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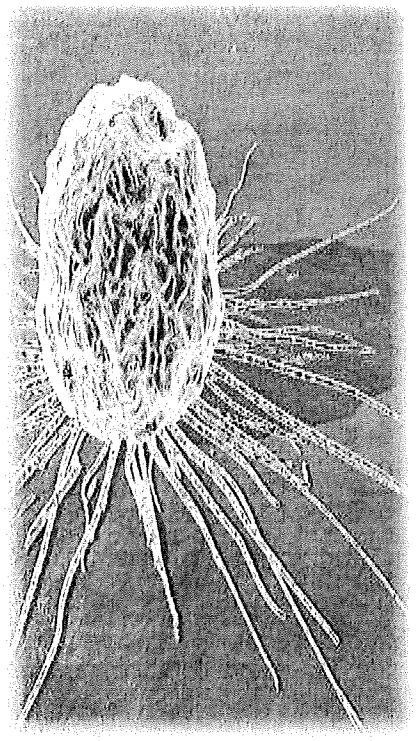
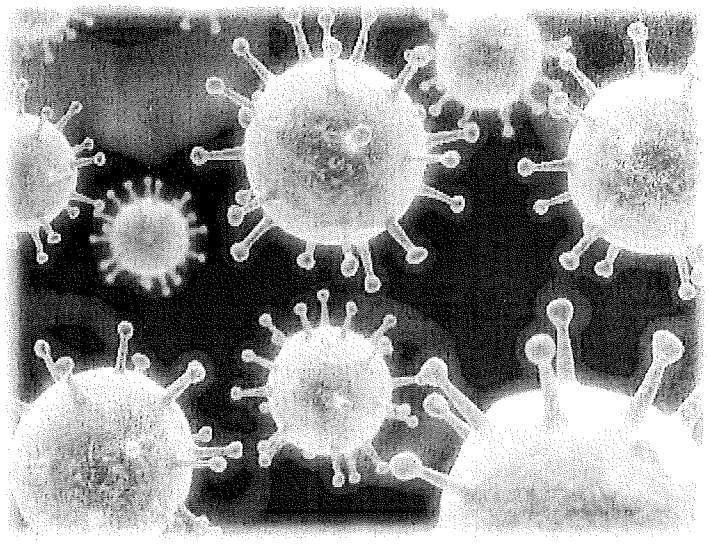


WHO IS YOUR CHILD REALLY SPENDING TIME WITH AT SCHOOL?



Contents

Abstract: Environmental surfaces, though they may appear clean, can harbour vast quantities of harmful pathogenic microorganisms. Therefore, it is imperative that proper cleaning and sanitation measures are in place to reduce the number of these microorganisms. This becomes even more important when those exposed to these environmental surfaces are a vulnerable group, such as elementary school children. To protect these children, surface sanitizers can be utilized to drastically reduce the amount of microorganisms present. One such sanitizer, SHC 50 General Sodium Hypochlorite Cleaner, was investigated to determine its effectiveness at reducing the microbial count on the desks of a grade on class. Twice a month the janitorial staff uses the sanitizer to clean the grade one desks. Within forty eight hours before and after the desks were cleaned, ten desks were swabbed and plated on 3M Aerobic Count Plate media and the resulting bacterial colonies enumerated. This was repeated for four separate cleaning periods. The final result was that the mean colony count of microbes on the school desks for the '**before**' sampling period was 42 cfu/50cm^2, while for the '**after**' sampling period it was 18 cfu/50cm^2. A chi squared analysis of the **'before'/'after'** mean colony counts was used to evaluate whether the desks tested for before and after samples produced bacterial colony counts exceeding the recommended guidelines of 100cfu/50cm^2. Of the eighty samples, only one **'before'** cleaning sample exceeded the guidelines. The result was a p value of 0.314267 was obtained, resulting in the null hypothesis not being rejected and implying that there was no association between the mean aerobic plate count before and after sanitation of the desks. A one tailed independent samples t test was also performed, providing a p value of 0.0000 (99.9%). These result showed that there was a statistically significant difference in mean colony counts between the **'before'** and **'after'** cleaning periods. It could therefore be concluded that the SHC 50 General Sodium Hypochlorite cleaner was effective at statistically reducing the microbial load on the desks.

Literature Review

Introduction: When a situation arises that involves maintaining and protecting the health of children, society as a whole, and in particular parents, regard this as an important priority. Yet, many of the environments children are placed in for extended periods of time of each day, such as daycares and schools, increase the probability of a child becoming ill[1,2,3]. This is due in large part to the children being in close physical contact with each other, permitting the easy transfer of many common and infectious pathogens[1]. Person to person transmission has, for many years, been recognized as a leading cause of acquiring harmful microorganisms in a multitude of environments[4]. However, indirect transmission must also be addressed. Can these same disease causing microorganisms be transferred indirectly from person to person via fomites such as desks? If so, what are effective ways of limiting, or altogether eliminating, the accumulation and transfer of these microorganisms from inanimate objects to a person? .

Environmental Surfaces and our Health: As our knowledge of how harmful bacteria and viruses increases, it follows that the public becomes more concerned about issues regarding their health and safety. Furthermore, such a concern typically leads to a greater understanding and appreciation among the general public of methods to improve public health. An obvious example is that it is not just medical practitioners and public health officials who understand the importance of hand washing to reduce the spread of infectious diseases[6,7]. In fact, washing ones hands more frequently, and not just after using the washroom, further reduces the likelihood of becoming ill[8,9,10]. Such a hygiene practice is important in environments where people are coming into frequent contact with one another and with surfaces that may harbour any number of microorganisms, such as in a crowded office[11,12,13], a day care centre[2], or school environment[10,14,3]. The concern and importance placed on ensuring certain high risk surfaces-like bathroom toilet seats-are free from harmful microorganisms is very much at the forefront of people's minds[9,15,16]. Evidence of this can readily been seen in the proliferation of products on store shelves claiming to be antimicrobial disinfectants. Such products range from surface cleaners used in the kitchen and bathroom to

antibacterial facial tissue. Moreover, just as many of us in society today have grown to trust and depend upon the health benefits of medicinal antibiotics for curing us when we are sick, so too have we become more dependent upon a variety of household cleaners to supposedly keep the surfaces we come into contact with free from germs.

Although the public is engaging in more rigorous hand washing and thoroughly disinfecting environmental surfaces, many individuals are still becoming ill, whether from the common cold or from more harmful illnesses[9]. The result is employees being absent more frequently from work[8], students missing more class time at school[13,10], and an overall greater burden placed upon our health care system. This is occurring even when environmental surfaces such as toilets and doorknobs are commonly recognized as having the potential of harbouring high microbial counts[2]. Furthermore, these areas are typically the most frequently sanitized[14], and have consistently shown to contain low microorganism counts[8,12,13,14] when routinely sanitized. What then could potentially be facilitating illness, even when those surfaces most feared for their potential to harbour harmful microorganisms, including fecal coliforms, are actually the areas most frequently sanitized[14]? Studies are pointing to a gap in the knowledge regarding what sort of environmental conditions are required for these harmful microorganisms to proliferate, and what are effective ways of destroying these microorganisms. As mentioned earlier, it is generally known that proper hygiene and sanitation is essential in washroom facilities to reduce the spread of disease, but not that it is also required for other areas and surfaces outside the bathroom. As a result, areas such as office and school desks, telephones, computer keyboards and mice, drinking fountains, cafeteria trays and bus seats can contain significant concentrations of harmful microbes[8,11,12,13,14]. Various studies have alluded to such surfaces being much less frequently sanitized, for instance desks, and containing anywhere from twenty one thousand[13] to up too two point seven million[14] microbes per square inch, while frequently cleaned surfaces, such as toilets seats, can contain from forty nine[8] to three thousand two hundred microbes per square inch[12,14]. People are coming into contact with these less sanitized surfaces and then picking up and transferring harmful microorganisms that are present[2,13,14]. This is very important, as evidence has shown that for many illnesses only a small number of organisms are

required to cause an infection[2,4,18,] and that many microorganisms, especially viruses, can survive extended periods of time on environmental surfaces [2,17,18].

Another question arises as to how these surfaces become so contaminated, as it surely could not simply be due to them being cleaned less frequently. In regards to desks, one obvious explanation is the amount of time individuals are in contact with these surfaces. Whether it be office workers or students, there is a greater likelihood of touching, and thus transferring, harmful microorganims from oneself, or other objects, to the desk[8,11,13]. Another contributing factor relates to the activities people engage in at their desks. Eating lunch at one's desk, storing food in one's desk, coughing and sneezing around the desk[8] itself, and leaving office or school supplies in or on one's desk all provide a food source and environment that encourages continued microbial growth[3,11,12,13]. Therefore, understanding that the above factors contribute to how quickly, and to what extent, one's desks becomes populated by microorganisms, will help limit them from becoming over contaminated. However, people still need to be able to work and function at their desks, and practically only so much can be done to initially avoid contamination of desks with bacteria, viruses and fungi. Hence, another solution is required.

Though we may recognize that all environmental surfaces need to be considered as breeding grounds for microbes, how are these harmful pathogens to be removed? Furthermore, there is a difference between cleaning a surface, which involves the use of soap or other agents to remove dirt, and disinfecting or sanitizing, which involves the use of chemicals such as bleach that have been proven to kill harmful microorganisms. Thus, even when these surfaces are assumed to be cleaned, they may not be effectively sanitized. This may be due to improper cleaning procedures being followed[19], or because the product itself is not an effective sanitizer[15,16]. A proper and effective disinfection routine then becomes essential in keeping in check microbial growth, and should combine both an effective disinfectant, and a proper and frequent cleaning routine[14, 20,21]. Numerous studies, conducted at various schools, have borne this statement to be true by showing that effective disinfecting plans can significantly reduce harmful microorganisms present on common surfaces[2,3,9,14].

Case Studies: One particular study performed at an elementary school involved daily wiping of surfaces with ammonium based sanitation wipes[3]. Teachers at the school were each given a container of Clorox™ disinfecting wipes with quaternary ammonium chloride as its active ingredient and instructed to clean the students' desks daily after lunch for eight consecutive weeks[3]. Combined with a proper hand washing routine, the desk cleanings were shown to substantially decrease the amount of microorganisms present on the surfaces of the desks[3]. Subsequently, this resulted in a significant reduction of the absenteeism rate for gastrointestinal illness at the elementary school for the classrooms involved[3]. This example demonstrates the importance of cleaning surfaces daily, and adds to other research that shows how a consistent, daily cleaning regime, whether it be with simple disinfectant wipes[13], or a solution based sanitizer/cleaner[8,12], is very effective in neutralizing the acquisition and spread of microorganisms.

Another research study conducted involved the in vitro comparison within a lab of various sanitizers/cleaners containing an assortment of active ingredients[2]. Several of these cleaners were commercially available, while some were typically only used in a hospital setting. The household sanitizers/cleaners included: Clorox Bleach™ (with sodium hypochlorite as its active ingredient), Arm and Hammer™ Baking Soda, Lysol™ antibacterial kitchen cleaner, Lysol™ disinfectant spray and Mr. Clean™[(2)]. The hospital sanitizers/cleaners included: the phenolic based cleaner Vesphene IIse™, the quatz based TBQ™, and alcohol in the form of ethanol[2]. The results of this study were based upon laboratory assays and showed that the most effective cleaners were TBQ™, Vesphne Isle™, ethanol, the Lysol™ Antibacterial Spray, and most relevant to the author's study, the Clorox™ Bleach[2]. What is more, the Clorox™ cleaning product showed excellent inactivation of antibiotic resistant strains of *S. aureus* and *Enterococcus*, and was one of only two disinfectants-the other being the Lysol disinfectant-to show excellent effectiveness for inactivating poliovirus[2]. These results reiterated the benefits of hypochlorite as a strong sanitizer against a variety of microorganisms. Furthermore, though cleaners/sanitizers with quaternary ammonium compounds as their active

ingredient are steadily becoming more popular because of their touted anti-microbial effectiveness[22] being similar to that of sodium hypochlorite[3], the American Centre for Disease Control still recommends the use of bleach based compounds such as Clorox[2]. This is because of the simple fact that quats has not been substantially proven to inactivate or destroy viruses such as norovirus or poliovirus[2,3].

Concerns with Sanitizers: Though an effective sanitizer/cleaner can be very useful in the war against harmful microorganisms, greater concern is emerging among the general public in regards to the harmful effects of the cleaners themselves. This is because many cleaners contain a litany of chemical compounds, with many already shown to contain harmful carcinogens[23] that are also found in pesticides[23,24]. Yet, "people assume that if it's on the shelf it's been tested, it's safe. And you can't make that assumption all the time. Not with the regulatory framework we have in place[24]". Fortunately, sodium hypochlorite based cleaners are favoured not only because of their effectiveness as sanitizers, but because of their relative harmlessness nature towards humans. The International Agency for Research in Cancer classifies sodium hypochlorite as a category 3 carcinogen, which implies it is 'not classifiable as to the carcinogenicity to humans, and is not considered a reproductive toxin[25]'. In addition, the MSDS sheet for sodium hypochlorite states that it contains no hazardous ingredients above 1.0%, and no carcinogenic ingredients above 0.1% [26]. It should be noted that though this product is not considered hazardous, it is a chemical, and can cause eye irritation or stomach problems if ingested[25,26]. As well, if sodium hypochlorite is mixed with an acid or a base, the production of chlorine gas may occur[25]. Finally, during some in vitro experiments, sodium hypochlorite has displayed evidence of mutagenic effects in both bacterial and mammalian cells[26,27]. Thus, as with any chemical cleaning product, proper care and safety precautions should always be taken when being used.

Purpose of Proposed Study: With this background information taken into consideration, the purpose of the proposed research study was two fold: First, an investigation of whether the sodium hypochlorite based cleaner SHC50 General Sodium Hypochlorite Cleaner "ready to use", which is used at the elementary school, was

effective at reducing the total microbial level on the student desks. Second, an attempt to ascertain whether the frequency of desk cleaning was sufficient in preventing them from containing more than the recommended microbial concentration of 100 cfu/50cm^2 at any one time[5,28]. This amount is prescribed to by the United States Public Service Standards, and followed by Health Canada's Compendium for Analytical Methods. This recommended concentration is also viewed as a good indicator with respect to the general sanitation level of the surface tested. Exceeding it indicates a higher general microbial concentration, and a greater likelihood that pathogenic microorganisms, such as fecal coliforms or norovirus, may be present in sufficient amounts to cause an illness. Such a potential health concern has implications for both Medical Health Officers (MHO) and Public Health Inspectors (PHI) in regards to the British Columbia School Act[29]. Section 90 (2) of the School Act states that if it is believed by the MHO or PHI that the health of the students are at risk, a school may be closed, while section 91 (1) outlines how it is the obligation of the MHO to examine the health of the students of a school. Conversely, section 61(1) of the Health Act[30] allows a PHI to inspect a school to ascertain whether a health hazard exits. Based upon previous research, a daily cleaning routine of environmental surfaces is required to achieve a microbial concentration below 100cfu/50cm^2, although this may not be practically feasible for most school janitorial staff. Currently, the elementary school tested for this project cleans its students' desks approximately twice a month.

Methods and Materials

Introduction: For the purpose of this research project samples were only taken from student desks in the specified grade one classroom, which consisted of twenty one male students and nine female students. Samples were not taken from the desks of a student who had been ill within the last two weeks, or from a desk contaminated by vomitus or body excrement. Environmental swab samples designated as **'before'** cleaning were taken within forty eight hours before school desks were scheduled to be cleaned.

Environmental swab samples designated as **'after'** cleaning were taken within forty eight hours after scheduled cleaning of the desks occurred.

To determine which desks would be sampled, all grade one students were randomly assigned a specific number. Numbers were randomly drawn and the corresponding student desks sampled. This procedure was repeated until forty desks for both **'before'** and **'after'** cleaning had been sampled. This equated to four **'before'** and **'after'** sampling sets, comprised of ten desks in each set (see table 1). Some desks, as expected, were sampled more than once, and some desks were not sampled at all. For consistency and to reduce bias, the same period of time **'before'** and **'after'** cleaning for all four sample sets was sought when sampling.

Materials:

Swabbing Materials	Culturing Materials	Growth and Enumeration
Test Tubes **Test Tube Rack** **Sterile Cotton** **Sponges** **Buffer Solution** **Tape** **Pens** **Pencils**	**3M Aerobic Count Plates** **3M flattening disc** **Pippetter** **1 ml pipette tips** **Laminar Flow Hood** **Vortex**	**Autoclave** **Parafilm** **Incubator** **Digital Camera** **Refrigerator** **Computer** **Excel** **NCSS**

Technique: Physically taking samples from the surface of an object, followed by laboratory analysis and testing of the samples, is regarded as the best way to determine if a surface is acceptable in respect to microbial contamination[5]. Surface sampling and testing provides not only a rough approximation of the microbial concentration of the surface, but when repeated, becomes more powerful[5,31] in its ability to judge the sanitary conditions present on the surface.

To properly sample environmental surfaces, several techniques are widely employed and utilized[32,5,33]. The three main techniques are the RODAC plate sampling method, the 3M Petrifilm method and swabbing[32,33]. The swabbing method itself can be further broken down into two main types. The first simply involves the use of sterile swabs and self made buffer/detergent solution[28,32,34], while the second involves the use of a pre-packaged, all in one swab and buffer solution kit made by 3M[35,36].

A RODAC plate is simply a pre-made agar plate with a cross-section grid delineated on the non agar side of the plate[32]. Each individual plate is relatively cheap, as it consists of only a small, specifically designed petri dish and **'in lab'** made agar. It is best suited for sampling the surface of flat, impervious surfaces[32,5]. It is a one step sampling and testing method, as sampling and culturing occur at the same time, with no transferring of solution to culture media required[32,33]. Though it has been shown to be quite useful for sampling for particular microorganisms, such as *Bacillus anthracis*[34] and *E.coli* [37], there are limitations. The maximum amount of area a RODAC plate samples is $25cm^2$. This means that if the surface to be sampled was one metre by one metre, a RODAC plate would not be a very representative sample at $25cm^2$, unless a lot of RODAC plates were used[32]. This however, can become quite expensive and cumbersome. A final drawback to RODAC plates is that typically there are some wasted plates, even when prepared by someone with experience[28].

The 3M petrifilm APC strip method is similar to RODAC plates in that the strip performs both surface sampling and transfer to media in one step[32,33]. Similar to the drawbacks with the RODAC pate, the 3M petrifilm APC strip can only sample $20cm^2$ per strip[32]. Lastly, a drawback present in both RODAC and 3M petrifilm APC strip sampling methods is that since the microorganisms sampled grow on the sampling media, there is no possibility of diluting the sample if concentrations are too high for proper enumeration[28].

The final method to be discussed is swabbing, which is the most common[2,5,38,3,42] and recommended[33] surface sampling technique.

Reasons for this include:

- Swabbing is a versatile surface sampling technique, particularly for irregular surfaces
- Can typically sample surfaces up to $1m^2$[5,33]
- Allows for repeated sampling of the same designated part of a surface and thus increases amount of microorganisms picked up by swab[32]
- Swab solutions contain buffers, detergents and surfactants[28,39] which help increase microorganism pickup and also help to neutralize any residual cleaning chemicals on the surface, such as quats or bleach, that may affect results[35,2]
- Swabbing allows for dilutions to be performed if concentrations of microorganisms will be too high for proper enumeration[28,32,5]

The first swab method to be discussed, the 3M pre-made environmental swabs, have become very popular in recent years. They come pre-made, require no preparation of buffer/detergent solution[32,35,36] and are reasonably priced for what they provide (1.25$ approximately for each swab[28]). Nevertheless, they do have limitations which contributed to them not being used for the research project. Though the price per swab kit may seem reasonable, when a lot of surface sampling needs to be performed, the amount of swabs required can quickly add up, along with the price. It was expected eighty to ninety swab samples would be used, and at 1.25$ per swab, that would have consumed the majority of the research budget. Another, more important reason why the 3M swabs were not used was that they do not allow for as much flexibility in regards to the surface area that can be sampled. Each 3M kit[28,32,40] only allows for a limited amount of surface area to be swabbed (max $100cm^2$).

After conducting a pilot study it was decided that sterile swab sponges would be used. The benefits of using sterile sponge swabs with **'in lab'** made buffer solution include:

- Swabbing is a technique the researcher is familiar with
- Swab solutions can be made **'in lab'** at no cost and will contain a buffer/detergent solution which is similar to that found in the 3M swabs[28]
- **'In lab'** created buffer solutions combined with the use of sponge swabs allow for the entire desk to be sampled.

- Sponge swabs are very effective at microbial recovery from environmental surfaces[32]

A drawback to using sponge swabs is that, unlike sterile fabric tipped swabs, they cost approximately one dollar per swab[41].

Summary of swabbing technique[32,5,33]: See Appendix A

Swab Solution: The **'in lab'** created swab solution contained a peptone buffer along with a surfactant to increase the yield when sampling. Thiosulfate was added to the solution to neutralize any chlorine remaining on the desks from the cleaning product. Residual chlorine can potentially destroy the microorganisms picked up during swabbing, especially if the sample cannot be plated right away, thus affecting the accuracy of the results[28].

Swab solution Recipe[42]: See Appendix B

Growth media: For growing and enumerating the microorganisms there were several options available. The first was preparing agar plates from scratch using standard ingredients, such as tryptic soy broth, inoculating the plates with the surface sampling solutions, incubating the plates at 35°C for approximately forty eight hours, and then counting the colonies[32,43]. Several advantages to this method were that various types of ingredients can be used to create the media, allowing for greater versatility to select the optimum ingredients to encourage microbial growth. More plates could have been prepared for the same equivalent price as buying pre-made plates[28]. Some drawbacks of self made agar plates were that they would have been much more labour intensive, and they would have increased the likelihood of spreading occurring, thus rendering counting the plates difficult. Spreading is where (typically because of water) individual colonies are not isolated from one another but instead grow together forming a large mass which is impossible to enumerate[28].

3M Aerobic Count Plates were used as a growth media for microorganism enumeration.

Reasons included:

- Money was available for purchase
- Easy to use, and ideally suited for storage in incubators[32]
- Required no media preparation
- Designed for enumeration[28] as they encourage the growth of individually isolated colonies and are designed to facilitate counting[43,44]
- Required only 48hrs to produce growth[39]

Control: For each sample set, a blank sterilized buffer solution was plated out on 3M ACP media to ensure that the buffer solution was actually sterile and would not affect results.

Growth of Microorganisms: Plating technique - See Appendix C

Statistical Analysis: The type of data obtained consisted of numerical data in the form of aerobic plate counts. It was expressed as colony forming units (cfu) per surface area (cm^2) swabbed. Descriptive statistics were performed on the colony counts recorded for the **'before'** cleaning plates, and for the **'after'** cleaning plates. The Number Cruncher Statistical System[45] was the statistical package used.

The mean cfu/cm^2 values obtained for the **'before'/'after'** cleaning plates were compared by way of inferential statistics using a one tailed independent samples t test. A one tailed independent samples t-test was proposed for assessing the numerical data because it was expected that the colony count of the plates from samples obtained **'after'** cleaning would be considerably lower than the colony counts of the plates of the **'before'** cleaning samples[28]. Because there was some variability as to how much time before and after the desks were cleaned they were actually swabbed, a paired t test was not utilized[31].

One tailed independent samples t-test: See Appendix D

Ho: Mean aerobic plate count after sanitization was equal to or greater than before sanitization

H_A: Mean aerobic plate count after sanitization was lower than before sanitization

In analysis of the t-test sampling information, it was seen that for the Tests of Assumption, normality was rejected in numerous categories. Therefore, the Aspen-Welch Unequal Variance Test results were utilized. The p value for whether mean cfu count '**before**' differs from mean cfu count **'after'** was 0.000000, with a five percent probability that chance played a role in the results[46]. Thus, the null hypothesis could be rejected and it could be concluded that there was a statistically significant difference in the results between **'before'** and **'after'** cleaning, implying that the mean aerobic plate count after sanitization was lower than before sanitization. The power of the results was high (99.9%), providing confidence in the validity of the results. As a result of this low p value, an alpha error was unlikely.

There was also nominal data generated from the numerical data obtained. Each cfu/cm^2 value recorded for every plate was compared to the standard guideline amount[5] of 100 cfu/50cm^2 and was assessed as to whether they were above or below this value. Those that fell below the recommended guideline were designated as **yes**, while those that exceed the recommended guideline were designated as **no**. The amounts of **yes's** and **no's** obtained from the '**before**' and '**after**' cleaning swabbing were then compared. This was done by way of a chi squared test. A chi squared test was performed on the nominal data in regards to how many plates of both **'before'** and **'after'** contain colony counts that exceed the recommended guidelines of 100cfu/50cm^2.

Chi squared test: See Appendix E

Ho: There was no association between the mean aerobic plate count before and after sanitation of the desks

H_A: There was an association between the mean aerobic plate count before and after sanitization of the desks

In performing the chi squared test the exact same data was used as for the one tail, two sample t test. The result was that one **'before'** cleaning sample exceeded the recommended guidelines, while the remaining thirty nine fell below the recommended guidelines. Zero **'after'** cleaning samples exceeded the recommended guidelines, thus all forty had less than the recommended guideline. The results from the test showed a p value of 0.314267, therefore H_0 was not rejected, and it was implied that there was no association between the mean aerobic plate count before and after sanitation of the desks. Due to the p value being significantly above 0.05, one should be cognizant of beta errors, where the null hypothesis has been accepted even though it should have been rejected.

	Before	After
Below guideline 'Yes'	39	40
Above guideline 'No'	1	0
Total	40	40

Data and Results: For pilot study results see Appendix F

Results Table 1: Bacterial counts on APC plates before Converting to CFU/50cm^2 factor. Bracket number represents Student ID Number

Please see attached excel document Appendix G 'Results" for Results Table 2 to 4

Period 1	Period 1	Period 2	Period 2	Period 3	Period 3	Period 4	Period 4
Before	After	Before	After	Before	After	Before	After
46 (4)	41 (4)	165 (1)	61 (1)	175 (3)	57 (3)	99 (2)	18 (2)
78 (7)	37 (7)	138 (5)	109 (5)	148 (7)	28(7)	81 (7)	24 (7)
177 (12)	18 (12)	102 (6)	33 (6)	41 (8)	16 (8)	167 (12)	38 (12)
71 (9)	9 (9)	147 (10)	86 (10)	123 (9)	17 (9)	125 (15)	32 (15)
89 (17)	46 (17)	298 (13)	201 (13)	32 (13)	27 (13)	48 (16)	28 (16)
53 (20)	10 (20)	109 (14)	67 (14)	43 (15)	30 (15)	219 (19)	20 (19)
27 (23)	52 (23)	81 (18)	55 (18)	93 (18)	49 (18)	48 (20)	38 (20)
86 (25)	49 (25)	99 (20)	26 (20)	66 (22)	37 (22)	77 (23)	66 (23)
217 (26)	93 (26)	74 (23)	81 (23)	184 (23)	70 (23)	138 (27)	17 (27)
69 (29)	28 (29)	87 (30)	83 (30)	133 (26)	102 (26)	104 (30)	39(30)

Desks not sampled: 11, 21, 24, 28

The student on desk number 15 was not entered into the randomization draw for the first two '**before**' and '**after**' sampling periods as this student was away from school during this period. However, they were entered into the final two randomization draws for '**before**' and **'after'** cleaning.

Sample calculation to convert colony count on 3M ACP media to CFU/50cm^2: See Appendix H

Discussion: The results of the research performed indicated that the SHC50 General Sodium Hypochlorite Cleaner combined with the current cleaning program was effective in significantly reducing the bacterial colony count on the school desks. The mean colony count after the desks were cleaned was observed to be less than half of what it was before cleaning. Though the cleaning program was effective, the results obtained were surprising in several ways. First, it was expected that more than the observed one 'before' cleaning samples would have a mean cfu/50cm2 count over 100cfu/50cm2, based upon the desks only being cleaned twice a month, and the desks being used by six year old children. Second, it was expected that the sanitizer and cleaning program utilized would not only statistically reduce the mean cfu/50cm2 count, but reduce it to a much lower number than the observed 18 cfu/50cm2.

There are several reasons that could explain the above results being observed. To begin with, the janitorial staff may only have cleaned part of the desks by giving them a quick wipe. Contrarily, the researcher swabbed the entire desk and possibly covered surface areas that may have not been cleaned/sanitized.

Another reason could be the contact time the sodium hypochlorite cleaner was allowed to sit on the desks, which was usually no more than a minute, according to observations of the teacher of the grade one classroom. It has been well documented that the longer a sanitizing agent is allowed to rest on an environmental surface-thus increasing its exposure time to microorganisms-the more microorganisms that will be destroyed[1].

The results obtained from the research conducted agreed with other research performed on evaluating the effectiveness of sodium hypochlorite based cleaners to reduce the amount of microorganisms residing on environmental surfaces[2,8,12]. Previous research reiterated how at recommended dilutions and contact time, bleach based cleaners are still highly effective at destroying a myriad of potential harmful microorganisms found on environmental surfaces[47]. As anticipated, use of such a cleaner was effective in reducing the amount of bacterial microorganism on the desks' surfaces. However, what varied for the results obtained in the current experiment from results described in other research was the SHC50 General Sodium Hypochlorite Cleaner and/or the cleaning routine failed to drastically reduce the amount of microorganisms present on the desks[2,3].

Limitations: One advantage to ACP plates as outlined in the materials and method section of the paper was that they reduce the likelihood of spreading occurring when compared to standard petri dish media plates. However, a few plates still experienced some spreading, which made counting the exact number of bacteria colonies more difficult, but still possible. Another limitation was that the **'after'** cleaning samples for each sample period were not taken at the exact same time frame after the **'before'** cleaning samples. For example, the final sample period (Feb 20^{th} -21^{st}) had approximately 24 hrs between the **'before'** and **'after'** sampling periods, while the third sampling period (Feb 6^{th} -8^{th}) had approximately 48 hrs between the **'before'** and **'after'** sampling periods. Due to the third sampling period having a longer time difference from when the desks were cleaned to when they were sampled it could have potentially allowed the desks to become re-contaminated to a greater extent.

Conclusion: The findings of the research performed showed that the sodium hypochlorite solution tested was successful in reducing the amount of microorganisms present on the surface of the desks. The mean bacterial count was reduced from 42 cfu/50cm^2 for **'before'** cleaning to 18 cfu/50cm^2 for **'after'** cleaning. Yet, based on previous research[2,3,8,12], the cleaner was expected to reduce the bacterial count more than was observed.

Recommendations to improve procedure: The first recommendation would be to use more ACP plates per each desk. By inoculating more 3M ACP plates for each desk sampled, a more representative colony count per desks can be obtained as multiple plates would be utilized for each swab sample. Another suggestion would be to use sponge swabs that already contain premade surfactant containing swab solution. This would minimize the likelihood of contamination being introduced from having to measure out separately made swabbing solution into each plastic bag containing a sponge swab. The procedure could also be improved upon by swabbing more desks for each sampling period, and continuing to sample throughout the entire school year. This increase in sample size would have allowed for more representative data to be collected.

Lastly, another way to improve the research procedure would be to ensure the desks are cleaned thoroughly and properly, thus reducing the likelihood that the data obtained would be due to an improper cleaning procedure. For the current research, the cleaning staff was not notified that testing would be done, nor were they instructed by the researcher on the proper way to clean and sanitize the desks. Therefore, it was difficult to state with any degree of certainty whether the higher than expected results obtained for **'after'** cleaning was due to the cleaning product, the cleaning routine, or a combination of both. If a precise and consistent cleaning routine was followed, the results obtained could have had more power to state that they are due to the cleaning product alone. This could be accomplished by having the researcher conduct the cleaning personally.

Significance to public health: The presence of excessive microorganism growth on environmental surfaces is a good indicator of not only the general sanitation level of the surface, but of the potential for pathogenic microorganisms such as viruses and bacteria to be living on the surface. High bacterial plate counts over the recommended guideline of 100 cfu/50cm^2 suggest that further, and more specific, testing may be warranted to determine whether harmful bacterial pathogens such as fecal coliforms are present. Such colony count results also suggest that better programs for both sanitizing environmental surfaces and proper hand washing would help to reduce the contamination

of these environmental surfaces. This is especially pertinent in an institutional setting, such as an elementary school. The reasons being it is easier for contagious pathogens to spread in such physical environments, and young children are typically more susceptible to illnesses, while at the same time not as personally hygienic as adults. Thus, having a proper sanitation and hygiene program becomes even more important.

Future Research: Finally, an area of pursuit for future research could involve testing the chemical cleaning product itself to determine if it is harmful. Questions such as does it contain carcinogenic compounds, or does it emit volatile organic compounds (VOC's), could be investigated. The results could then be compared to other common environmental surface sanitizers/cleaners to ascertain which is more 'safe' to use. Such information would be useful in the realm of public health because the most effective cleaner may not be the healthiest and safest choice. One may then have to decide how important an effective cleaner really is if the cleaner itself may be harmful to one's health.

Another area of future research could involve acquiring microbial samples from the desks daily after they have been sanitized to determine how much time elapses before the microbial load on the desks exceeds the recommended value of $100cfu/50cm^2$

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Appendix:

Appendix A: Environmental Swabbing Procedure[5]

•Desks were randomly chosen to be sampled.

•The entire desk was sampled.

•Sterile sponge swabs were opened and moistened in buffer solution. Excess solution on swab/sponge head was rinsed off.

•Holding the swab at a 30-45 degree angle it was rubbed across sampling surface area completely in one direction. This was repeated twice, each new time rubbing in a different direction.

•Upon finishing designated area, swab was rinsed in solution again, and any excess solution on swab head was squeezed out.

•Shake vigorously and plate.

•If cannot be plated, then store below 4°C until can be plated.

3.512 Sampling procedure

To sample equipment surfaces, open the sterile swab container, grasp the end of a stick, being careful not to touch any portion that might be inserted into the vial, and remove the swab aseptically.

Open a vial of buffered rinse solution, moisten the swab head, and press out the excess solution against the interior wall of the vial with a rotating motion.

Hold the swab handle to make a 30°-angle contact with the surface. Rub the swab head slowly and thoroughly over approximately 50 cm^2 of surface three times, reversing direction between strokes. Move the swab on a path 2 cm wide by 25 cm long or other dimensions to cover an equivalent area. Return the swab head to the solution vial, rinse briefly in the solution, then press out the excess. Swab four more 50-cm^2 areas of the surface being sampled, as above, rinsing the swab in the solution after each swabbing, and removing the excess.

After the areas have been swabbed, position the swab head in the vial, and break or cut it with sterile scissors or other device,[9] leaving the swab head in the vial. Replace the screw cap, put the vial in a waterproof container packed in cracked ice or other suitable refrigerant, and deliver to the laboratory. Analyze the sample within 24 hr after collection.

Appendix B: Swabbing solution Recipe

- 1.25 ml of stock phosphate buffer solution
- 5 ml of 10% aqueous solution thiosulfate
- 4 g of asolectin
- 10 g of tween 80 (or similar surfactant)

Appendix C: Use of 3M ACP[44]

1. Place the Petrifilm Aerobic Count plate on a flat surface.

2. Lift top film. Hold pipette perpendicular to the plate and carefully dispense 1 ml of sample onto the center of bottom film.

3. Release top film down onto sample.

4. Distribute sample evenly using a gentle downward pressure on the center of the (recessed side). Do not slide the spreader across the film. Remove spreader and leave plate undisturbed for ore minute to permit solidification of the gel.

5. Incubate plates in a horizontal position, with the clear side up in stacks not exceeding 20 plates. Follow current total plate count standards for incubation temperature. Temperatures above 37C are not recommended. Incubate plates 48 $\pm$ 3 hr.

6. Petrifilm Aerobic Count plates can be counted on a standard colony counter. The reduction of the tetrazolium indicator dye will cause the colonies to become red. All red dots regardless of size or intensity should be counted as colonies. The circular growth area is approximately 20 cm2. Estimates can be made on plates containing greater than 250 colonies by counting a representative number of squares and multiplying by the appropriate number to obtain an estimated count for the total 20 cm2 growth area. The presence of very high concentrations of colonies on me plates will cause the entire

growth area to become red or pink in color; record results as "too numerous to count (TNTC). Occasionally, on overcrowded plates, the center may lack visible colonies but many small colonies will be seen on the edges. When this occurs, record results as TNTC. Some organisms can liquefy the gel, allowing them to spread out and obscure the presence of other colonies. If a liquifier interferes with counting, an estimated count should be made by counting the unaffected areas.

7. To isolate colonies for further identification, lift the top film and pick the colony from the gel.

Appendix F: Pilot Study Results:

The pilot study involved testing both fabric tipped swabs and sponge tipped swabs. Both were used to sample a desk twice **'before'** it has been cleaned. For the fabric tipped swabs 1ml of broth solution was pipetted into nine millilitres of another buffer solution to create a 10^{-1} dilution, giving both standard and 10^{-1} dilutions to plate. Swabbed solutions of 1 millilitre were then pipetted onto both 3M ACP and self made TSB agar plates, and then allowed to incubate for forty eight hours at 35 degrees Celsius. For the sponge swabs, 1ml of the 20ml broth solution used with each sponge was pipetted onto 3M ACP plates only. After forty eight hours all plates were enumerated to gauge whether there was a difference between environmental sampling methods, whether one media type was better than the other, and whether the solutions had to be diluted first before plating. The results indicated poor recovery by the fabric tipped swab as compared to the sponge swab, even when considering the sponge swab sampled the entire desk. This added further credence to the researcher's decision to use sponge swabs as opposed to fabric tipped swabs.

Appendix H: Sample Calculation

- Dimension of school desks = 59cmx44cm=2596 cm^2
- 1 ml pipetted onto ACP plates from 20ml solution used to sample each desk=1ml/20ml=1/20

- For week 1 desk number 4, 46 colonies were counted on the incubated ACP plate. This was sampled over the entire desk which was 2596cm^2. For a 50cm^2 sample must divide 2596cm^2/50cm^2=51.92
- Only 1ml of the 20 ml used to swab each desk was pipetted, so this implies that only $1/20^{th}$ of the actual bacteria present in the solution was incubated.
- With this in mind the formula for determining colony count per 50cm^2 is (46 cfu*20)/51.92=18

Appendix D: One tailed independent samples t-test

Two-Sample Test Report

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Variable Plate_Count

Descriptive Statistics Section

Variable	Count	Mean	Standard Deviation	Standard Error	95% LCL of Mean	95% UCL of Mean
Cleaning_Period=1	40	41.925	22.63465	3.578853	34.68608	49.16391
Cleaning_Period=2	40	18.675	13.68958	2.164512	14.29686	23.05314

Note: T-alpha (Cleaning_Period=1) = 2.0227, T-alpha (Cleaning_Period=2) = 2.0227

Confidence-Limits of Difference Section

Variance Assumption	DF	Mean Difference	Standard Deviation	Standard Error	95% LCL of Mean	95% UCL of Mean
Equal	78	23.25	18.70471	4.182499	14.92328	31.57672
Unequal	64.16	23.25	26.45245	4.182499	14.89491	31.60509

Note: T-alpha (Equal) = 1.9908, T-alpha (Unequal) = 1.9976

Equal-Variance T-Test Section

Alternative Hypothesis	T-Value	Prob Level	Decision (5%)	Power (Alpha=.05)	Power (Alpha=.01)
Difference <> 0	5.5589	0.000000	Reject Ho	0.999792	0.997902
Difference < 0	5.5589	1.000000	Accept Ho	0.000000	0.000000
Difference > 0	5.5589	0.000000	Reject Ho	0.999944	0.999140

Difference: (Cleaning_Period=1)-(Cleaning_Period=2)

Aspin-Welch Unequal-Variance Test Section

Alternative Hypothesis	T-Value	Prob Level	Decision (5%)	Power (Alpha=.05)	Power (Alpha=.01)
Difference <> 0	5.5589	0.000001	Reject Ho	0.999779	0.997721
Difference < 0	5.5589	1.000000	Accept Ho	0.000000	0.000000
Difference > 0	5.5589	0.000000	Reject Ho	0.999942	0.999074

Difference: (Cleaning_Period=1)-(Cleaning_Period=2)

Tests of Assumptions Section

Assumption	Value	Probability	Decision(5%)
Skewness Normality (Cleaning_Period=1)	2.6860	0.007232	Reject normality
Kurtosis Normality (Cleaning_Period=1)	1.7038	0.088424	Cannot reject normality
Omnibus Normality (Cleaning_Period=1)	10.1173	0.006354	Reject normality
Skewness Normality (Cleaning_Period=2)	4.4948	0.000007	Reject normality
Kurtosis Normality (Cleaning_Period=2)	3.8308	0.000128	Reject normality
Omnibus Normality (Cleaning_Period=2)	34.8786	0.000000	Reject normality
Variance-Ratio Equal-Variance Test	2.7338	0.002225	Reject equal variances
Modified-Levene Equal-Variance Test	7.3288	0.008334	Reject equal variances

Two-Sample Test Report

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Variable Plate_Count

Mann-Whitney U or Wilcoxon Rank-Sum Test for Difference in Medians

Variable	Mann Whitney U	W Sum Ranks	Mean of W	Std Dev of W
Cleaning_Period=1	1349	2169	1620	103.8859
Cleaning_Period=2	251	1071	1620	103.8859

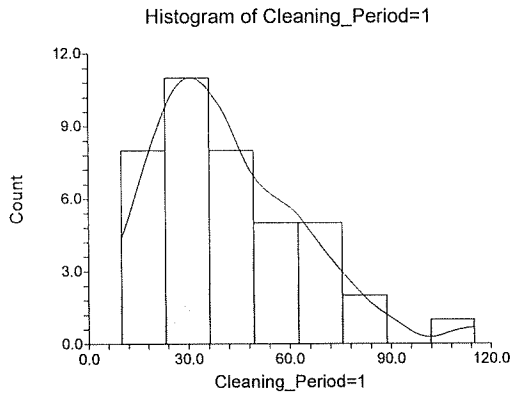
Number Sets of Ties = 22, Multiplicity Factor = 366

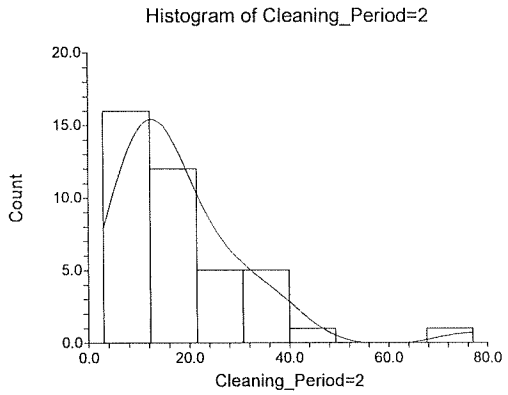
	Exact Probability		Approximation Without Correction			Approximation With Correction		
Alternative Hypothesis	Prob Level	Decision (5%)	Z-Value	Prob Level	Decision (5%)	Z-Value	Prob Level	Decision (5%)
Diff<>0			5.2846	0.000000	Reject Ho	5.2798	0.000000	Reject Ho
Diff<0			5.2846	1.000000	Accept Ho	5.2895	1.000000	Accept Ho
Diff>0			5.2846	0.000000	Reject Ho	5.2798	0.000000	Reject Ho

Kolmogorov-Smirnov Test For Different Distributions

Alternative Hypothesis	Dmn Criterion Value	Reject Ho if Greater Than	Test Alpha Level	Decision (Test Alpha)	Prob Level
D(1)<>D(2)	0.575000	0.3041	.050	Reject Ho	0.0000
D(1)<D(2)	0.000000	0.3041	.025	Accept Ho	
D(1)>D(2)	0.575000	0.3041	.025	Reject Ho	

Plots Section





Two-Sample Test Report

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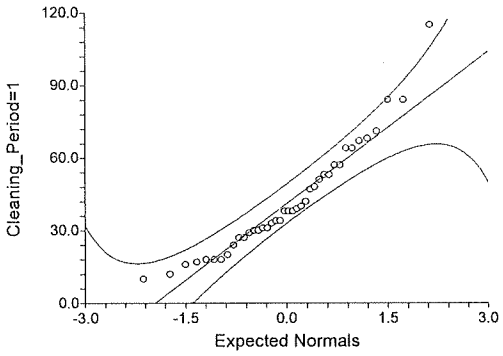
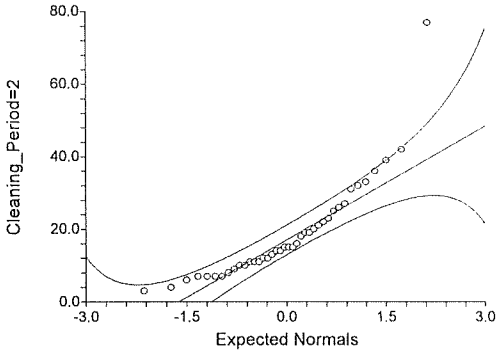
Variable Plate_Count

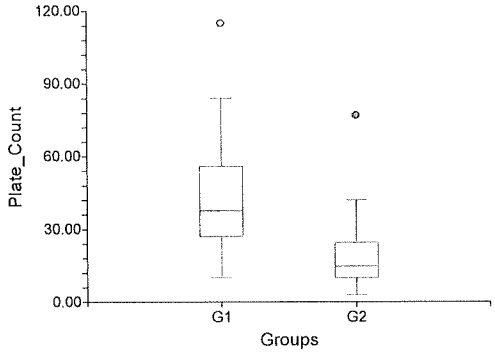
Normal Probability Plot of Cleaning_Period=1



Normal Probability Plot of Cleaning_Period=2



Box Plot



Appendix E: Chi Squared Analysis

Cross Tabulation Report

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Counts Section

	Before_After		
Above_Below	**0**	**1**	**Total**
0	1	0	1
1	39	40	79
Total	40	40	80

The number of rows with at least one missing value is 0

Chi-Square Statistics Section

Chi-Square	1.012658	
Degrees of Freedom	1	
Probability Level	0.314267	Accept Ho

WARNING: At least one cell had an expected value less than 5.

Fisher's Exact Test Section

	P1	P2
Proportions	0.025000	0.000000
Difference (D0 = P1-P2)	0.025000	
Correlation Coefficient	0.112509	

Hypothesis	Prob Level	Test Type	Calculation Method
Ho: P1=P2			D=P1-P2 for a table
Ha: P1<P2	1.000000	One-Tailed	Sum of prob's of tables where D<=D0
Ha: P1>P2	0.500000	One-Tailed	Sum of prob's of tables where D>=D0
Ha: P1<>P2	1.000000	Two-Tailed	Sum of prob's of tables where |D|>=|D0|

Appendix G: Results

Results Table 2:
ACP colony count

Sample Period 1		Sample Period 2		Sample Period 3		Sample Period 4	
Before (Jan 09)	After (Jan 11)	Before Jan 23)	After (Jan 26)	Before (Feb 6)	After Feb 8)	Before Feb 20)	After (Feb 21)
46	41	165	61	175	57	99	18
78	37	138	109	148	28	81	24
71	9	102	33	41	16	167	38
177	18	147	86	123	17	125	32
89	46	298	201	32	27	48	28
53	10	109	67	43	30	219	20
27	52	81	55	99	49	48	38
86	49	99	26	63	37	77	66
217	93	74	81	184	70	138	17
69	28	87	83	133	102	104	39
5	0	0	1	0	0	0	0

Control

Results Table 3:
ACP count converted to CFU/50cm2

CFU/50cm2 Sample Period 1		CFU/50cm2 Sample Period 2		CFU/50cm2 Sample Period 3		CFU/50cm2 Sample Period 4	
Before(Jan 09)	After (Jan 11)	Before (Jan 23)	After (Jan 26)	Before (Feb 6)	After (Feb 8)	Before (Feb 20)	After (Feb 21)
18	16	64	23	67	22	38	7
30	14	53	42	57	11	31	9
27	3	39	13	16	6	64	15
68	7	57	33	47	7	48	12
34	18	115	77	12	10	18	11
20	4	42	26	17	12	84	8
10	20	31	21	38	19	18	15
33	19	38	10	24	14	30	25
84	36	29	31	71	27	53	7
27	11	34	32	51	39	40	15

Results Table 4: Before and After Cleaning

Mean = 42 cfu/50cm2

All 'Before' Cleaning
CFU/50cm^2
18
30
27
68
34
20
10
33
84
27
64
53
39
57
115
42
31
38
29
34
67
57
16
47
12
17
38
24
71
51
38
31
64
48
18
84
18
30
53
40

Mean = 18 cfu/50cm2

All 'After' Cleaning
CFU/50cm^2
16
14
3
7
18
4
20
19
36
11
23
42
13
33
77
26
21
10
31
32
22
11
6
7
10
12
19
14
27
39
7
9
15
12
11
8
15
25
7
15

Before Cleaning Descriptive	
Mean	41.925
Standard Error	3.578853
Median	38
Mode	18
Standard Deviation	22.63465
Sample Variance	512.3276
Kurtosis	1.446823
Skewness	1.077845
Range	105
Minimum	10
Maximum	115
Sum	1677
Count	40

After Cleaning Descriptive	
Mean	18.675
Standard Error	2.164512
Median	15
Mode	7
Standard Deviatic	13.68958
Sample Variance	187.4045
Kurtosis	7.39315
Skewness	2.229137
Range	74
Minimum	3
Maximum	77
Sum	747
Count	40