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The Evaluation of Water Quality and Weather Patterns as Indicators for Escherichia Coli in Slaters Creek Watershed in Millersville, Tennessee

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THE EVALUATION OF WATER QUALITY AND WEATHER PATTERNS AS INDICATORS FOR ESCHERICHIA COLI IN SLATERS CREEK WATERSHED IN MILLERSVILLE, TENNESSEE.

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

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Honors College Graduate Distinction at Western Kentucky University

By

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ABSTRACT

Water quality sampling was conducted in conjunction with the city of Millersville, Tennessee in order to assess levels of Escherichia coli (E. coli) in Slaters Creek. The city of Millersville is under a storm water National Pollutant Discharge Elimination System (NPDES) permit that requires compliance monitoring. In the past, monitoring of E. coli has resulted in noncompliance with state water quality regulations. A water quality assessment, including E. coli and water quality parameters, was conducted to determine if E. coli levels varied between dates within the study area. Statistical methods were utilized to determine if variations existed between the sampling strata, potential sources of pollution, and external influences, such as rainfall. Results indicated that principal components analysis is a viable tool for elucidating water quality changes and explaining variability in the data. Future watershed monitoring in Millersville should include biological sampling to determine the chronic effect of pollutants from storm water runoff.

Keywords: Water Quality, Public Health, E. coli, Storm Water, Principal Components Analysis

Dedicated to the people of Millersville and Goodlettsville, Tennessee and to all the professors and students who have helped me along the way. I couldn't have done this project without all of your wisdom and support.

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PUBLICATIONS

1. Taylor, Ritchie; Golla, Vijay; Myatt, Bob; Advani, Shailesh; Gole, Pragati; Nair, Rasmi; Brown, Jacqueline; Willis, Nate; and Barringer, Ellen, "Warren County, Kentucky Hazardous Materials Commodity Flow Analysis, Final Report" (2010). *Public Health Faculty Publications.* Paper 1. http://digitalcommons.wku.edu/public_hlth_fac_pub/1

FIELDS OF STUDY

Major Field: Environmental Health Science

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CHAPTER 1

INTRODUCTION

The intent of this research was to determine if water quality parameters or weather patterns could be indicators for Escherichia coli (E. coli) in Slaters Creek. Slaters Creek is located in Millersville, Tennessee in the Cheatham Lake Watershed. A map showing the location of the Cheatham Lake Watershed is shown on the following page in Figure 1.1. Slaters Creek is designated as a Municipal Small Storm Sewer System (MS4) covered under the general permit #TNS077887 (Smith, 2009). In 2008 the Environmental Protection Agency (EPA) added Slaters Creek to the 303(d) list as being impaired for E. coli and siltation. This was determined after the EPA had taken samples from Slaters Creek from 2001 to 2005. On August 18, 2005 a total of 4600 CFU/100mL of E. coli was reported in Slaters Creek, which is three times higher than the maximum target of 941. Other excess amounts were 2400 CFU/100mL and 1700 CFU/100mL reported in May and June of 2001.

The reason elevated levels of E. coli are a concern in Slaters Creek is because E. coli and Fecal Coliform indicate that harmful bacteria from fecal waste may be present in the water (EPA, 2009). When harmful bacteria are present, health effects to humans such as diarrhea, nausea, cramps, headaches or other symptoms, may occur (EPA, 2009). These symptoms may be more severe in young children, infants and people with compromised immune systems (EPA, 2009). In an effort to create a safer environment for

Figure 1.1 Cheatham Lake Watershed in Tennessee

http://www.tn.gov/environment/watersheds/five/cheatham/

the residents near Slaters Creek the city of Millersville has partnered with students and faculty of Western Kentucky University. The joint goal of this partnership is to determine the cause of E. coli in Slaters Creek and to allow research to be done in an effort to help solve water quality problems, not only in Slaters Creek, but in other locations as well.

This study focused on water quality parameters and weather patterns in order to learn the effects these variables have on E. coli concentrations. Previous studies have been conducted similar to the study completed in Slaters Creek. These studies have found that certain water quality parameters and weather conditions can indicate when E. coli levels are more likely to be elevated. One such study was conducted in the Yverdon karst aquifer system located between the Jura Mountains and Swiss Plateau (Pronk, Goldscheider & Zopfi, 2007). This study looked at particle-size distribution (PSD) and any relationship that might be present between PSD and E. coli. Pronk et al. (2007) concluded that by monitoring PSD continuously an early-warning system might be established for fecal contamination in spring water.

A study conducted on two creeks in Indiana found other water quality parameters to be correlated with the presence of E. coli. The study found that E. coli concentration and loading was correlated significantly with the 7-d antecedent precipitation (7dP) and turbidity. (Vidon, Tedesco, Wilson, Campbell & Casey, 2008). This same study also found that at high flow conditions a very poor correlation existed between E. coli and 7dP and turbidity. Due to this finding, the study was able to determine that different flow rates may affect the concentration of E. coli in different ways (Vidon et al., 2008).

In regards to weather patterns, it has been found that sunlight effects the concentration of E. coli in water bodies. One study conducted at Lake Michigan found

that on cloudy or partly cloudy mornings E. coli concentrations frequently exceeded swimming criteria, while on sunny mornings the swimming criteria for E. coli concentrations were rarely exceeded (Whitman, Nevers, Korinek & Byappanahalli, 2004). Whitman et al. (2004) also determined that a decreased effect of insolation was seen as water depth increased. While the Whitman et al. study was done in standing water this can still be related to Slaters Creek because of shallow depths and minimal flow in many areas of the creek.

It has also been found that wind can have an effect on E. coli concentrations in water bodies. A study conducted in Ringkobing Fjord on the coast of Jutland, Denmark studied the association between recontamination of water due to wind causing resuspension of sediment particles (Roslev, Bastholm & Iversen, 2008). In the study Roslev et al. (2008) sited that sediments may act as a reservoir for fecal indicator bacteria which would include E. coli. The final conclusion of Roslev et al. (2008) was that resuspension of sediment particles may cause bodies of water to become non-compliant due to fecal indicator bacteria. Jeng, England, and Bradford (2005) found similar results when they conducted a study in the Jahncke Canal on the shore of Lake Pontchartrain. This study found that that storm water runoff increased the presence of E. coli substantially in estuarine sediments of the Jahncke Canal (Jeng et al., 2005). The estuarine sediments were found to prolong the life of indicator organisms, such as E. coli, for at least seven days (Jeng et al., 2005). This conclusion made by Jeng et al. (2005) could help explain why the study conducted by Roslev et. al (2008) showed that resuspension caused by wind can cause higher concentrations of E. coli.

CHAPTER 2

METHODOLOGY

The first step for this project was to perform compliance sampling for the cities of Millersville and Goodlettsville. Compliance sampling was done in order to satisfy the requirements of the NPDES permit. Compliance sampling included eleven different sampling locations throughout the two cities. These sampling locations were located in three different creeks; Lumsley Fork, Manskers Creek, and Slaters Creek. For this research, data was only used from Slaters Creek, which incorporated four of the eleven sampling locations. Compliance samples were analyzed for E. coli at the Waters Laboratory located on Western Kentucky University's campus. Compliance samples were taken during the months of September and October in 2011. A total of five samples were obtained from each sampling location.

Once compliance sampling was completed a sampling regime began and ran from November 2011 to March 2012. In this time frame water quality samples were taken using a YSI Water Quality Meter that tested pH, total dissolved solids (TDS), temperature, turbidity, dissolved oxygen (DO), conductivity and oxidative reduction potential (ORP). Weather conditions and time of day were recorded during every sampling event and at each sampling location. Finally water samples to test for E. coli were collected. Water samples taken during this time period were tested for E. coli using Coliquant EZ Kits which utilize the coliscan membrane filter method, an EPA approved

method. Appendix A gives a detailed description of this method. In order to ensure accuracy of the study, field blanks and duplicate samples were taken during each sampling event.

After all the data was collected results were analyzed using SPSS, which is a predictive statistical software. Using SPSS a principle component plot was created to determine if any water quality parameters or weather conditions correlated with the E. coli levels. The principle component plot was used because it is a non-parametric technique and the data from this study was not a normal distribution. SPSS was also used to determine if seasonal trends existed within the data. This information will also help determine if weather conditions are good indicators for E.coli.

CHAPTER 3

RESULTS

The results of this study indicate that a point source pollution problem exists within the Slater's Creek watershed. Figure 3.1 shows that the E.coli results were not closely related to any of the water quality measurements. This indicates that runoff from rainfall is not causing the higher E.coli levels and therefore a point source pollution input is the likely cause of the E. coli.

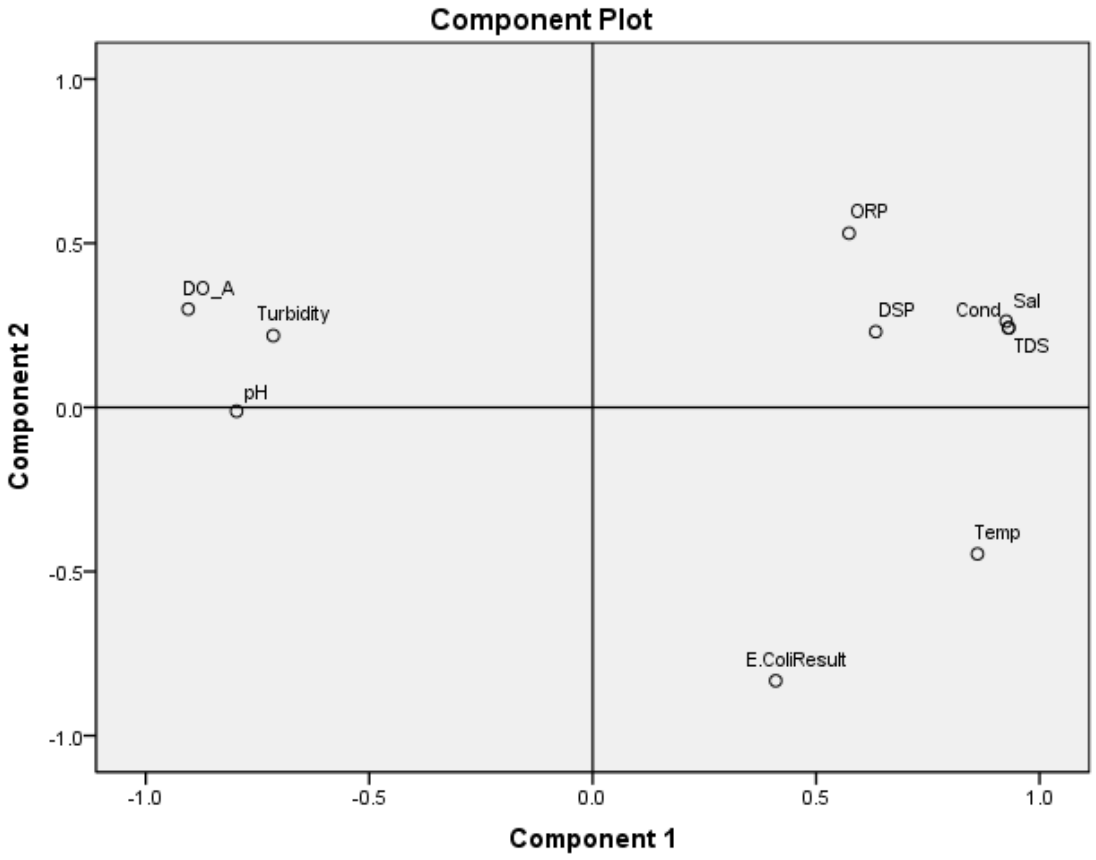

Figure 3.1: SPSS Principle Component Plot

Results from SPSS also show a seasonal trend in the E. coli levels, which is represented in Figure 3.2. Figure 3.3 is the amount of precipitation that occurred each month in Millersville according to the National Oceanic and Atmospheric Administration (NOAA). The precipitation total for March 2012 is not given because at the time of the data analysis the month had not ended, therefore data for the whole month wasn't available. Generally months with less precipitation have higher E. coli levels. This is another indicator of a point source pollution problem because generally E.coli levels are higher when precipitation is higher.

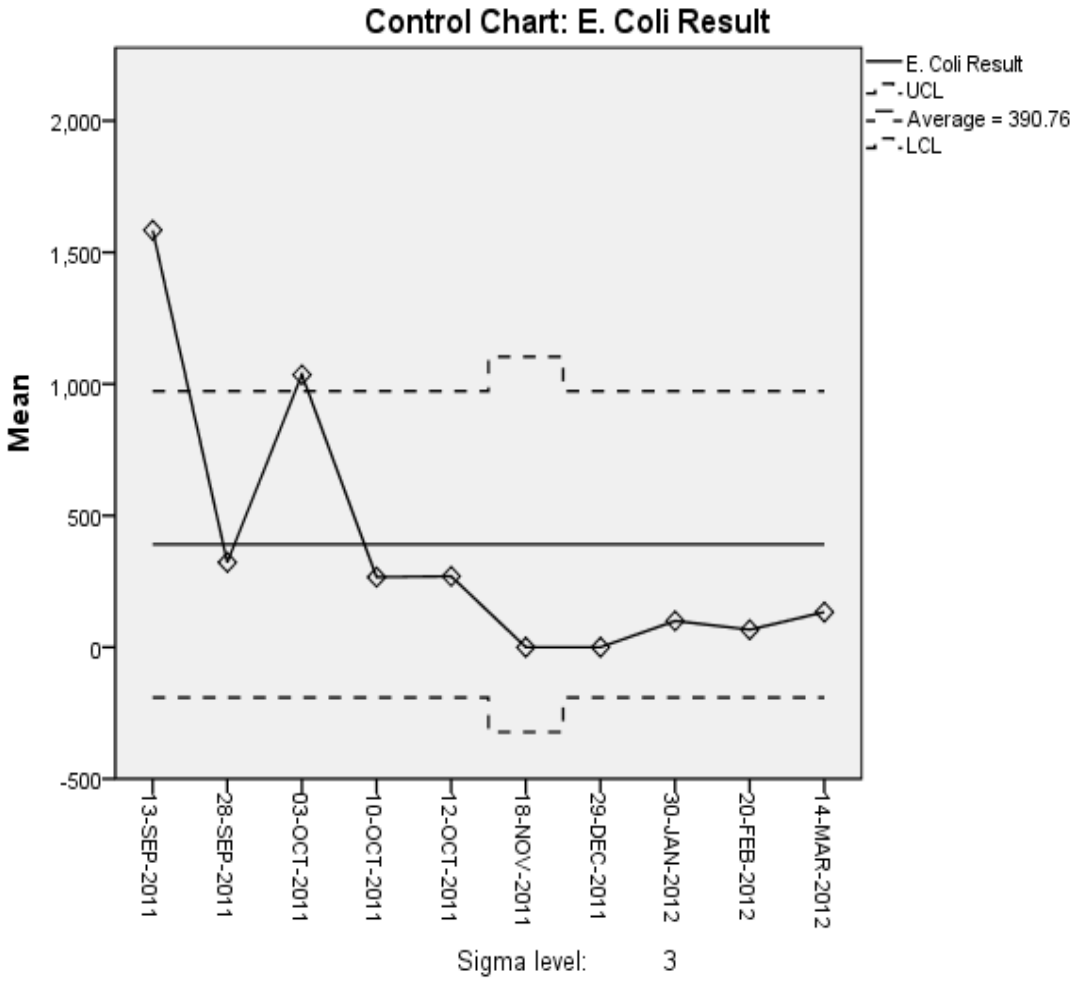

Figure 3.2: SPSS Seasonal Trend Graph

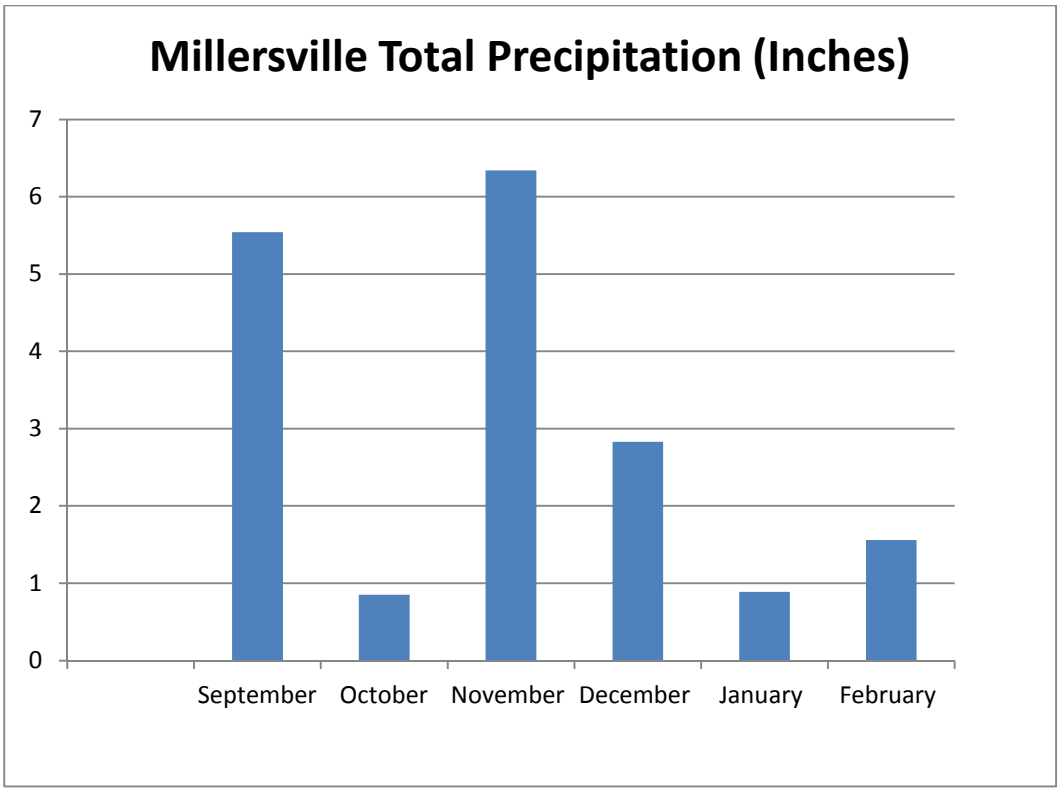

Figure 3.3: NOAA Monthly Precipitation Totals in Millersville, TN

The full results for the E. coli data are given in Figure 3.4 on the following page. The highlighted portions of the data are samples that exceeded the maximum daily target of 941 CFU / 100 mL. These results support the seasonal trend graph given in Figure 3.2 because the dates when E.coli exceeded the maximum was during September and October.

The location SBSC was a portion of Slaters Creek but it was not within the immediate study area of Millersville. It was decided that the results from this area might skew the data more than assist in finding the problem. Therefore only compliance sampling was performed at this location and not the additional sampling conducted from November 2011 to March 2012.

Location	Date	Time of Sample	E. Coli Result (cfu)
LDSC	9/13/2011	14:05	2187
LDSC	9/28/2011	7:38	187
LDSC	10/3/2011	7.55	586
LDSC	10/10/2011	10:21	233
LDSC	10/12/2011		292
LDSC	11/18/2011	12:47	0
LDSC	12/29/2011	12:41	0
LDSC	1/30/2012	11:53	0
LDSC	2/20/2012	13:52	0
LDSC	3/14/2012	13:34	100
CCSC	12/29/2011	12:12	0
CCSC	1/30/2012	11:26	0
CCSC	2/20/2012	14:15	0
CCSC	3/14/2012	13:23	200
CDSC	9/13/2011	10:42	2187
CDSC	9/28/2011	7:38	309
CDSC	10/3/2011	6:30	2359
CDSC	10/10/2011	10:41	556
CDSC	10/12/2011		384
CDSC	11/18/2011	13:10	0
CDSC	12/29/2011	12:27	0
CDSC	1/30/2012	11:41	300
CDSC	2/20/2012	14:02	200
CDSC	3/14/2012	13:07	100
SBSC	9/13/2011	9:52	379
SBSC	9/28/2011	10:37	471
SBSC	10/3/2011	6:49	160
SBSC	10/10/2011	11:52	10
SBSC	10/12/2011	11:50	132

Figure 3.4: E.coli Results for Slaters Creek

CHAPTER 4

DISCUSSION

The purpose of this study was to determine if water quality parameters and weather conditions could be correlated with E. coli levels. It was not possible to correlate E. coli levels with either of these factors because of the conditions within Slaters Creek. The results indicate that a point source pollution problem is the likely cause for the elevated E. coli levels in Millersville, Tennessee. The United States Geological Service (USGS) defines point source pollution as "water pollution coming from a single point such as a sewage-outflow pipe" (Perlman, 2012). The data indicates that rainfall is not correlated with the E. coli contamination and non-point source pollution is a product of rainfall. If non-point source pollution was the problem then the data could have been drastically different and correlations may have been possible.

At this time the data indicates that during warmer weather the E. coli levels are generally higher. This means that in the spring and summer, when people are more likely to be near or in the creek, the E. coli levels may be higher. The city of Millersville needs to educate the public about E. coli contamination in order to ensure the safety of the citizens. This could be done through programs within the schools and through educational handouts given out at city functions. If people do come into contact with the creek it is possible illnesses may start to be seen within the general public.

In the future Slaters Creek will need to continue to be monitored to determine if the seasonal trends observed within this data continues throughout the rest of the year. Biological assessments also need to be taken to determine if the increased E. coli levels during portions of the year are affecting the biological integrity of the creek. Both of these future projects will give a better idea of the overall health of Slaters Creek.

CHAPTER 5

CONCLUSION

While correlations were not able to be made between E. coli levels and weather conditions or water quality parameters other conclusions were made possible from this study. The data obtained from Slaters Creek indicated that seasonal trends may be an effective way to determine if a point source pollution problem exists within streams. The data also indicated that by monitoring water quality within a stream it may be possible to determine if point source or non-point source pollution is occurring.

Both of these conclusions could help other communities determine what the source of E. coli may be within their creeks. By using water quality parameters to determine if point source or non-point source pollution is occurring, communities will be able to narrow down the likely causes of the pollution. This will mean monetary savings for the communities because resources will not be used to search for pollution in places it is not occurring. The future of determining the source of E. coli could be through first observing the water quality parameters.

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APPENDIX A

EPA Approved Method for E. Coli Determination

The COLISCAN® MEMBRANE FILTER METHOD Developed

for ESCHERICHIA COLI AND TOTAL COLIFORMS

USEPA Approved for the determination of E. coli and total coliforms for use in National Primary Drinking Water regulations (NPDWR) monitoring.

1.0 Scope and Application

1.1 The Coliscan® Membrane Filter method consists of a medium which detects the presence or absence of E. coli and total coliforms simultaneously and also allows the enumeration of each. It is approved for use in analyzing potable water by certified drinking water laboratories. It is also useful in the analysis of other waters, clinical applications, veterinary and agricultural applications, pharmaceutical applications and in the area of food and beverages.

1.2 The method allows the detection and enumeration of E. coli and other coliforms in 24 hours or less and does not require a confirmation step.

1.3 The detection limit is one target CFU/sample.

2.0 Summary of the Method

2.1 The Coliscan® MF medium determines the presence or absence (and enumeration) of E. coli and other coliforms in any size water sample (100 mL is required for drinking water). The sample (diluted or not) is passed through a 0.45um pore size, 47 mm

diameter membrane filter using standard equipment and methodology. The filter is then placed into a 50 mm plate containing a pad saturated with the medium (if in broth form) or a layer of the medium which has been solidified with an added agar gelling agent. Incubate for 24 hr at 35°C±0.5°C. E. coli CFUs will appear as blue/purple and other coliform CFUs will appear pink/magenta.

2.2 Coliscan® MF medium (broth or agar) contains nutrients to assure the growth of the target organisms, buffers to maintain appropriate pH, and inhibitors to reduce the growth of nontarget organisms. It is similar to the modification of m-TEC described by Duncanson and Cabelli (1986 paper presented at the National Meeting of AWWA). E. coli colonies growing on the medium appear blue to purple due to the combination of the enzymes glucuronidase and galactosidase affecting their respective substrates, 5-Bromo-4-Chloro-3-Indolyl-B-D-glucuronide (X-gluc) and 6-Chloro-3-Indolyl-B-D-galactoside (Red-Gal®). The teal green product of X-gluc hydrolysis combines with the pink/magenta product of the Red-Gal® hydrolysis to produce the blue to purple appearance of the E. coli colonies. Other coliform colonies (than E. coli) are colored pink/magenta as a result of producing only the galactosidase which acts on the Red-Gal® only.

3.0 Definitions

3.1 Escherichia coli - Those bacteria which grow as blue/purple colonies on the Coliscan® MF medium as a result of the production of both glucuronidase and galactosidase enzymes. These bacteria are of fecal origin.

3.2 Total Coliforms - Those bacteria which make up the sum of the E. coli (blue/purple colonies) and other coliforms. The other coliforms will appear as pink/magenta colonies

on the Coliscan® MF medium because they produce galactosidase, but not glucuronidase and so cleave only the Red-Gal® substrate. Species of the genera Citrobacter, Enterobacter, Escherichia, and Klebsiella are the main groups (other than Escherichia) of coliform bacteria.

3.3 Non Coliforms - Bacteria that form colonies which are not blue/purple or pink/magenta on Coliscan® MF medium.

4.0 Interferences

4.1 No known chemical substances normally encountered in drinking water or source waters have been observed to affect the color of E. coli or other coliform colonies on the Coliscan® MF medium. If particulate or colloidal materials are suspended in water samples, they may interfere with filtering efficiency by clogging filter pores and they may cause some spreading of bacterial colonies as they grow on the filter surface during incubation. However these materials would very rarely prevent the accurate determination of the bacterial population.

4.2 Colonies exhibiting the color(s) of the target organisms should not be included in the E. coli or other coliform counts if they are less than 0.5 mm diameter (except when the entire colony population is small due to excessive crowding on the plate. In such a case the sample should be rerun at a higher dilution.). Small colored colonies of this nature should not be counted unless they are isolated into pure culture and then verified by approved procedures.

4.3 Colonies exhibiting a teal green color which is indicative of the production of glucuronidase without the production of galactosidase should not be counted as E. coli without isolation into pure culture and verification by approved procedures.

4.4 It can not be safely assumed that colonies can be picked directly from the surface of the filter and used to inoculate confirmatory media directly, particularly if the colonies are blue/purple or teal green, as they may be contaminated by cells from adjoining colonies that have traveled on the filter surface. Therefore, questionable colonies should be picked and streaked onto the surface of a differential medium such as Coliscan® Easygel® to ensure their purity before further testing.

4.5 Unlike media which utilize a fluorogen (such as MUG or MUGal) and a chromogen, where the fluorogen tends to diffuse rapidly into the surrounding medium, thus making the timing of reading the test results critical (before excessive diffusion occurs which may make neighboring colonies appear as false positives), the chromophores of the chromogens used in Coliscan® MF are quite insoluble and little or no diffusion away from the target colonies occurs.

5.0 Safety

5.1 Standard safety practices should be observed by persons using these materials in the laboratory.

5.2 Any materials containing living or viable microbes should be disinfected or sterilized by accepted standard methods before being discarded.

5.3. Refer to the MSDS for specific product information.

6.0 Equipment and supplies

6.1 Incubator set at 35°C±0.5°C with provision for maintaining materials at above 80% humidity.

6.2 Filter funnel apparatus for 47 mm membrane filters, with a vacuum source.

6.3 Dissecting microscope (10-15X) with built-in light sources.

6.4 Sterile disposable or properly cleaned (by well known standard methods) glass or plasticware including 1 and 10 mL pipettes, sample collection containers, flasks, graduated cylinders, and diluent containers.

6.5. Sterile forceps

6.6 Sterile 50 mm diameter petri dishes with absorbent pads

6.7 Sterile 0.45µm pore size, 47 mm diameter micropore filters for sample filtering

6.8 Sterile Dilution and Rinse water prepared in accordance with standard methods.

6.9 Biohazard bag

7.0 Reagents and Standards

7.1 Coliscan® MF medium of Micrology Laboratories LLC is provided in a broth for using 2 mL/dish, or it may be obtained in dehydrated agar based form. The media are provided without the cefsulodin antibiotic (for the elimination of some non-target organisms). The medium should be kept frozen (2-6°C) and has an expiration time of 6 months.

7.2 Preparation of the Medium for use: The broth medium should be thawed and freshly prepared cefsulodin solution should be added so that the cefsulodin concentration equals 5 µg/mL of medium. The prepared medium can be kept refrigerated for up to 2 weeks. The agar medium should be mixed with reagent-grade water according to instructions and autoclaved for 15 min/15 lb pressure, tempered to below 80º C and then freshly prepared cefsulodin solution should be added (5µg/mL of medium). The medium should then be dispensed into 50 mm petri dishes (5 mL/dish). Store at 4°C for up to 2 weeks.

7.3 Preparation of the Cefsulodin solution: For Coliscan® MF broth, pure cefsulodin is provided in a sterile tube to which 10 mL of sterile reagent-grade water (provided) is

added. Shake until the cefsulodin is completely dissolved and dispense 0.5 mL of this solution into each 20 mL bottle of Coliscan® MF broth.

For Coliscan® agar medium, pure cefsulodin is provided in a sterile tube to which 10 mL of sterile reagent-grade water (provided) is added. Shake until the cefsulodin is completely dissolved and dispense the 10 mL aliquot into 1 liter of hot liquid autoclaved (tempered) Coliscan® agar, mix well and dispense into 50 mm petri dishes (5 mL/dish). Cefsulodin solutions will be sterile if carefully prepared to avoid the introduction of contaminating microbes. The prepared media should be affirmed as sterile by incubating a control plate of each lot and verifying no growth.

If operators prefer, they can filter sterilize their cefsulodin solutions to ensure sterility, but testing the prepared medium with a control is still recommended.

7.4 Make a 10% solution of sodium thiosulfate using reagent-grade water.

8.0 Sample collection, Dechlorination, Preservation, Shipment and Storage 8.1 Collect samples in a sterile, clean wide mouth glass or heat resistant plastic bottle with a leakproof closure, all of which is non-toxic in use. A presterilized, sealable, non-toxic plastic bag may also be used for sample collection.

8.2 For potable water, open the tap and allow the water to run for 2-3 minutes and then collect the sample using aseptic technique to avoid contamination. For other sample types, aseptically collect water that is representative of the source.

8.3 Samples with residual chlorine should be neutralized at the time of collection by adding 1 mL of a 10% solution of sodium thiosulfate (or the equivalent) per liter of sample.

8.4 Samples should be tested as soon as possible after collection. If processing is not done within 1 hour, the sample should be held on ice or refrigerated at 2-8°C. Potable water samples should be tested or processed within a maximum holding time of 30 hours of collection and non-potable samples should be tested or processed within a maximum holding time of 8 hours of collection.

9.0 Quality Control

9.1 Coliscan® MF is tested for quality control at the time of manufacture and is certified to meet specifications. Each lot should be tested by the using laboratory by preparing three plates of the medium, one to serve as a positive control, one to serve as a negative control, and one to serve as an uninoculated control.

Prepare 24 hour tryptone broth cultures of typical E. coli, Enterobacter aerogenes or Klebsiellsa pneumonia, and Pseudomonas aeruginosa or Salmonella typhimurium. Prepare serial dilutions of E. coli and Enterobacter or Klebsiella combined so that the combined inoculum will result in 20-80 CFU/100 mL and filter. Place the filter on the surface of one of the plates of Coliscan® MF medium. Prepare serial dilutions of the Pseudomonas or Salmonella so that the inoculum will result in 20-80 CFU/100 mL and filter. Place the filter on the surface of the second plate of Coliscan® MF medium. Filter 100 mL of sterile diluent and place the filter on the surface of the third plate of Coliscan® MF medium. Use sterile filter apparatus for each prep.

Incubate the plates 24 hr. at 35°C±0.5°C. The E. coli/Enterobacter or Klebsiella control should have both blue/purple (E. coli) and pink/magenta (Enterobacter or Klebsiella) colonies, the Pseudomonas or Salmonella control should have colorless colonies, and the diluent blank control should have no colonies.

Colonies from the controls may be picked and tested further with various diagnostic media if desired.

10.0 Calibration and Standardization

10.1 Coliscan® MF calibration or standardization is not required.

10.2 Incubators should be tested daily to assure maintenance of proper temperature. Thermometers used should be tested at least annually against an NIST certified thermometer.

11.0 Procedure

11.1 Test Procedure

11.1.2 Using proper technique, filter the sample through a 47 mm, 0.45µm pore size membrane filter. Rinse the filter funnel twice with at least 20 mL of sterile diluent/rinse to complete the filtration. Transfer the filter to a petri dish containing a pad saturated with 2 mL of the Coliscan® MF broth, invert the dish and incubate at 35°C±0.5°C for 24 hours.

11.2 Interpretation

11.2.1 Check the filters for colony forming units. Generally, colonies are obvious and can be observed with the unaided eye in normal room or daylight. However, the use of a 10-15X magnifying device is recommended for critical analysis.

11.2.2 The sum of blue/purple and pink/magenta colonies is the Total Coliform Positive count. Blue/purple colonies are counted as E. coli. Pink/magenta colonies are counted as other than E. coli coliforms. Clear or white colonies are counted as non-coliforms.

12.0 Data Analysis, Calculation, Interpretation and Reporting Results

12.1 Presence/Absence Test

12.1.1 The presence of at least one blue/purple or pink/magenta colony at least 0.5 mm in diameter indicates the sample is total coliform positive. The presence of at least one blue/purple colony indicates the sample is positive for E. coli. The presence of at least one pink/magenta colony indicates the sample is positive for general coliforms.

12.2 General Coliform (excludes E. coli) - Quantitative Test

12.2.1 Count the number of pink/magenta CFUs present on the membrane filter and record as the number/amount of sample used for that test. For example, if the amount of sample was 10 mL and there were 20 pink CFUs, record as 20 per 10 mL. Then translate to the number of CFUs/100 mL. In this case, the 10 mL sample is 0.1 of 100, so the 20 CFUs should be multiplied X10, giving 200 CFU/100 mL sample. All pink/magenta CFUs should be counted as general coliforms. (Colonies should be at least 0.5 mm diameter to be counted.)

12.3 E. coli (Fecal) - Quantitative Test

12.3.1 Count the number of blue/purple CFUs present on the membrane filter and record as the number of E. coli/amount of sample used for that test. Then translate to the number of E. coli CFUs/100 mL of sample (see 12.1.1). All blue/purple CFUs should be counted as E. coli. (Colonies should be at least 0.5 mm diameter to be counted.)

12.4 Total Coliforms - Quantitative Test

12.4.1 The sum of the number of general coliform CFUs and the number of E. coli CFUs from one sample equals the number of total coliforms in that sample. That is, the total number of pink/magenta CFUs and blue/purple CFUs = the total coliforms for that sample.

13.0 Method Performance Characteristics

13.1 Specificity - In a study done to compare Coliscan®MF to the m-TEC Method for the detection and enumeration of E. coli from disinfected wastewater effluent, the false positive error was 3.8% and the false negative error was less than 1.0%. That is, of 105 CFUs judged to be E. coli (blue/purple) which were picked and subjected to Enterotube® analysis, only 4 were identified as other than E. coli. And of 131 CFUs judged to be coliforms other than E. coli (pink/magenta) which were picked and subjected to Enterotube analysis, only 1 was identified as other than a true coliform.

13.2 Comparability - The Pearson Coefficient for the parallel analyses on the Coliscan®MF and the m-TEC methods within the same laboratory was 0.928. T- test analyses indicated no significant differences between the methods at the 95% confidence level.

13.3 The membrane filter medium known as MI Agar was developed by the USEPA and was approved for use with drinking water as stated and published in the Federal Register Vol. 64, No. 230, Dec. 1, 1999. On the basis that the Coliscan MF® medium is based upon very similar, equally effective technology, the EPA has granted it official approval for the determination of total coliforms and E. coli for use in drinking water monitoring also. The EPA reported the false-positive and false- negative rates for E. coli to both be 4.3% with the MI Agar method. The specificities for E. coli and total coliforms were reported to be 95.7% and 93.1%, respectively. Because of the parallels in the methodology between the MI Agar and the Coliscan®MF, testing results should be the same for both media.

14.0 Pollution Prevention

14.1 Laboratory personnel should use pollution control techniques to minimize waste generation wherever possible. Where this is not possible at the source, recycling should be practiced.

15.0 Waste Management

15.1 Each laboratory is responsible to comply with all federal, state and local regulations governing waste management. Special emphasis should be placed on hazardous waste identification rules and land disposal restrictions and to protecting the air, water, and land by minimizing and controlling all release from fume hoods and bench operations. Compliance is also required with any sewerage discharge permits and regulations. Federal, state or local authorities should be contacted for further specific information.

16.0 References

16.1 APHA (1995) Standard Methods for the Examination of Water and Wastewater. Edition 19.

16.2 Brenner, K.P., et al. (2000) Membrane Filter Agar Medium Containing Two Enzyme Substrates Used for the Simultaneous Detection of Total Coliforms and E. coli. United States Patent #6,063,590.

16.3 Brenner, K.P., et al. (1993) New Medium for the Simultaneous Detection of Total Coliforms and Escherichia coli in Water. Appl. Environ. Microbiol. 59: 3534-3544.

16.4 Roth, J.N., W.J. Ferguson. (1993) Method Test Media and Chromogenic Compounds for Identifying and Differentiating General Coliforms and Escherichia coli Bacteria. United States Patent #5,210,022.

16.5 Umble, A.K., et al. (1999) Elkhart, Ind., Tests an Improved, Simplified Membrane Filtration Method for Escherichia coli Detection and Enumeration. Water Environment Tech. Vol.11, No.4, 57-59.

16.6 USEPA. National primary and secondary drinking water regulations: Analytical methods for regulated drinking water contaminants: Proposed rule, F6. Federal register 58(129): 65626. Wash., D.C., Office of the Federal Register. Dec. 15, 1993.

16.7 USEPA. National primary drinking water regulations: Analytical methods for certain pesticides and microbial contaminants: 40 CFR Part 141. Federal register Vol. 63, No. 147, July 31, 1998. Proposed rules.