port Franks beach had the poorest bacterial water quality since 33.3 percent of the days during the study were unsatisfactory. In contrast, the *E. coli* levels exceeded the standard in only 5.6 percent of the days of the study at the Parkhill Reservoir beach. It is interesting to note that the Ipperwash and Port Franks beaches are the two shallowest beaches. The slope of the Port Franks beach is 0.04 and the slope of the Ipperwash beach is 0.02. It is speculated there is less mixing of the littoral zone water with the lake water, which is of better bacterial water quality further from the shore, at these shallow beaches.

It is obvious that other factors are contributing to the bacterial water quality. The discharge of creeks or rivers into Lake Huron in the proximity of the beaches is potentially the major factor affecting the *E. coli* concentrations.

The following factors have been documented to affect the water quality of bathing beaches:

1. Sewage plant discharge;
2. Combined sewer overflow;
3. Septic tank discharge;
4. Agricultural runoff;
5. Seagulls and other wildlife;
6. Climatic factors such as: rainfall, wind speed, wind direction, hours of bright sunlight and water and air temperature;
7. Bathers;
8. Power and sailboats — black and grey water discharge.

Of the above factors, agricultural runoff has been shown to be a major source of the fecal bacteria that affects the bacterial water quality. Seagulls were also identified as an additional source of fecal bacteria. Of the climatic factors, wave height, wind speed and direction also contribute to the fluctuations in the bacterial water quality of the beaches studied.

The correlations between the *E. coli* levels of each beach and water temperature, wave height, bathers, pets and seagulls were studied and the only significant correlation to be found was with wave height. This was also the case in the 1995 study.

It has been demonstrated by Palmateer and Huber, 1985, that, as the wave height increases, bottom sediments at the sediment-water interface become resuspended. These sediments have been identified as having elevated levels of fecal bacteria, including *E. coli,* associated with them. Consequently, as the sediment containing the *E. coli* bacteria became resuspended by increased wave action, as indicated by

increased wave height, the levels of *E. coli* in the water column also increased. As seen in Table 14, the highest correlation was shown at the Ipperwash Beach (Pearson R = 0.77). The wave action at a beach is the net result of wind speed and direction and the natural littoral currents.

TABLE 14: List of correlation coefficients between daily *E. coli* levels and corresponding wave height for each Lake Huron Beach

BEACH LOCATION	CORRELATION COEFFICIENT (Pearson R)
Grand Bend (a.m.)	0.61
Grand Bend (p.m.)	0.60
St. Joseph's	0.57
Port Blake	0.63
Port Franks	0.14
Pinery Provincial Park	0.54
Parkhill	-0.06
Ipperwash	0.77
Highland Glen	-0.09

The statistical analysis of the data comparing *E. coli* concentrations and bathers, pets, seagulls and wave height are displayed graphically as wind velocity component scatter plots. The wind velocity component was defined as the distance that the wind has travelled directly to the beach during the six hours before the sampling took place.

The wind velocity component has direction and the most relevant direction for each beach was calculated. The direction for each beach is as follows: St. Joseph's — west; Port Blake — west; Grand Bend — northeast; Pinery — west; Port Franks — west; Ipperwash — west; and Highland Glen — north.

It can be observed in Figure 18 that the wind velocity component from the northwest, blowing on shore, increased in size with the logarithm of *E. coli* levels at the beaches on Lake Huron as well as showing the impact on bathers. When the wind came from the opposite or off-shore direction, *E. coli* concentrations declined.

The effect of seagulls is demonstrated in Figure 19 for all beaches except the Parkhill Reservoir. Although the wind component has an effect on the *E. coli* levels, the seagull numbers at a beach had little or no effect when compared to the same beach without seagulls. By definition, beaches with seagulls had greater than five birds and beaches without seagulls had none.

The effect of pets on the beach was tested for an impact on the *E. coli* levels as shown in Figure 20. There is obviously no effect, as pets were present with low levels of *E. coli* and absent with high *E. coli* levels. Beaches with pets were defined as having greater than 0.4 pets present.

The effect of wave height was dramatically demonstrated in Figure 21. Wave action is defined as waves greater than 0.4 metres in height and calm water was defined as having a 0.0 metre wave height. The wave height for all beaches was measured and has proven to have a significant effect on *E. coli* levels. All data points showing significant wave action had elevated *E.* coli levels occurring with the positive wind velocity component for each beach.

The rainfall and water temperature did not show a relationship with increasing *E. coli* levels.

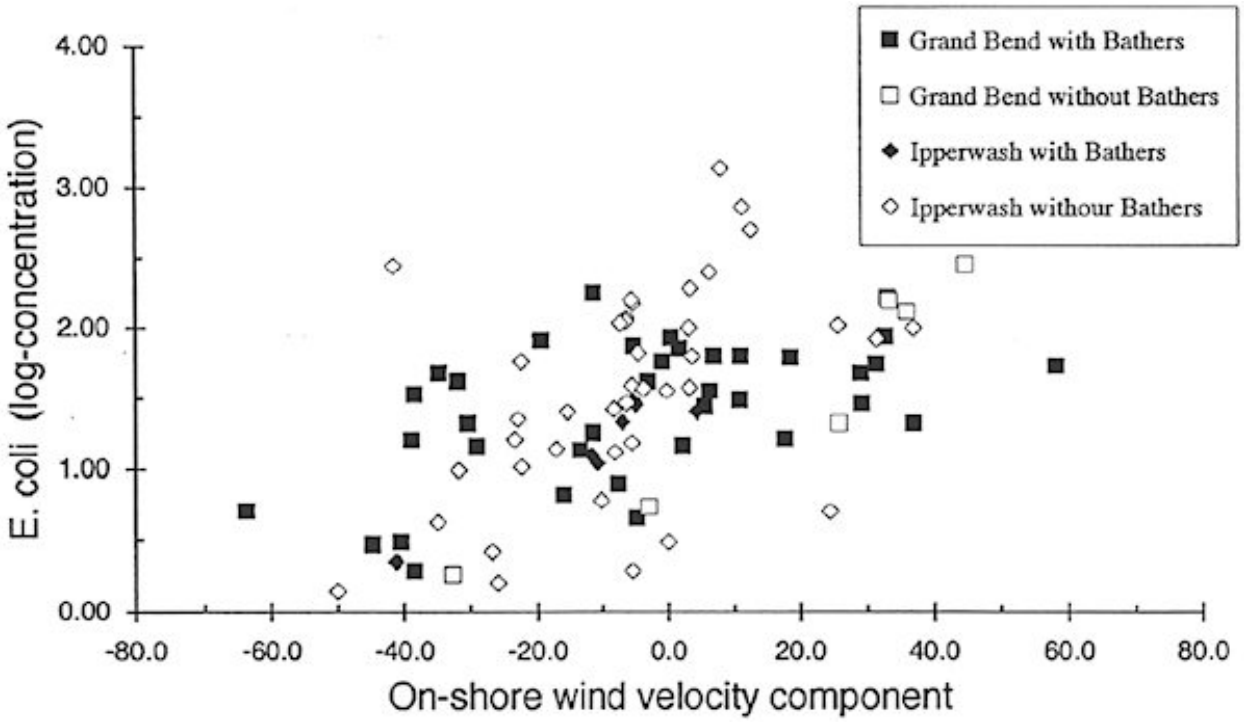


Figure 18: Effect of bathers on *E. coli* levels at the beach with the wind velocity component.

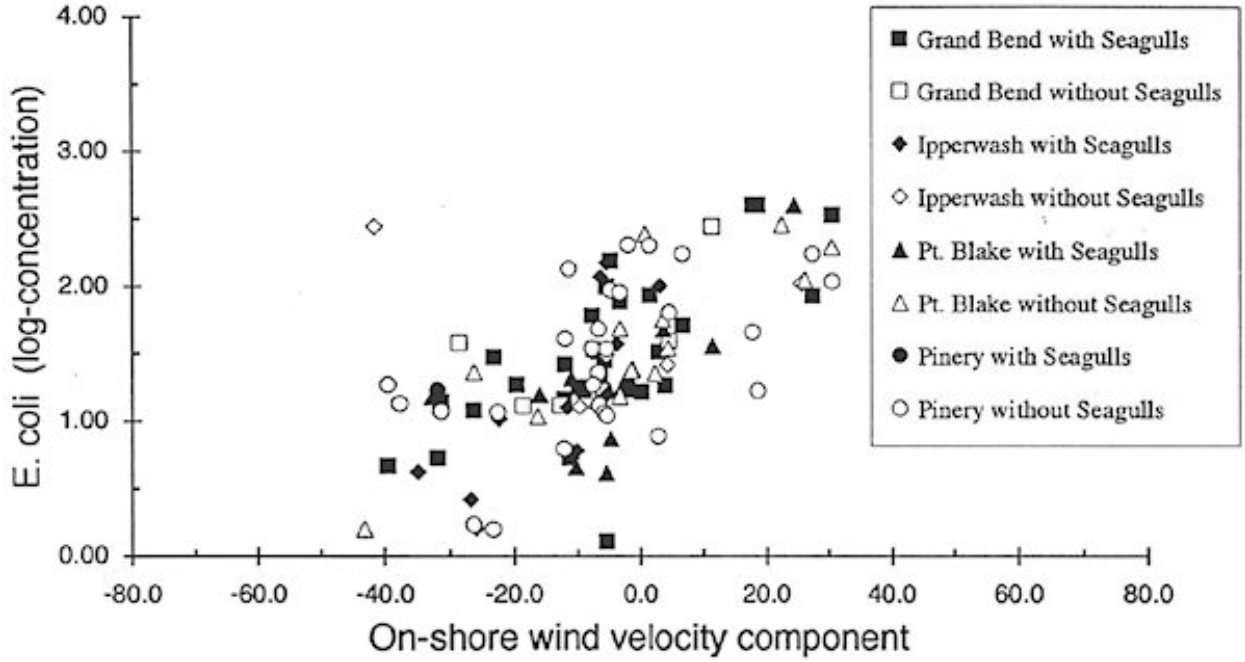


Figure 19: Effect of seagulls on *E. coli* levels at the beach with the wind velocity component.

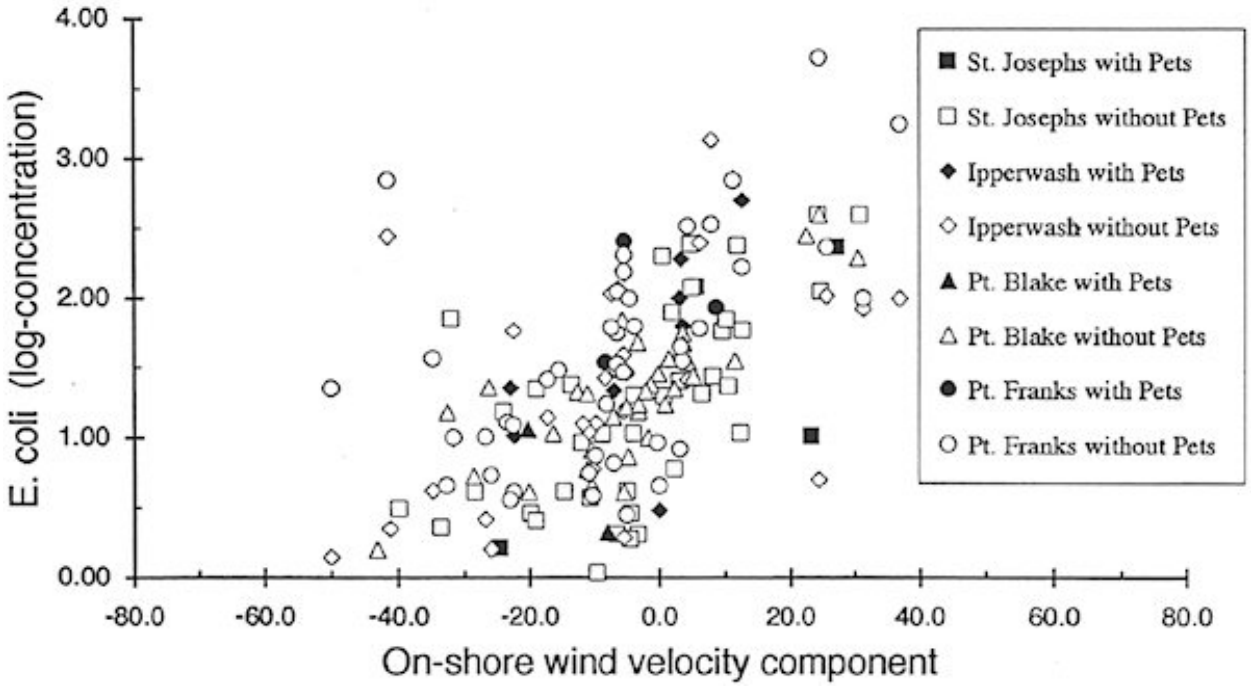


Figure 20: Effect of pets on *E. coli* levels at the beach with the wind velocity component.

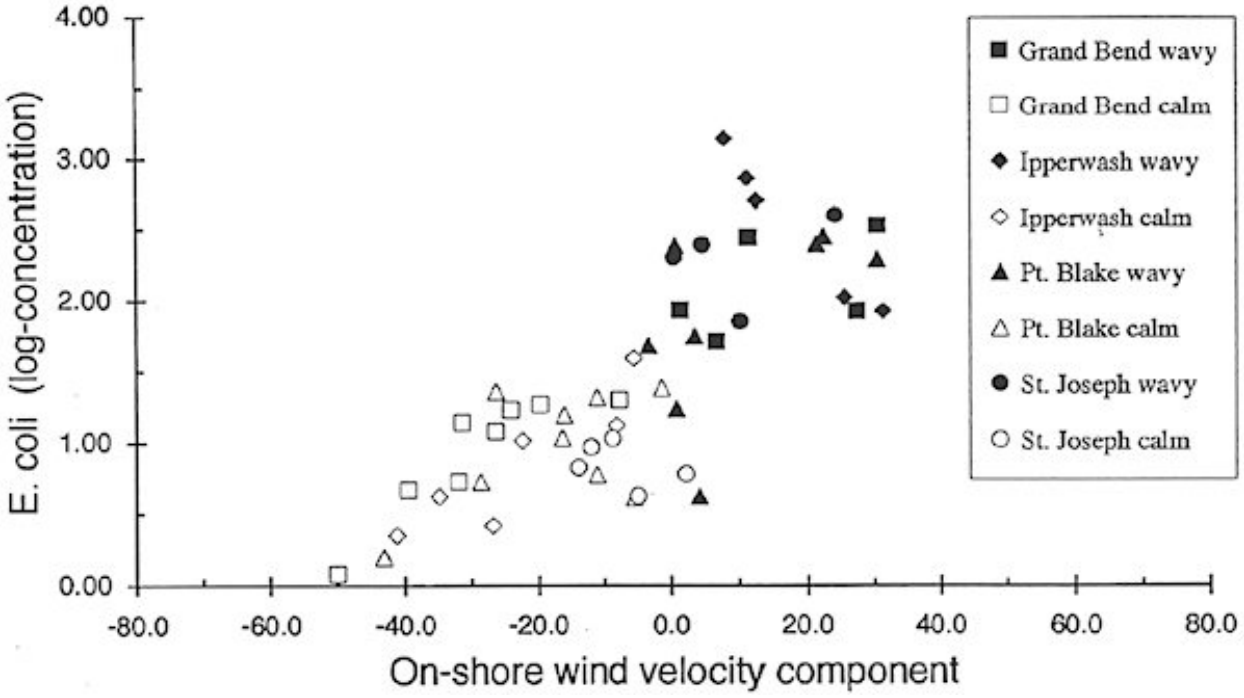


Figure 21: Effect of wave height on *E. coli* levels at the beach with the wind velocity component.

Comparison with the 1995 Study

The beach bacterial water quality of 1996 showed a significant improvement over the 1995 bathing season, as shown in Table 15.

TABLE 15: Comparison of geometric means and percentage of days when the means exceeded the 100 *E. coli* standard for 1995 and 1996.

Beach	*E. coli* Geometric Mean 1995	*E. coli* Geometric Mean 1996	Percentage Above Standard 1995	Percentage Above Standard 1996
St. Joseph's	234.7	75.8	30.2	20
Port Blake	108.6	59.6	26.9	17
Grand Bend	132.8	66.2	30.2	15.1
Pinery	258.7	57.4	36.5	19.6
Ipperwash	149.3	117.8	34.9	32.1

The improvement is interesting. The only beach to have a geometric mean concentration greater than 100 *E. coli* per 100 mL was Ipperwash, which was elevated in 1995, however, the other beaches showed significant improvement.

The rapid *E.* contesting demonstrated a significant improvement in accuracy as compared to the membrane filtration standard method. The percentage of agreement above and below the *E. coli* standards were substantially higher than in 1995. The galactosidase assay and glucuronidase assay performed better than the 1995 study; however, the 1995 study used glucuronidase assay almost exclusively.

The concept of the test is to provide a prediction of the bacterial water quality before the peak daily swimming period. It is evident that with the use of the Rapid Testing,

predictions can be made accurately and safely, so that a real protection of public health can be achieved.

In further assessing the accuracy of the rapid method, the coefficient of determination of R^2 for both assays indicates the improvement in test protocol and reagents as compared to last year. A further refinement to be considered is to improve the filtration of a very turbid sample by using a special filter from Gelman Sciences, which contains a 25 µm pre-filter of 10 µm pore size, under which lies the 25 µm diameter, 0.45 µm pore size bacterial filter. The pre-filter should reduce the impact of the particulates in turbid samples.

Appendix A

Sample Site Descriptions for Creeks and Rivers

St. Joseph's Creek:

Turn left on Highway 21 onto St. Joseph's Shore, follow the road to the end. Go down the stairs, follow the wooden path to the wooden bridge. Collect the sample off the bridge using the dipping pole.

Grand Bend Harbour — Ausable River:

The first sample is taken off the middle of the dock at the boat launch. The second sample is collected from the bridge across from Steve's Garage and before Monroe's Marine, down the stairs by the gate. Take the sample off the dock.

Stephen "B" Line Creek — Desjardin Drain:

Turn left off Highway 81 from Grand Bend onto Stephen "B" Line and collect the sample at the first creek site encountered. Collect from the east side of the creek.

Port Franks — Mud Creek:

From Grand Bend, follow Highway 21 travelling south into Northville, turn right on the Port Franks Road behind MacPherson's Restaurant, turn left on Riverside Drive, turn right into the Windsor Park Residential Area and follow Bond Road to the beach site. Mud Creek is on the south side of Port Franks beach. The first sample site is at the mouth of the river, 3 to 5 feet. The second site is upstream, across from the white house.

Jericho Creek:

Travel out from Windsor Park, turn right (south) on Riverside Drive, collect the sample on the left side of the road. On the right side is Camp Ipperwash. The landmarks are the Savannah Trail signs on the left side of road. The creek is not

marked with a road sign.

Highland Glen River:

Collect the sample at the river mouth, north of the beach.

Ausable River Site — Drainage Ditch:

Turn off Highway 21 onto Highway 79 (east bound), turn left (north) onto Bog Line Road (County Road 18). The river is the one with a bridge and grey station house. On the left side of the road is a house with a satellite dish. Collect the sample on the left side of the road.

Parkhill Creek Site:

Approximately 4 to 5 kilometres (north east) up the road from the Ausable River site on Bog Line Road (County Road 18). Collect the sample on the left side of the road.

Tri-County Bridge:

Turn onto Greenway Road (Lambton - Middlesex County Road 5) from Highway 21 travelling east. Stop at the river site at the bridge with county and township signs. Collect the sample on the left side of the bridge (north). Note, the right side of the bridge (south) is full of thorn bushes.

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