Determining the Relationship Between Drinking Water Quality of Personal Water Bottles and Cleaning Practices Followed by the Public.

By

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"The views expressed in this paper are those of the author and do not necessarily reflect the official policy, position or views of BCIT, the Environmental Health Program or its faculty."

Abstract

It is becoming very common to see people carrying around personal water bottles throughout the day wherever they go, whether it be to school, work, or the gym (Rydings, 2004). People may have a false sense of security that the water they are drinking is safe because it is from the tap or from a commercial water bottle or cooler, but this may not be the case once you put it in a personal water bottle. Initially, there are guidelines in place to ensure safe drinking water from the tap and safe bottled water (Health Canada, 1996; CFIA, 2002), but there are no water quality guidelines once you put it in a water bottle.

The purpose of this research project was to determine if a relationship exists between drinking water quality found in personal water bottles and the general cleaning practices followed by the public. The microbiological values obtained were compared to the Canadian Drinking Water Quality Guidelines to determine if they met the guidelines. Finally, it was assessed if public education was needed regarding cleaning practices for personal water bottles users.

Both a short survey and microbiology testing were conducted. The survey was carried out by means of in-person interviews. Ninety participants were randomly selected by voluntarily responding to a posted sign requesting their participation. Approximately 110 ml sample of water from their personal water bottle was collected using a sterilized sample bag. Microbiological analysis was conducted within 30 hours of sample collection by means of Membrane Filtration and Heterotrophic Plate Count (HPC) using standard m-HPC agar.

All microbiological and survey data collected was entered into NCSS in order to statistically analyze the results. The analysis of variance (ANOVA) was used to determine the differences between the types of cleaning methods (soap and water, rinsing with tap water and other methods) and microbiological counts. The results indicated that there was a significant difference between the types of cleaning. Tap water rinsing resulted in the lowest average microbiological counts and the post hoc test revealed that

the greatest difference between the types of cleaning methods used were with soap and water. Correlational/Regression statistics were used to determine the relationship between the timeframe of cleaning and microbiological counts. The results indicated that as the timeframe between cleaning increases, so did the microbiological counts, however the relationship was weak. The microbiological counts found in this study, exceeded the Canadian Drinking Water Quality Guideline HPC maximum limit of 500 cfu/ml 74.4 % of the time. Based on all the results of the study, government agencies or personal water bottle manufacturers should consider developing and disseminating to the public the importance of regular cleaning of personal water bottles and recommend the best methods to use.

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INTRODUCTION

It is becoming very common to see people carrying around personal water bottles throughout the day wherever they go, whether it be to school, work, or the gym (Rydings, 2004). How many of these people ever think about the quality of water they are consuming or if their cleaning practices are sufficient enough to prevent continuous contamination of the water? Most likely not many. People may have a false sense of security that the water they are drinking from the tap, out of a commercially bottled water or water cooler must be safe, but this may not be the case once you put it in a water bottle. The reality is that guidelines are in place initially to ensure safe drinking water and safe bottled water (Canadian Food Inspection Agency (CFIA), 2002; Health Canada, 1996), but there are no measures for water quality guidelines once you put it in a water bottle.

Over the years there has been an increase in awareness of both the health benefits of drinking water and the importance of the quality of drinking water being consumed (Rydings, 2004). Most important are the lessons learned about drinking water quality from outbreaks in Canada over the years, such as *Cryptosporidium* in Kelowna and Cranbrook, BC in 1996 (CBC News Indepth, 2004) and *E. coli* in Walkerton, Ontario in 2000 (Rydings, 2004). This has lead to improved monitoring and guidelines of water quality all over the country and an increased consumption of bottled water (Rydings, 2004). For example, sales of bottled water has increased over the years, which now exceeds $5.7 billion worldwide (Rosenberg, 2003).

There appears to be substantial research on bacterial contamination of commercially bottled water and water coolers. However, there is limited research addressing the quality of drinking water in personal water bottles, but as this practice is becoming more common, this issue needs to be addressed.

PURPOSE OF THE STUDY:

The purpose of this research project was to determine if a relationship exists between drinking water quality found in personal water bottles and the cleaning practices followed by the public. In addition, the microbiological values obtained were compared to the Canadian Drinking Water

Quality Guidelines to determine if they met the guidelines. Finally, it was assessed if public education was needed regarding cleaning practices for personal water bottles users based on the results obtained. In this study, water coolers are defined as units that dispense and hold large quantities of water; commercially bottled water was defined as water bottled by a company, including Dasani and Evian; and personal water bottles were defined as re-used commercial bottled water or sports bottles, such as Nalgene. Cleaning practices were measured by determining the most common cleaning method used (ex. soap and water, tap water rinse, other) and also by determining the timeframe between cleaning.

The Literature Review attempts to justify using HPC to measure drinking water quality. As well as, to compare commercially bottled water and water coolers to personal water bottles by means of bacteriological contamination through poor hygienic practices, temperature and storage abuse and lack of regular cleaning and sanitizing.

LITERATURE REVIEW:

HPC is commonly used to indicate the overall bacterial quality of drinking water (Oliphant, Ryan & Chu, 2002; Ehlers, van Zyl, Pavlov & Muller, 2004). HPC measures the presence of heterotrophic bacteria, which are classified as a broad range of non-photosynthetic microorganisms commonly found in both natural and treated water (Takeo Yoshimura, 1999), whether it be tap, commercially bottled water or water coolers (Ehlers, van Zyl, Pavlov & Muller, 2004). The problem is, these bacteria can multiply under suitable conditions, which can result in even higher numbers of bacteria (Takeo Yoshimura, 1999). Tap water and bottled water using municipal water sources may meet bacteria water quality guidelines. However, if the water is stored for long periods of time at room temperature or there is a lack of a disinfectant residual it may result in elevated HPC bacteria counts by the time it is consumed (Takeo Yoshimura, 1999).

Over the years, researchers have commonly found the following heterotrophic bacteria in various water sources, including *Achromobacter, Acinetobacter, Aeromonas, Alcaligenes, Arthrobacter, Caulobacter, Corynebacterium, Flavobacterium* and *Pseudomonas* (Ehlers, van Zyl, Pavlov & Muller, 2004; Takeo Yoshimura, 1999). However, the question of the public health significance

of bacterial counts in water has been raised ever since 1883, when Robert Koch introduced plate counts to assess water quality. (Exner, Vacata & Gebel, 2003). This is because most of the bacteria are nonpathogenic to humans (Nsanze, Babarinde & Al Kohaly, 1999). Yet, some of these members of bacteria that are found in drinking water have species that are known to produce virulence factors and act as opportunistic pathogens (Ehlers, van Zyl, Pavlov & Muller, 2004; Takeo Yoshimura, 1999). These pathogens may be the cause of both hospital- and community-acquired infections (Exner, Vacata & Gebel, 2003). People who are most at risk of infections caused by opportunistic pathogens include the very young and the elderly with weakened immune systems, pregnant women, organ transplant and chemotherapy patients and those with immunocompromising diseases such as AIDS (Ehlers, van Zyl, Pavlov & Muller, 2004). Although nonpathogenic HPC bacteria have been considered harmless, several epidemiological studies suggest that there are potential health risks associated with HPC bacteria in drinking water when it meets water quality guidelines (Ehlers, van Zyl, Pavlov & Muller, 2004). These include associations between high numbers of HPC bacteria in tap water and gastroenteritis (Ehlers, van Zyl, Pavlov & Muller, 2004).

Over the years there have been new developments in the support of colony counting for the purpose of assessing drinking water quality. They include the improvement of nutrition composition of agars, which support the growth of a wider variety of bacteria found in water; the discovery of biofilms in the late 1960's, where a large number of microorganisms can be found and which contributes to the levels of microorganisms in water; and new procedures which have lead to improved means of identifying a wider variety of bacteria found in water (Exner, Vacata & Gebel, 2003).

Many people believe that bottled water is better then tap water because it does not contain bacteria (Rydings, 2004). This however, is not the case. As mentioned before, any source of drinking water naturally contains bacteria (Ehlers, van Zyl, Pavlov & Muller, 2004). However, the bacteria in these bottles can survive and multiply (Rydings, 2004). Studies have shown that bacterial counts in water coolers and bottled water often exceed water quality guidelines long after bottling (Rydings, 2004). For example, some bottled water has contained bacterial counts between 1,000-100,000 cfu/ml, which exceeds the 500 cfu/ml as recommended by the

Guidelines for Canadian Drinking Water (Takeo Yoshimura, 1999). A lack of a disinfecting residual in any source of water, whether it be tap, bottled or coolers can make the water susceptible to contamination by heterotrophic bacteria (Takeo Yoshimura, 1999).

Research has determined that water coolers and bottled water are at risk of contamination from poor handwashing practices, temperature and storage abuse and the lack of thorough cleaning and sanitizing followed by those who consume the water through these sources (University of Edinburgh Health & Safety Department, nd).

Outbreaks of disease from bottled water have been associated with unsanitary practices (Erickson, 2002), which caused the introduction of bacteria (Rosenberg, 2003). Bacterial contamination can be introduced every time the water bottle is changed, since the reservoir is exposed to both bacteria and dust in the air; and from touching the faucet opening with hands or with the mouth of drinking containers (Rydings, 2004). In order to prevent contamination, it has been recommended to thoroughly wash hands with soap and warm water after washroom breaks, and after handling dirty items such as money, using disposable gloves before handling the bottle and by preventing the faucet from contacting the container (Erickson, 2002).

Water stored in the temperature range between 4 and 42°C can also contribute to the multiplication of the naturally occurring bacteria, especially around room temperature (21°C) (Nsanze, Babarinde & Al Kohaly, 1999). Bacteria can grow to levels that are harmful to health under either improper or prolonged storage of bottled water (Ehlers, van Zyl, Pavlov & Muller, 2004). The reason that the number of bacteria increases rapidly in source waters, regardless of treatment, is that bottled water creates a closed system (Rosenberg, 2003). In a very short period of time, bacteria will attach to the inside of the bottle and multiply using the organic matter present in the water as a food source (Rosenberg, 2003). A rapid increase in bacterial counts will occur until all the organic material in the water has been consumed. Studies have demonstrated that within only a few days, bottled water sitting at room temperature have measured counts of bacteria in the range of 10^4 and 10^5 cfu/ml (Rosenberg, 2003).

Water coolers have become common in many workplaces and in homes (Perceptive Instruments, 2005). Nearly one-third of cold-water samples from these water coolers do not meet drinking water guidelines (Perceptive Instruments, 2005). It is important to control the amount of heterotrophic bacteria in water coolers (Wells, 2001). This can be accomplished by regular cleaning and sanitizing schedules performed on the water contact surfaces of the cooler (Wells, 2001). Sanitizing is an essential step, but it is not effective on surfaces that contain biofilms since biofilms shield bacteria from sanitizers (Wells, 2001). If the interior of the cooler is not cleaned before it gets sanitized bacteria levels will not decrease and may, in fact, increase. This is because biofilms form on plastic and rubber-like surfaces, which can serve as a food source, contributing to bacteria growth (Automatic Vending Association, nd; Wells, 2001). Sanitizer residual gets used up when trying to breakdown the biofilm, without ever reaching the bacteria (Wells, 2001). Bacteria can only be killed when the sanitizer comes into direct contact with the microorganisms (Wells, 2001).

Health Canada recommends that cleaning and sanitizing be performed at each bottle change (Rydings, 2004). A thorough cleaning and sanitizing process would consist of appropriate contact time and concentration of the sanitizer and allowing for complete air-drying (Rydings, 2004). Air-drying is a very effective means of killing bacteria. Since water droplets in the bottle evaporate, the concentration of the sanitizer increases, therefore eliminating the remaining bacteria (Wells, 2001). Sanitizing water coolers on a regular basis is an easy, inexpensive way of preventing bacterial growth (Wells, 2001).

Studies have now determined that the contamination of bacteria in water coolers and bottled water come from naturally occurring bacteria and/or from bacteria being introduced by human means (Ehlers, van Zyl, Pavlov & Muller, 2004). What about personal water bottles? They can be improperly handled, they often sit out at room temperature for long periods of time without sufficient cleaning, could they not be subject to contamination as well? There appears to be only one study that examines bacterial water quality in personal water bottles, which was conducted at an elementary school in Alberta. The problem identified during the study was that students were encouraged to bring water bottles to school to keep at their desks, but were not encouraged to take them home to be cleaned. Some students did not take their water bottles home to get

cleaned for months on end and continued to refill them. Analysis of the water determined that 64.4% of the water collected exceeded the maximum of 500 cfu/mL as outlined by the Guidelines for Canadian Drinking Water Quality (Oliphant, Ryan & Chu, 2002).

Personal water bottles, like water coolers, can also fall victim to fecal contamination by improper and insufficient hand washing practices, especially after using washroom facilities (Oliphant, Ryan & Chu, 2002). Constant contact of unclean hands to the mouth of the water bottles can introduce pathogenic contaminants that can lead to multiplication on and into the bottle that is later consumed. Proper and sufficient hand washing and cleaning of personal water bottles are important to ensure safe bacterial quality of drinking water (Oliphant, Ryan & Chu, 2002).

Bacteria thrive in warm, moist environments and any drinking container can become a suitable environment for bacterial growth (American Plastics Council, 2004). Leaving water standing at room temperature for long periods of time can create this environment leading to significant bacterial contamination (Oliphant, Ryan & Chu, 2002). Even treated, chlorinated water has been shown to support significant bacteria re-growth after only 8-24 hours at room temperature (Oliphant, Ryan & Chu, 2002).

As in the case of bottled water and water coolers, bacteria can also adhere to parts of any water bottle and build up overtime, leading to both the formation of a biofilm and high counts of bacteria (Exner, Vacata & Gebel, 2003; Rydings, 2004). Personal water bottles that are not properly cleaned, sanitized and air-dried lead to further colonization of biofilms, that remain on the inside surface of the bottle (Exner, Vacata & Gebel, 2003).

Some people re-use commercially bottled water bottles day after day refilling them with their own water supply. These bottles are constructed out of a non-durable plastic that are designed for one-time use (University of Minnesota-Extension Service, 2003). It has been suggested that if consumers insist on re-using these types of bottles, they should wash it daily with hot, soapy water using a bottlebrush to clean in around the neck and lid and allow the bottle and lid to dry completely between uses in conjunction with proper hand washing practices, especially after using the washroom (University of Minnesota-Extension Service, 2003). However, studies have

indicated that thorough washing of these bottles may cause the plastic to breakdown at a fast rate, causing chemicals to leach into the water making it chemically unsafe to drink (Source Weekly, 2003). Nalgene, a personal water bottle manufacturer, offers an alternative to the soft plastic water bottles. These bottles are made with Polycarbonate plastic that claims to be durable, resistant to staining, resistant to retention of odors and can be safely dishwashed using the top rack (Nalgene, Nunc. International, 2005). They appear to be a better alternative to reusing commercially bottled water since they are meant to be used repeatedly. Nalgene also produces bottles with wide-mouth openings making it easier for cleaning (University of Minnesota-Extension Service, 2003). The problem with more durable plastic bottles is that they do not include cleaning instructions with them, so it is up to the consumer to decide how to handle this. Nalgene has a website offering cleaning instructions that includes using only warm soapy water, lemon or baking soda or by using a dishwasher (on the top shelf only) (Nalgene Nunc. International, 2005). The only situation they recommend using a sanitizer is for removing stubborn stains (Nalgene Nunc. International, 2005). There are no directions when and how often to clean, or even when it is appropriate to replace the bottle. Daily cleaning using proper methods is a vital part of making sure personal water bottles do not become a breeding ground for bacteria.

This study attempted to determine the relationship between bacteriological quality of water in personal water bottles and cleaning practices followed by the public. The primary goal of this research project was to determine if it is necessary to educate the public on the importance of appropriate handling, cleaning and sanitizing of personal water bottles in order to maintain potable drinking water.

METHODOLOGY

This research project consisted of conducting both microbiological testing and a survey.

Survey:

The survey was conducted by means of in person interviews. A script was utilized to facilitate consistency of information communicated to each participant. The randomly selected

participants were asked to read a short cover letter to familiarize themselves with the study and pertinent confidentiality information. They were also given the opportunity to receive information on the results of the study, by filling out a contact information form. A copy of the script, survey, cover letter and contact information form is included in Appendix A. Each participant filled out the survey that was numbered or lettered, which corresponded to the same number or letter on the drinking water sample bag.

Microbiological:

Approximately 110 ml sample of drinking water from participants personal water bottle was collected using a sterilized filter bag containing a sodium thiosulfate tablet and placed in a cooler filled with ice packs to keep the water samples cool. Next, the samples were taken to the microbiology lab at BCIT where microbiological analysis was be conducted by means of Heterotrophic Plate Count (HPC) using Membrane Filtration (MF). The samples run by MF were divided in one -100ml sample and one -10 ml in order to count colonies with ease by means of dilution. If the 100 ml sample gave results that were 'to numerous to count' (TNTC) than the 10 ml sample produced results that were more countable. The drinking water samples were analyzed within 30 hours of collection and then compared to the recommended HPC colonies of <500 CFU/ml according to the Guidelines for Canadian Drinking Water Quality (Health Canada, 1996). The results from this study can be generalized to all students who use personal water bottles at BCIT. Although the results cannot be generalized to the entire general population, time and sampling collection convenience were factors taken into consideration.

In order to ensure the reliability and validity of the study, a pilot test was conducted at the beginning in January, 2006 prior to the official study.. The pilot study consisted of testing 5 people to ensure that the methods work (Heacock & Chiodo, 2005), determined the simplicity and length of the survey (Haworth, 2005) and to practice microbiological techniques.

Participants:

In order to randomly select participants, a few signs were placed at various locations at BCIT. The sign stated, "Want to know what's growing in your water bottle and have a chance to win $50? Then bring your filled personal water bottles (Example: re-used commercially bottled water

(ex. Evian) or Nalgene) to (a specific location) on (specified dates and times)" (Appendix B). Ninety water samples and corresponding surveys were collected from participants, in order to increase the validity and reliability of the study.

Other ways that the validity and reliability of the study was increased was by excluding certain members of the public and/or certain types of water samples. This study excluded anyone who did not use a personal water bottle, which was defined as a sports bottle, a re-used commercial water bottle (ex. Evian-soft plastic) or a hard plastic water bottle (ex. Nalgene). Excluding these people was accomplished by the information provided on the posted sign, which indicated that the participant bring their personal water bottle to the study location. The survey also excluded anyone whose drinking water source was well water. Homeowners with well water are only encouraged to have their water supply tested, so the safety of the water source cannot be assumed. Whereas, untreated and treated municipal water sources are tested by the GVRD daily and weekly (depending on the water type) (GVRD, 2004), so it can assumed that the water source was up to acceptable standards. In order to exclude people who did not clean their water bottles the second question on the survey asked if people accomplished this task. In addition, ENVH 8400 students were also excluded from the study since they were aware of the researchers anticipated outcome. The first two questions in the survey were not used in the statistical analysis portion of the research project, they were only used for the purpose of data exclusion (Haworth, 2005). Participants had the opportunity to enter into a draw to win $50, in attempts to achieve a minimum of 90 participants.

Ethical Considerations:

Since each participant was informed, in the cover letter, that the study was completely voluntary and confidential and that hard copies of the survey and contact information would be destroyed upon completion, there was no need for this study to be approved by the Ethics Review Board (Heacock & Chiodo, 2005). Heacock & Chiodo (2005) also checked to make sure BCIT policy on human subjects and research was being adhered to. In addition, the participants were not sought out, they came on their own free will in response to the posted signs.

Chosen Survey Method & Microbiological Method:

The best-fit survey method for this study was an in-person interview since it gave the researcher the ability to collect a drinking water sample and a conduct a survey with each participant at the same time. By recommendation of Kim Cummings (2005), Membrane filtration (MF) and Heterotrophic plate counts (HPC) using standard m-HPC agar was the method used in this study, in order to determine microbiological counts (see Figure 1 and 2). This method was recommended since it was an acceptable standard method commonly used to test drinking water quality in the field (Standard Methods for the Examination of Water and Wastewater, 1998). Membrane Filtration also produces highly reliable and reproducible numerical results, has the ability to test large volumes of sample water with low-counts in a short period of time and it doesn't expose the bacteria to heat shock (Standard Methods for the Examination of Water and Wastewater, 1998). This method gave a snap-shot of the total amount of live heterotrophic bacteria in a drinking water sample at one particular period of time (K. Cummings, personal communication, November 7, 2005). In general, this method was selected based on time and cost constraints, convenience and availability of materials since the lab and materials, including the media was supplied by the Microbiology lab at BCIT.

Alternate Microbiological Methods:

Other methods in the field to determine coliform counts, include Most Probable Number, however it was not used since this method produces results at a slower rate than MF and it is not as accurate as MF (Heacock & Chiodo, 2005-Appendix; Food Technology Laboratory Manual, 2002).

Heterotrophic plate counts can be determined by two other methods, which are the pour plate method and the spread plate method. The pour plate method was not chosen, since this method can affect the resulting counts since it exposes bacteria to significantly high temperatures causing heat shock and since colonies can grow throughout the media, they often grow at a slower rate. The spread plate method was also not chosen since the agar can only absorb a small volume of the water sample (only 0.1 to 0.5 ml), which was not appropriate for this study (Standard Methods for the Examination of Water and Wastewater, 1998).

Experimental Methods:

For complete experimental methods on drinking water sample collection, Heterotrophic Plate Count (HPC) and Membrane Filtration see Appendix C.

Figure 1: Example of a Membrane Filtration set-up (Leboffe & Pierce, 1999).

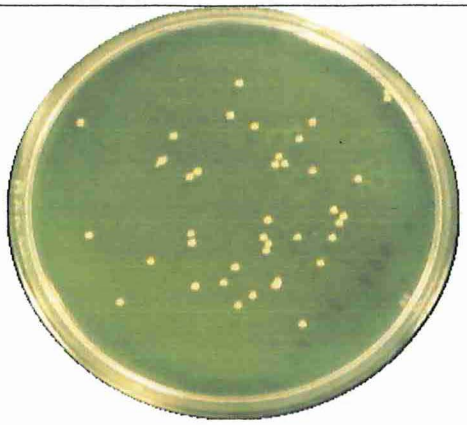

Figure 2: Heterotrophic Plate Count (Leboffe & Pierce, 1999). Live heterotrophic bacteria form clusters enabling the researcher to count the total number present.

STATISTICAL ANALYSIS

All quantitative numerical microbiological and survey data collected was entered into NCSS in order to statistically analyze the results using differential and inferential statistics (NCSS, 2001). Ninety water bottles from ninety subjects were tested in order to approximate 30 subjects per group of cleaning methods in order to increase the probability that the data was normally distributed.

The types of numerical descriptive statistics that were used to analyze the data included the mean, median, standard deviation and range, in order to measure the central tendency (mean & median), and the spread of data (standard deviation and range). The two inferential statistical tests that were used were ANOVA and Correlational/Regression. ANOVA was used to determine the differences between groups, which were the types of cleaning methods (soap and

water, rinsing with tap water and other methods) and microbiological counts. Correlational/Regression Statistics was used to determine the relationship between the timeframe of cleaning and the microbiological counts (Heacock & Chiodo, 2005).

RESULTS

Survey Results:

The results from the survey indicate that the majority of people used soap and water (40%) to clean their water bottles, followed by other methods (31%) (which included dishwasher as the majority) and the least common method used was tap water rinse (29%). In regards to the timeframe between cleaning, the majority of people cleaned their water bottles within 1 month (40%), 38% cleaned within 1 day, 14% cleaned within 1 week and finally only 8% cleaned their water bottles within the last 6 months. See Figure 3 for graphical representation and Appendix D for full Descriptive Statistical data.

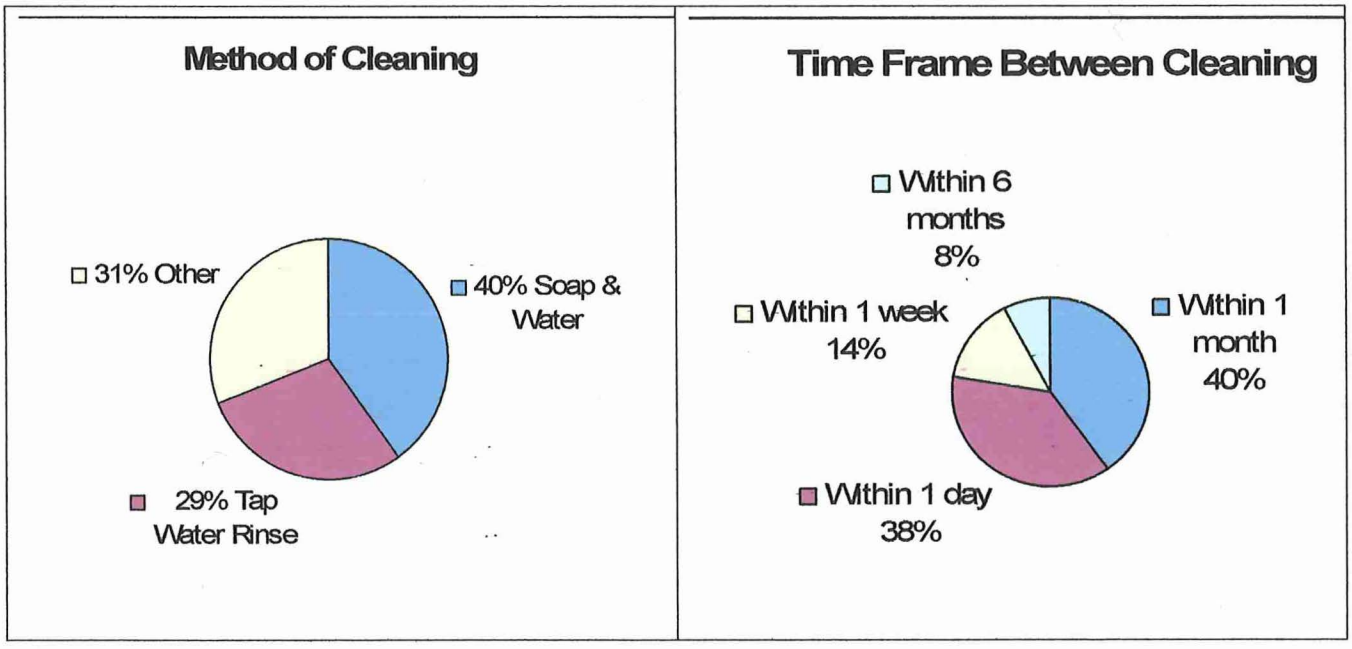

Figure 3: Pie Chart of Results for Method of Cleaning and Timeframe Between Cleaning

Microbiological Results:

Descriptive Statistics of Cleaning Methods (cfu/100ml):

	Mean	Standard Deviation	Median	Range
Soap & Water	19318	33317	2445	151980
Tap Water Rinse	3305	5247	1300	18159
Other	27370	84785	624	420000

Descriptive Statistics of Time Frame Between Cleanings (cfu/ml):

	Mean	Standard Deviation	Median	Range
One Day	8851	18809	900	86700
Within 1 Wk	16375	40691	1215	165999
Within 1 Mos	8944	10986	2300	30000
Within 6 Mos	79436	154221	7800	419000

Inferential Statistics:

ANOVA:

Refer to Appendix E for raw data and Appendix F for the results print-out. According to the Tests of Assumptions Section for Normality, all assumptions were rejected since all p values were <0.05 ($p=0.00$). The results indicated that the data is not normally distributed and the results from the non-parametric test were then examined. The non-parametric test used was the Kruskal-Wallis One-Way ANOVA on Ranks. The results indicated that the p value was <0.05 ($p=0.0225$) and the decision was to reject H_o. As a result, there was a statistically significant difference between the microbial counts and the different methods of cleaning. In order to determine the difference between the methods of cleaning, the Post hoc test was examined using the Kruskal-Wallis Multiple-Comparison Z-Value Test. The results of this test indicate that group 0 (cleaning with soap and water) was different than both group 1 (tap water rinse) and group 2 (other cleaning methods) and both group 1 and group 2 are different than group 0. (H. Heacock, personal communication, March 2, 2006).

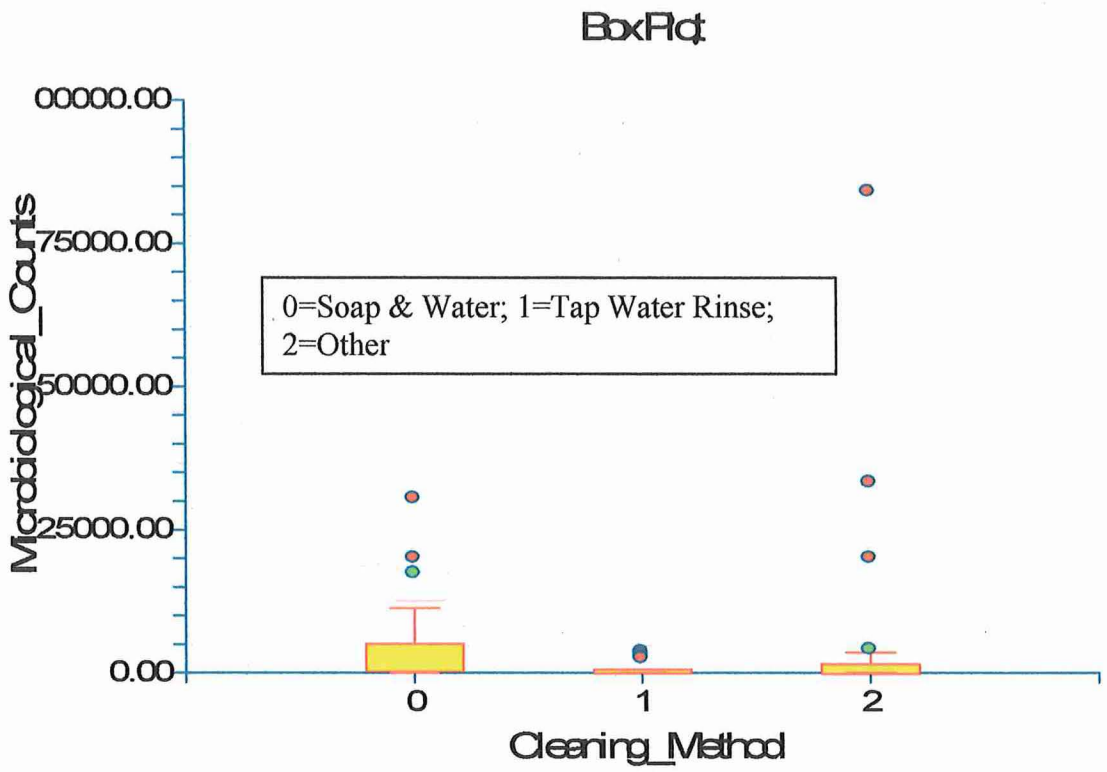

Figure 4: ANOVA Box Plot Results

Correlation/Regression:

Refer to Appendix E for raw data and Appendix G for the results print-out. According to the NCSS linear regression report the equation of the resulting line was y=13988x+4607. The interpretation is that for every unit increase in the timeframe between cleaning the microbiology counts increased by a slope of 4607 cfu/100ml. The results also indicated that the correlation was r=0.2482 (Figure 5). Since the results lie within the 0-0.25 range, the relationship between cleaning frequency and microbiological counts indicates little or no relationship (H. Heacock, personal communication, March 2, 2006).

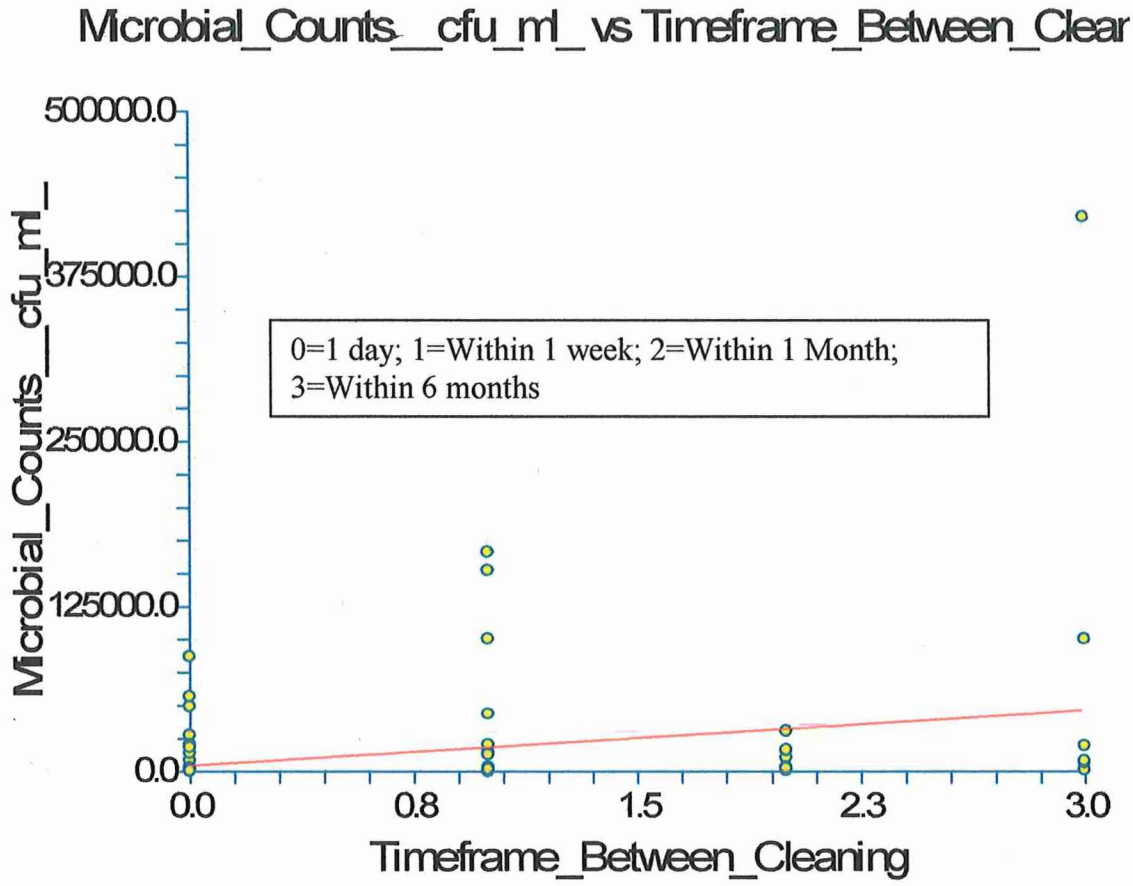

Figure 5: Correlation/Regression Results

In addition, the results from the t-test for the intercept indicate that the relationship was not significant and that the intercept is in fact close to 0, since p=0.5399 and H_o fails to be rejected. However, the t-test for the slope indicates that there was a significant difference since p=0.0183 H_o can be rejected. This means that as the timeframe between cleanings increased (ie. From 1 day to 6 months), so did the microbiolgical counts.

Canadian Drinking Water Quality Guidelines Comparison Results:

Finally, the microbiological counts were compared to the Canadian Drinking Water Quality Guidelines to determine if they met the guidelines. The results indicated that the guideline of <500 cfu/ml was exceeded 74.4% of the time (63/90 samples).

Type I (alpha) and Type II (Beta) Errors:

In both statistical tests, alpha was set at 0.05 in order to determine statistical significance and determine the likelihood the results were due to chance. The results of the test gave a p value of 0.0225, therefore H_o was rejected and the likelihood the results were due to chance was low. This makes Type I errors in this study unlikely. The results also show that the power = 31%, and Beta = 0.69 (since power = 1-Beta). This indicates that Beta errors are high and the power of this study is low since Beta errors of 0.69 exceeds the desired 0.2 and power is <80%. This may be due to the large spread of microbiological counts obtained and would therefore, require a larger sample size to reduce Beta errors and increase the power of the study (H. Heacock, personal communication, March 2, 2006).

DISCUSSION

The purpose of this research project was to determine if a relationship existed between drinking water quality found in personal water bottles and the cleaning practices followed by the public. The cleaning practices were determined by surveying what types of cleaning methods were used and the timeframe between the cleanings. The relationship between the types of cleaning methods and microbiological counts was measured using the ANOVA analysis of variance test, whereas the relationship between the timeframe between cleaning and microbiological counts was measured using correlation/regression.

The results of the ANOVA test indicated that there was a difference between the microbiological counts obtained and the different methods of cleaning. The descriptive statistics on the results of the different cleaning methods revealed that the tap water rinse had the lowest average microbial counts of 3305 cfu/ml, whereas other cleaning methods resulted in average microbial counts of 27,370 cfu/ml. This would mean that tap water rinsing may be a better method of cleaning over soap and water and/or other methods since it gave the lowest microbiological counts. However, these results may be misleading since there were not equal amounts of participants for each cleaning method. For example, only 29% of the participants used soap and water (average count 3305 cfu/ml), whereas 40% of participants used other methods of cleaning (average count 27,370 cfu/ml) , which may relate to the higher average counts obtained. The results of the Kruskal-

Wallis Multiple-Comparison Z-Value Test post hoc test revealed that cleaning with soap and water resulted in microbial counts that were the most different from both tap water rinse and other methods used. There was no other information that could be deduced from this test or any other post hoc test (H. Heacock, personal communication, March 2, 2006).

The results of the correlation/regression test indicated that as the timeframe between cleaning increased the microbiological counts also increased. However, the correlation between the relationship was weak. This may be due to the large range of microbiological counts that were obtained as indicated by the large standard deviation, which ranged from 10,000 to 154,000 cfu/ml. In addition, it is also possible that the weak relationship may have been due to recall bias of the water bottle owner and interviewer bias on behalf of the researcher. Firstly, the owner of the water bottle may not have been able to recall exactly when they last cleaned their water bottle and may have guessed or even lied about when they completed this task. Secondly, the interviewer if asked for clarification by the water bottle owner regarding the question about the timeframe between cleaning, may have been answered in a way that would have lead the participant to answer differently then they would have without anyone's guidance.

The microbiological values obtained were then compared to the Canadian Drinking Water Quality Guidelines to determine if they satisfied the appropriate HPC parameters. The microbiological results obtained in this study exceeded the maximum HPC limit of 500cfu/ml 74.4% of the time. This result is consistent with those obtained by the study performed at the elementary school in Alberta since analysis of the water there determined that 64.4% of the water collected from bottles exceeded the maximum limit (Oliphant, Ryan & Chu, 2002).

Based on all the results found in this study, it may be necessary for government agencies and/or personal water bottle manufacturers to consider educating the public on recommended cleaning practices of personal water bottles by means of pamphlets, newspapers or the media to ensure the integrity of the water the public drinks even after it comes out of the tap. This would include the importance of regular cleaning and recommending cleaning methods, such as soap and warm water, using a bottle scrubber especially around the mouth of the bottle, rinsing and possibly even sanitizing with 100ppm bleach solution and allowing to completely air dry.

Limitations

One of the limitations of this study was the sample size. Since the microbiological counts obtained varied greatly from 0 to over 400,000 cfu/ml, it firstly made it very difficult to count the colonies, but it also contributed to the type II errors in this study. In addition, due to time and budget constraints, only BCIT teachers, students and visitors were sampled, therefore the generalizability of the study was limited to only the previously listed participants. Furthermore, performing a study with HPC bacteria counts only gave a picture of the general level of sanitation and doesn't necessarily indicate if there were harmful bacteria present in the water at that time. Lastly, participant recall bias and interviewer bias may have contributed to results that may not have given an accurate picture of the actual study results.

Conclusions

Overall the findings from this study indicated that the counts of heterotrophic bacteria were higher the longer the timeframe between cleaning and that large numbers of microbiological counts were obtained. As well, it appeared that there was a significant difference between cleaning methods, although it was not definitive on which one since tap water rinsing resulted in the lowest average microbiological counts, but the post hoc test revealed that washing with soap and water resulted in microbiological counts was different then the other two cleaning methods. In general, the counts of heterotrophic bacteria greatly exceeded the drinking water guidelines. Therefore, according to the Canadian Drinking Water Guidelines, the water in 74.4% of the personal water bottles was not safe to drink.

Recommendations

Based on the results of this study, the following recommendations would include:

- Educating the public on the importance of cleaning and replacing their water bottles on a regular basis since there are no guidelines in place to protect the water they drink from their personal water bottles.
- Having hard plastic water bottle manufacturers provide suggested cleaning methods with every water bottle product, which may include washing with warm, soapy water, rinsing, sanitizing (with for example: bleach) every few days or recommending daily dishwashing and allowing the bottle to completely air dry.

- Disseminating the information to Environmental Health Officers about the potential contamination of personal water bottles, so that if questioned by the public regarding this issue, they will have the information to give them about the importance of regularly cleaning their personal water bottles and can even provide recommended cleaning methods.

Future research suggestions that could be made to improve on this research project would include:

- Surveying and sampling a wider variety of subjects (ie. Gyms, workplaces, general public) in order to increase the generalizability
- Increase the sample size to reduce the beta errors in the study
- Testing for specific opportunistic bacteria, such as pseudomonas, and/or indicator organisms such as total and fecal coliforms
- Perform more dilutions: 100 ml, 10 ml, 1 ml and 0.1 ml to improve counting ability
- Test only hard plastic personal water bottles or soft plastic personal water bottles or compare the differences between the two
- In a laboratory setting test the effects of different cleaning methods on counts of specific bacteria of only one water source or possibly even contaminating that water source to test the effectiveness of the cleaning methods

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APPENDIX A

Survey Script

Surveyor:

Hello, my name is Vanessa Ouellette and I am a student at BCIT in the Environmental Health Program. As part of my program requirements, I am conducting a test of the drinking water quality of personal water bottles and cleaning practices/cleaning frequency followed by the public by means of microbiological testing and a survey. The survey will take approximately 3-5 minutes and while you are filling out the survey I will collect 110 ml sample of water from your personal water bottle. Are you still interested in participating in the study?

- If the answer is "NO," then ask them if you can record a reason why they do not wish to participate? and thank them for their time.

- If the answer is "YES," then thank them and continue:

Surveyor:

Please take a moment to read the cover letter for more information on the study and thoroughly read the instructions on the survey before answering the questions. Do you have any questions before you begin?

Surveyor:

This concludes the survey and sampling. Thank you for your time and consideration.

Cover Letter

Title: Determining the Relationship Between Drinking Water Quality of Personal Water Bottles and Cleaning Practices/Cleaning Frequency Followed by the Public.

Purpose: To determine if there is a relationship between counts of bacteria and common cleaning practices/cleaning frequency followed by the public.

Notice: Participation in this study is voluntary. All information obtained will be kept strictly confidential. All hard copies submitted (including the survey and contact information form) will be destroyed after data is compiled. You may withdraw from the survey at any time if you feel uncomfortable without penalty.

If you would like to receive a summary of the findings of this study, please fill out the separate contact information form and hand it in to the research team member.

This survey will take approximately 3-5 minutes.

Thank you for participating!

Questionnaire

Instructions: Regarding the personal water bottle you are drinking from today, please read both the questions and answers before selecting the most appropriate answer:

1. What is the source of the drinking water in your personal water bottle?

Municipal___
Well___

2. Do you clean your water bottle?

Yes___
No___

3. Regarding the last time you cleaned your water bottle, what would best describe the method you used to clean it?

Soap and water___
Tap water rinse___
Other (ex. dishwasher, lemon, baking soda, bleach and water)_____

4. Recall back to the two times you last cleaned your water bottle. What following selection would best describe the length of time between those two cleanings?

One day___
Within one week___
Within one month___
Within six months___
Don't know___

This concludes the survey, thank you very much for your time and participation!

Contact Information Form

To receive a copy of the findings of this study please fill out the contact information below and return it to the research representative.

1. First Name and phone #: _____
   _____
   _____
   _____

   OR.....

2. Email Address: _____

APPENDIX B

DO YOU WANT TO KNOW WHAT'S GROWING IN YOUR WATER BOTTLE & HAVE A CHANCE TO WIN $50?

Then bring your filled personal water bottle (Example: re-used commercial water bottles (ex. Evian), or Nalgene)
to_____
on_____ and
participate in a student-coordinated study.

APPENDIX C

Heterotrophic Bacteria

Methods 8241 and 8242

Pour Plate and Membrane Filter Methods

Plate Count Agar*, m-HPC, m-TGE, m-TGE with TTC, and m-TSB/USP

Scope and Application: For water and wastewater

* This method meets or exceeds the specification criteria stated in *Standard Methods for the Examination of Water and Wastewater*, 19th edition, Method 9215 B. Pour Plate Method.

Introduction

The standard plate count attempts to provide a standardized means of determining the density of aerobic and facultatively anaerobic heterotrophic bacteria in water. Bacteria occur singly or in pairs, chains, clusters or packets, and no single method, growth medium, or set of physical conditions can satisfy the physiological requirements of all bacteria in a water sample. However, the heterotrophic plate count is a good measure of water treatment plant efficiency, aftergrowth in transmission lines, and the general bacterial composition of source water.

Technique is Important

Good laboratory technique is essential when accuracy is important, particularly in microbiological laboratory procedures. Care in sample collection and preservation, a clean laboratory or work surface, proper sterilization and inoculation practices, and close temperature control help assure reliable results.

Preparing the Work Area

To save time, start the incubator before preparing the other materials. Set the incubator for the temperature required in the procedure (usually 35 ± 0.5 °C).

Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray, or dilute iodine solution. Wash your hands thoroughly with soap and water.

Mark each pour plate, membrane filtration petri dish, or other sample container with the sample number, dilution, date, and any other necessary information. Take care not to contaminate the inside of the sample container in any way.

Preparing Sample Containers

Take care to prevent contamination when conducting bacterial tests. All materials used for containing or transferring samples must be sterile. To collect samples, use any of the following: sterilized plastic bags, sterilized disposable bottles, autoclavable glass bottles, or autoclavable plastic bottles.

Sterilized plastic bags or disposable bottles: Presterilized plastic bags and bottles are available with or without dechlorinating agent. The bottles are available with a 100-mL fill-to line.

Note: Dechlorinating reagent should be used with potable or chlorinated water samples. It is not necessary for unchlorinated or nonpotable water samples. However, dechlorinating reagent will not interfere with unchlorinated samples so, for simplicity, plastic bags containing dechlorineting reagent may be used for all samples.

Autoclavable glass or plastic bottles: Glass or plastic bottles (125-mL size) may be used instead of sterilized plastic bags or disposable bottles. These containers should be prepared as follows:

1. Wash in hot water with detergent.
2. Thoroughly rinse with hot tap water, followed by a distilled water rinse to make sure that all detergent is removed.
3. If dechlorinating agent is needed (for chlorinated, potable water), add the contents of one Dechlorinating Reagent Powder Pillow for each 125-mL of container volume. (A 250-mL sample container will require two powder pillows.)
4. Steam sterilize glass and autoclavable plastic containers at 121 °C for 15 minutes. Glass sample containers may be sterilized by hot air at 170 °C for one hour.
5. Store sterile containers, tightly capped, in a clean environment until needed.

Preparing Test Equipment

Use high-quality laboratory equipment and ready-to-use media to save time and minimize errors. Hach's prepared media helps eliminate contamination due to technique.

Preparing the Materials

Note: Disinfect the work bench or work area with a germicidal cloth, dilute bleach solution or dilute iodine solution. Wash hands thoroughly with soap and water.

Using Presterilized Equipment And Media

Bacteriological testing requires sterile materials, a disinfected work area and proper handling techniques, or contamination may give false results. To simplify technique and minimize the possibility of contamination, Hach offers membrane filters, disposable pipets, petri dishes with and without absorbent pads, inoculating loops, buffered dilution water, sampling bags and 2-mL prepared growth media. All have been presterilized.Hach offers presterilized and disposable pipets, petri dishes, with and without absorbent pads, inoculating loops, 99-mL bottles of buffered dilution water, sampling bags, and prepared growth media. When using these materials, an autoclave is unnecessary because only the filter funnel and forceps require sterilization. The funnel can be sanitized by immersion in boiling water for 5 minutes prior to use. (An optional, disposable sterile filter unit is also available.) The forceps can be sterilized by dipping them in alcohol and flaming.

14. Disconnect the syringe tip from the vacuum support tubing. Dispose of the liquid in the syringe.

Using an Autoclavable Filter Assembly for Membrane Filtration

Note: Disinfect the work bench or work area with a germicidal cloth, dilute bleach solution or dilute iodine solution. Wash hands thoroughly with soap and water.

1. After sterilization, remove the filter funnel assembly from the wrapping paper.
2. Do not contaminate the funnel by touching the inner surfaces that will be exposed to the sample.
3. Insert the funnel with rubber stopper into the filtering flask or filter funnel manifold and connect to the water trap and aspirator with rubber tubing.
4. Using sterile forceps, place a sterile membrane filter on the filter base and attach the filter funnel top.
5. Filter a small quantity of sterile Buffered Dilution Water through the funnel to assure a good seal on the filter and connections before filtering the sample.

Collecting and Preserving Samples

General Guidelines

Use proper sampling procedures to insure that seasonal variances are detected and that results are representative of the sample source. Using a sterile container, collect a sufficient volume of sample (usually 100 mL) for the guidelines to be met. The World Health Organization guidelines prescribe 200 mL per sample, while *Standard Methods for the Examination of Water and Wastewater* prescribes 100 mL per sample. Maintain at least 2.5 cm (approximately 1 inch) of air space to allow adequate space for mixing the sample prior to analysis.

Avoid sample contamination during collection. Carefully open each sample container just prior to collection, and close immediately following collection. Do not lay the lid or cap down and avoid touching the mouth or the inside of the container. Do not rinse the container.

No dechlorination is necessary if the sample is added directly to the medium on site. Otherwise, samples should be treated to destroy chlorine residual and immediately transported for analysis after collection. Sodium thiosulfate, sterilized within the collection container, is commonly used to destroy chlorine residual.

Failure to properly collect and transport samples will cause inaccurate results.

Analyze as soon as possible after collection. Allow no more than 6 hours to elapse between collection and examination for nonpotable water samples and 30 hours for potable water samples. For best results maintain the sample at or below 10 °C, but do not freeze. Failure to properly collect and transport samples will cause inaccurate results.

Membrane Filter Procedure

The Membrane Filter (MF) Heterotrophic Plate Count Method* is a fast, simple way to estimate bacterial populations in water. Since no single medium can satisfy the growth requirements of all bacteria, several types of media are offered for detecting heterotophic bacteria in water. The m-HPC medium, available in both the broth and agar formats, is a high-nutrient medium used to enumerate heterotrophs in treated potable water samples. The m-TGE broth, originally developed for use with dairy products, is now commonly used to determine bacterial counts in water by membrane filtration. The m-TGE broth with TTC contains a redox dye, triphenyltetrazolium chloride, which colors the colonies red, thus enhancing their visibility. The m-TSB/USP broth is a general purpose medium which was designed to conform with the formula specified in the USEPA's Code of Federal Regulations (21 CFR) for sterility testing of pharmaceutical products.

In the initial step, an appropriate sample volume is passed through a membrane filter with a pore size small enough (0.45 microns) to retain the bacteria present. The filter is placed either on an absorbent pad (in a petri dish) saturated with a culture medium or on an agar medium that is selective for heterotrophic bacteria growth. The petri dish containing the filter and pad is incubated, upside down, for 24 to 48 hours, depending on the medium used, at the appropriate temperature. After incubation, the colonies which have developed are identified and counted by using a low-power microscope.The MF method is especially useful for testing drinking water because large volumes of sample can be analyzed in a short time.

Diluting the Sample

The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter.

Generally, for finished, potable water, the volume to be filtered will be 100 mL. For samples which are suspected to have higher heterotrophic bacteria counts, use a smaller sample volume. Some sample types will require a very small volume to obtain the optimum 20 to 200 CFU. Because it is almost impossible to measure these small volumes accurately, as series of dilutions should be made. The following procedure describes one method of preparing a series of dilutions.

Dilution Technique

1. Wash hands.
2. Open a bottle of sterile, Buffered Dilution Water.
3. Shake the sample collection container vigorously, approximately 25 times.
4. Using a sterile transfer pipet, pipet the required amount of sample into the sterile Buffered Dilution Water.
5. Recap the buffer dilution water bottle and shake vigorously 25 times.

*Method 8242

Dilution Series

A. If 10-mL sample is required:

- Transfer 11 mL of sample into 99 mL of sterile buffered dilution water. Filter 100 mL of the dilution to obtain the 10-mL sample.

B. If 1-mL sample is required:

- Transfer 11 mL of the 10-mL dilution from sample A into 99 mL of sterile buffered dilution water. Filter 100 mL of the dilution to obtain the 1-mL sample.

C. If 0.1-mL sample is required:

- Transfer 11 mL of the 1-mL dilution from sample B into 99 mL of sterile buffered dilution water. Filter 100 mL of the dilution to obtain the 0.1-mL sample.

D. If 0.01-mL sample is required:

- Transfer 11 mL of the 0.1-mL dilution from sample C into 99 mL of sterile buffered dilution water. Filter 100 mL of the dilution to obtain the 0.01-mL sample.

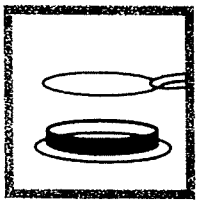
E. If 0.001-mL sample is required:

- Transfer 11 mL of the 0.01-mL dilution from sample C into 99 mL of sterile buffered dilution water. Filter 100 mL of the dilution to obtain the 0.001-mL sample.

F. If 0.0001-mL sample is required:

- Transfer 11 mL of the 0.001-mL dilution from sample D into 99 mL of sterile buffered dilution water. Filter 100 mL of the dilution to obtain the 0.0001-mL sample.

Membrane Filter Method for Heterotrophic Bacteria — Method 8242



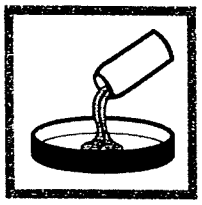
1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish.

Note: Do not touch the pad or the inside of the petri dish.

Note: To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.

Note: Alternatively, a prepared m-HPC agar plate may be used.

Note: For ease of use, petri dishes containing pads are available.



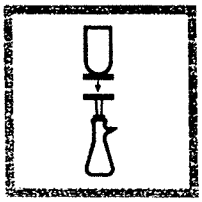
2. Invert ampules two or three times to mix broth. Open an ampule of m-HPC, m-TGE with TTC, or m-TSB/USP Broth, using an ampule breaker if necessary. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

Note: For broth prepared from dehydrated medium, pipet approximately 2.0 mL of broth onto the pad using a sterile pipet. Drain excess medium from the petri dish and replace the lid.

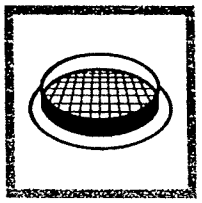
3. Set up the Membrane Filter Assembly as described under *Preparing the Materials* on page 2. Using sterile forceps, place a membrane filter, grid side up, in the assembly.

Note: Alternatively, a sterile, disposable filter unit may be used.

4. Shake the sample vigorously to mix. Filter the appropriate volume through the sterile 47 mm, 0.45µm, gridded membrane filter. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20 to 30 mL of sterile buffered dilution water.



5. Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

7. Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ± 0.5 °C for 48 hours for m-HPC, or 24 hours for m-TGE, m-TGE with TTC, or m-TSB/USP.

8. Remove the dish from the incubator. Count colonies on membrane filters using a 10 - 15X stereo binocular microscope.

Note: Bacterial colonies grown on m-HPC, m-TGE, or m-TSB/USP medium appear clear to cream. Colonies grown on m-TGE medium with TTC indicator appear red to aid visibility.

Counting, Computing, and Reporting Results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time, and the medium.
For example: 98 CFU/L, mL, 35 °C, 24 hours, m-TGE broth.

1 to 2, or fewer colonies per square — Count all of the colonies on the filter, and divide the results by the volume of original sample used.
For example: if there are 122 colonies on the filter, and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{\text{122 colonies}}{\text{10 mL sample}} = \text{12.2 CFU/mL}$$

3 to 10 colonies per square — Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.
For example: if you calculated an average of 8 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{8 colonies/square} \times 100}{\text{0.1 mL sample}} = \text{8000 CFU/mL}$$

10 to 20 colonies per square — Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.
For example: if there are an average of 17 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{17 \text{ colonies/square} \times 100}{0.1 \text{ mL sample}} = 17,000 \text{ CFU/mL}$$

More than 20 colonies per square — If there are more than 20 colonies per square, record the count as > 2000 divided by the volume of original sample used.
For example: if the original volume of sample used were 0.01 mL, results would be > 2000/0.01 or > 200,000 CFU/mL.

Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.

REQUIRED MEDIA AND REAGENTS

Description	Unit	Cat No.
Dilution Water, Buffered, sterile, 99-mL	each	14305-72
Dilution Water, Buffered, sterile, 99-mL	25/pkg	14305-98
m-HPC Agar Plates	15/pkg	26114-15
m-HPC Broth Ampules, plastic, 2-mL	50/pkg	28124-50
m-TSB/USP Broth Ampules, plastic, 2-mL	50/pkg	28126-50
m-TGE PourRite™* Ampules, glass, 2-mL	20/pkg	23738-20
m-TGE with TTC PourRite™ Ampules, 2-mL	20/pkg	24284-20

*PourRite is a Hach Company trademark

APPENDIX D

Descriptive Statistics for Cleaning Methods

Summary Section of Microbiological_Counts when Cleaning_Method=0

Count	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Range
36	19318.08	33317.67	5552.945	20	152000	151980

Means Section of Microbiological_Counts when Cleaning_Method=0

Parameter	Mean	Median	Geometric Mean	Harmonic Mean	Sum	Mode
Value	19318.08	2445	3566.231	268.2615	695451	112

Summary Section of Microbiological_Counts when Cleaning_Method=1

Count	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Range
26	3305.077	5247.54	1029.127	1	18160	18159

Means Section of Microbiological_Counts when Cleaning_Method=1

Parameter	Mean	Median	Geometric Mean	Harmonic Mean	Sum	Mode
Value	3305.077	1300	706.6304	21.25635	85932	1200

Summary Section of Microbiological_Counts when Cleaning_Method=2

Count	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Range
28	27370.14	84785.21	16022.9	0	420000	420000

Means Section of Microbiological_Counts when Cleaning_Method=2

Parameter	Mean	Median	Geometric Mean	Harmonic Mean	Sum	Mode

Descriptive Statistics for Timeframe Between Cleanings

Summary Section of Microbiological_Counts when Timeframe_Between_Cleaning=0

Count	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Range
36	8851.056	18809.22	3134.871	0	86700	86700

Means Section of Microbiological_Counts when Timeframe_Between_Cleaning=0

Parameter	Mean	Median	Geometric Mean	Harmonic Mean	Sum	Mode
Value	8851.056	900	892.4243	69.37928	318638	20

Summary Section of Microbiological_Counts when Timeframe_Between_Cleaning=1

Count	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Range
34	16375.82	40691.14	6978.474	1	166000	165999

Means Section of Microbiological_Counts when Timeframe_Between_Cleaning=1

Parameter	Mean	Median	Geometric Mean	Harmonic Mean	Sum	Mode
Value	16375.82	1215	1272.386	28.88886	556778	1200

Summary Section of Microbiological_Counts when Timeframe_Between_Cleaning=2

Count	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Range
13	8944.462	10986.09	3046.992	500	30500	30000

Means Section of Microbiological_Counts when Timeframe_Between_Cleaning=2

Parameter	Mean	Median	Geometric Mean	Harmonic Mean	Sum	Mode
Value	8944.462	2300	3967.607	2010.34	116278	1400

Summary Section of Microbiological_Counts when Timeframe_Between_Cleaning=3

Count	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Range
7	79436.14	154221.4	58290.21	1000	420000	419000

Means Section of Microbiological_Counts when Timeframe_Between_Cleaning=3

Parameter	Mean	Median	Geometric Mean	Harmonic Mean	Sum	Mode
Value	79436.14	7800	13039.35	3535.337	556053	

Cleaning Method	Micro Counts		Timeframe Between Clean	Micro Counts
0	2380	Cleaning Method:	0	2380
0	19250	0=Soap and water	3	19250
0	49000	1=Tap water rinse	0	49000
0	1120	2=Other	1	1120
0	86700		0	86700
0	27250		0	27250
0	43330	Frequency of Cleaning:	1	43330
0	56500	0=One day	0	56500
0	1200	1=Within one week	1	1200
0	2710	2=Within one month	0	2710
0	30500	3=Within six months	2	30500
0	152000		1	152000
0	20100		1	20100
0	30330		2	30330
0	20000		0	20000
0	21		1	21
0	2005		2	2005
0	14200		1	14200
0	3200		0	3200
0	1030		0	1030
0	78		1	78
0	1540		2	1540
0	100000		1	100000
0	1400		2	1400
0	1230		1	1230
0	2000		1	2000
0	1050		1	1050
0	10301		2	10301
0	570		0	570
0	99		0	99
0	1120		0	1120
0	2510		1	2510
0	1890		1	1890
0	8160		0	8160
0	657		1	657
0	20		0	20
1	12500		1	12500
1	122		1	122
1	15600		2	15600
1	2850		1	2850
1	2100		1	2100
1	3002		2	3002
1	2500		0	2500
1	1200		1	1200
1	18160		0	18160
1	14		0	14
1	1583		3	1583
1	13800		0	13800
1	270		0	270
1	1200		1	1200
1	1200		2	1200
1	1400		2	1400

1	1200		1	1200
1	3010		1	3010
1	1		1	1
1	16		0	16
1	1560		0	1560
1	1402		0	1402
1	1000		3	1000
1	102		0	102
1	120		0	120
1	20		0	20
2	201		1	201
2	70		1	70
2	524		1	524
2	166000		1	166000
2	20100		1	20100
2	920		0	920
2	117		0	117
2	330		0	330
2	85		0	85
2	708		0	708
2	880		0	880
2	0		0	0
2	17		1	17
2	18000		0	18000
3	540		0	540
3	2553		1	2553
3	16200		2	16200
3	420000		3	420000
3	5		0	5
3	100000		3	100000
3	129		1	129
3	110		1	110
3	500		2	500
3	6420		3	6420
3	1505		1	1505
3	350		0	350
3	7800		3	7800
3	2300		2	2300

APPENDIX F

Analysis of Variance Report

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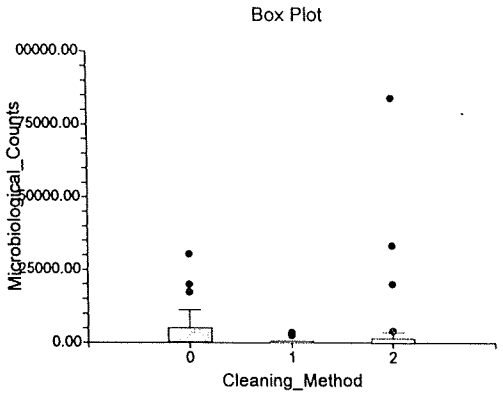
Database	C:\Documents and Settings\Va ... rch Real Data Due March 6.S0
Response	Microbiological_Counts

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	9.5187	0.000000	Reject
Kurtosis Normality of Residuals	7.1150	0.000000	Reject
Omnibus Normality of Residuals	141.2290	0.000000	Reject
Modified-Levene Equal-Variance Test	1.5360	0.221011	Accept

Box Plot Section



Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: Cleaning_Method	2	Yes	S(A)	S+sA
S(A)	87	No		S(A)

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Cleaning_Method	2	8.077387E+09	4.038693E+09	1.50	0.227974	0.312532
S(A)	87	2.336311E+11	2.685415E+09			
Total (Adjusted)	89	2.417085E+11				
Total	90					

* Term significant at alpha = 0.05

Analysis of Variance Report

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Database	C:\Documents and Settings\Va ... rch Real Data Due March 6.S0
Response	Microbiological_Counts

Kruskal-Wallis One-Way ANOVA on Ranks
Hypotheses
Ho: All medians are equal.
Ha: At least two medians are different.

Test Results

Method	DF	Chi-Square (H)	Prob Level	Decision(0.05)
Not Corrected for Ties	2	7.587965	0.022506	Reject Ho
Corrected for Ties	2	7.589527	0.022488	Reject Ho

Number Sets of Ties	6
Multiplicity Factor	150

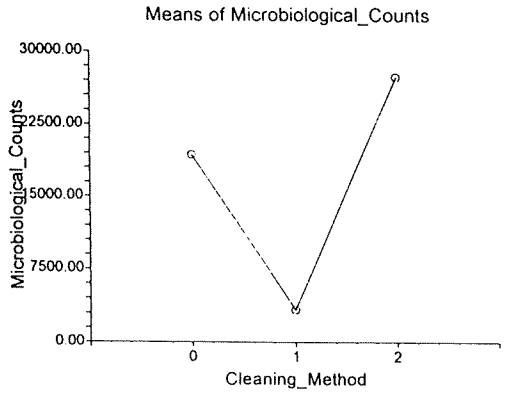
Group Detail

Group	Count	Sum of Ranks	Mean Rank	Z-Value	Median
0	36	1972.00	54.78	2.7509	2445
1	26	1036.00	39.85	-1.3086	1300
2	28	1087.00	38.82	-1.6298	624

Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	90	17197.19		16664.43
A: Cleaning_Method				
0	36	19318.08	8636.832	2653.649
1	26	3305.077	10162.93	-13359.36
2	28	27370.14	9793.247	10705.71

Plots of Means Section



Analysis of Variance Report

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Database	C:\Documents and Settings\Va ... rch Real Data Due March 6.S0
Response	Microbiological_Counts

Kruskal-Wallis Multiple-Comparison Z-Value Test

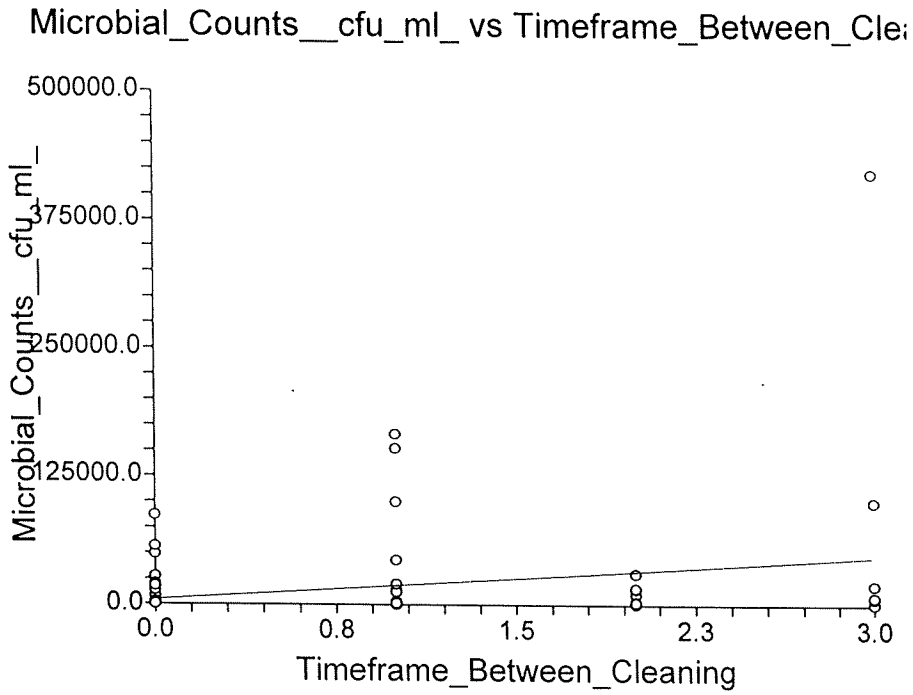
Microbiological_Counts	0	1	2
0	0.0000	2.2210	2.4242
1	2.2210	0.0000	0.1440
2	2.4242	0.1440	0.0000

Regular Test: Medians significantly different if z-value > 1.9600
Bonferroni Test: Medians significantly different if z-value > 2.3940

APPENDIX G

Database C:\Documents and Settings\Va ... rch Real Data Due March 6.S0
Y = Microbial_Counts__cfu_ml_ X = Timeframe_Between_Cleaning

Linear Regression Plot Section



Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	Microbial_Counts__cfu_ml_	Rows Processed	90
Independent Variable	Timeframe_Between_Cleaning	Rows Used in Estimation	90
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	4607.5880	Rows Prediction Only	0
Slope	13988.4455	Sum of Frequencies	90
R-Squared	0.0616	Sum of Weights	90.0000
Correlation	0.2482	Coefficient of Variation	2.9522
Mean Square Error	2.577472E+09	Square Root of MSE	50768.81

Summary Statement

The equation of the straight line relating Microbial_Counts__cfu_ml_ and Timeframe_Between_Cleaning is estimated as: Microbial_Counts__cfu_ml_ = (4607.5880) + (13988.4455) Timeframe_Between_Cleaning using the 90 observations in this dataset. The y-intercept, the estimated value of Microbial_Counts__cfu_ml_ when Timeframe_Between_Cleaning is zero, is 4607.5880 with a standard error of 7488.1840. The slope, the estimated change in Microbial_Counts__cfu_ml_ per unit change in Timeframe_Between_Cleaning, is 13988.4455 with a standard error of 5819.7540. The value of R-Squared, the proportion of the variation in Microbial_Counts__cfu_ml_ that can be accounted for by variation in Timeframe_Between_Cleaning, is 0.0616. The correlation between Microbial_Counts__cfu_ml_ and Timeframe_Between_Cleaning is 0.2482.

A significance test that the slope is zero resulted in a t-value of 2.4036. The significance level of this t-test is 0.0183. Since 0.0183 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 13988.4455. The lower limit of the 95% confidence interval for the slope is 2422.9073 and the upper limit is 25553.9837. The estimated intercept is 4607.5880. The lower limit of the 95% confidence interval for the intercept is -10273.6043 and the upper limit is 19488.7802.

Descriptive Statistics Section

Parameter	Dependent	Independent
Variable	Microbial_Counts__cfu_ml_	Timeframe_Between_Cleaning
Count	90	90
Mean	17197.1889	0.9000
Standard Deviation	52113.5865	0.9247
Minimum	0.0000	0.0000
Maximum	420000.0000	3.0000

Y = Microbial_Counts__cfu_ml_ X = Timeframe_Between_Cleaning

Regression Estimation Section

Parameter	Intercept B(0)	Slope B(1)
Regression Coefficients	4607.5880	13988.4455
Lower 95% Confidence Limit	-10273.6043	2422.9073
Upper 95% Confidence Limit	19488.7802	25553.9837
Standard Error	7488.1840	5819.7540
Standardized Coefficient	0.0000	0.2482
T Value	0.6153	2.4036
Prob Level (T Test)	0.5399	0.0183
Reject H0 (Alpha = 0.0500)	No	Yes
Power (Alpha = 0.0500)	0.0934	0.6618
Regression of Y on X	4607.5880	13988.4455
Inverse Regression from X on Y	-187155.4879	227058.5298
Orthogonal Regression of Y and X	-187155.4879	227058.5297

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(4607.58796904658) + (13988.4454664915) * (Timeframe_Between_Cleaning)

Analysis of Variance Section

Source	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (5%)
Intercept	1	2.66169E+10	2.66169E+10			
Slope	1	1.489099E+10	1.489099E+10	5.7774	0.0183	0.6618
Error	88	2.268175E+11	2.577472E+09			
Lack of Fit	2	1.564084E+10	7.820422E+09	3.1848	0.0463	
Pure Error	86	2.111767E+11	2.455543E+09			
Adj. Total	89	2.417085E+11	2.715826E+09			
Total	90	2.683254E+11				

s = Square Root(2.577472E+09) = 50768.81

Notes:
The above report shows the F-Ratio for testing whether the slope is zero, the degrees of freedom, and the mean square error. The mean square error, which estimates the variance of the residuals, is used extensively in the calculation of hypothesis tests and confidence intervals.

Linear Regression Report

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Database C:\Documents and Settings\Va ... rch Real Data Due March 6.S0
Y = Microbial_Counts__cfu_ml_ X = Timeframe_Between_Cleaning

Tests of Assumptions Section

Assumption/Test	Test Value	Prob Level	Is the Assumption Reasonable at the 0.2000 Level of Significance?
Residuals follow Normal Distribution?			
Shapiro Wilk	0.4916	0.000000	No
Anderson Darling	13.0574	0.000000	No
D'Agostino Skewness	9.2110	0.000000	No
D'Agostino Kurtosis	6.9520	0.000000	No
D'Agostino Omnibus	133.1728	0.000000	No
Constant Residual Variance?			
Modified Levene Test	2.6813	0.105106	No
Relationship is a Straight Line?			
Lack of Linear Fit F(2, 86) Test	3.1848	0.046311	No

No Serial Correlation?
Evaluate the Serial-Correlation report and the Durbin-Watson test if you have equal-spaced, time series data.

Notes:
A 'Yes' means there is not enough evidence to make this assumption seem unreasonable. This lack of evidence may be because the sample size is too small, the assumptions of the test itself are not met, or the assumption is valid.
A 'No' means the that the assumption is not reasonable. However, since these tests are related to sample size, you should assess the role of sample size in the tests by also evaluating the appropriate plots and graphs. A large dataset (say N > 500) will often fail at least one of the normality tests because it is hard to find a large dataset that is perfectly normal.

Normality and Constant Residual Variance:
Possible remedies for the failure of these assumptions include using a transformation of Y such as the log or square root, correcting data-recording errors found by looking into outliers, adding additional independent variables, using robust regression, or using bootstrap methods.

Straight-Line:
Possible remedies for the failure of this assumption include using nonlinear regression or polynomial regression.