Comparison of Aerobic and *E. Coli* Colony-Forming Units Isolated From Circulating Paper and Plastic $20 Canadian Banknotes

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Abstract

Introduction

Paper currency serves as an ideal breeding ground for microorganisms. Furthermore, the combination of its widespread use and its constant exchange make paper currency a likely agent for disease transmission. Recently the Bank of Canada has begun issuing plastic banknotes; theoretically, these plastic bills are less prone to contamination due to the inherent properties of plastic and specifically added antibacterial agents.

Purpose

The purpose of this study was to determine if the new plastic banknotes harbor fewer bacterium than comparable (in age and denomination) paper bills. The objective was to sample both paper and plastic bills, and statistically verify whether there is (or is not) a difference in microbial load.

Methods

Standard microbiological methods were followed to test paper and plastic bills. The sample bills were tested using 3M aerobic colony Petrifilm plates and *E. coli*/coliform Petrifilm plates. The number of colonies counted on the Petrifilm plates (both types) was used to indicate associated contamination levels.

Results

On average, the plastic bills had lower counts of aerobic bacteria and *E. coli* coliforms. However, there was not a statistically significant difference of contamination rates between plastic and paper bills (p-value: 0.090332). A low power (0.380125) indicates a beta error may have occurred and that a larger sample size is required to provide more accurate results.

Conclusions

The main conclusions resulting from this study include the following:

1. Contamination rates for plastic bills are statistically similar to those of paper bills.
2. On average (mean and median data), plastic bills had fewer aerobic bacteria and *E. coli* coliforms than paper bills.
3. Canadian bills have a similar contamination rate as US bills. This study showed a 6.6% rate of heavily contaminated Canadian bills, compared to 7% in the United States (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).
4. Contamination rates varied greatly. While the majority of bills had relatively low contamination rates, a select few had extremely high rates.

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1.0 Introduction

Money is the most widely used and sought after service on the planet. The transfer of paper currency has been the model of economic exchange since its introduction in China circa 1000 AD (Bernholtz, 2003). In the late 1800s and early 1900s, scientists began to theorize that the transmission of money was associated with the transmission of disease (Schaarschmidt, 1884) (Hilditch, 1908) (Morrison, 1910) (Boyer, 1921). Modern scientific techniques have confirmed these theories and have shown that viable pathogenic organisms (viruses, bacteria, and fungi) can be isolated on the surfaces of both paper and coin currency (Kuria, Wahome, Jobalamin, & Kariuki, 2009) (Lamichhane, Adhikary, Guatam, & Maharjan, 2009) (Thomas, Vogel, Wunderli, Suter, & Witschi, 2008).

Research has shown that paper currency serves as an ideal breeding ground for microorganisms for several reasons. First, the paper bills offer a large surface area for organisms and organic debris to collect (Ayandele & Adeniyi, 2011). Secondly, folds and/or deliberate depressions or projections specifically engineered into the bills' design as anti-counterfeiting methods serve as settling sites for both organisms and debris, which allow the microorganisms to live longer (Lamichhane, Adhikary, Guatam, & Maharjan, 2009). Lastly, banknotes weave their way through the population for many years before they come to rest. Studies indicate that the age and denomination of a bill have a direct correlation with the contamination observed (e.g., older bills had the most contamination while newer bills had the least) (Pradeep, Marulasiddaiah, & Chetana, 2012).

Physical transfer of material from hands, surfaces, and the environment can contaminate paper currency (Ahmed, Parveen, Nasreen, & Feroza, 2010) (Kuria, Wahome, Jobalamin, & Kariuki, 2009). Individuals from almost every socio-economic background routinely hold and

transfer paper currency. Any object that can spread communicable diseases throughout a diverse population should be considered a risk to public health. Therefore, paper currency has an important role in the transmission of pathogenic microorganisms and presents a moderate risk to public health.

The Bank of Canada has ceased making paper currency. Beginning in 2011, the bank began issuing new polypropylene (plastic) currency notes for $100 and $50 bills, with all other denominations scheduled for transition by 2013. The new bills have four advertised features to lessen the microbial load on circulating notes: less pore space for colonization, easier to physically clean (waterproof), absorb little to no moisture, and are impregnated by an antibacterial agent (Bank of Canada, 2012b). This study aims to test these claims by examining the microbial load on circulating paper and plastic Canadian currency.

2.0 Literature Review

2.1 Significance to Public Health

Paper currency and coins can serve as agents for transmission of microorganisms (fomites) and are frequently and freely passed from person to person. This section will limit its focus to the prevalence of contamination, common isolated pathogens from banknotes, risks associated with food establishments, and the introduction of the new plastic Canadian banknotes.

2.2 Prevalence

Two constant aspects of the studies researched show that denomination and age of a bill directly correlate with contamination. Currency notes of lower denominations were the most contaminated, presumably because lower denomination notes pass through more hands in their lifetime than the higher denomination notes (Lamichhane, Adhikary, Guatam, & Maharjan, 2009) (Ayandele & Adeniyi, 2011). Other studies demonstrated that the age of the currency note

had a positive correlation with microbial contamination. Increased contact time is presumed to escalate contamination (Barro, Bello, & Savadogo, 2006) (Igumbor, Obl, & Bessong, 2007).

2.2.1 Worldwide

Studies from around the world have reported high rates of microbial contamination of currency notes in circulation (Pradeep, Marulasiddaiah, & Chetana, 2012) (Barro, Bello, & Savadogo, 2006) (Ayandele & Adeniyi, 2011). Although every location contained endemic bacterium, the microorganisms most commonly isolated on paper money included members of the family *Enterobacteriacea*, *Mycobacterium tuberculosis*, *Vibrio cholerae*, *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp., and *Corynebacterium* sp. (Ahmed, Parveen, Nasreen, & Feroza, 2010). Common background contaminants of paper money were environmental organisms such as gram-positive flora (especially *Bacillus* sp.) and those arising from human normal skin flora such as *Staphylococcus aureus* (Ahmed, Parveen, Nasreen, & Feroza, 2010)

2.2.2 Developing Nations

Developing nations have the highest rates of currency contamination. After researching studies conducted around the world, it became clear that poor nations with large, impoverished populations were funding these studies (India, Nepal, Myanmar, Vietnam, several parts of Africa, and others) (Pradeep, Marulasiddaiah, & Chetana, 2012) (Igumbor, Obl, & Bessong, 2007) (Barro, Bello, & Savadogo, 2006).

One particular study conducted in the Venda region of South Africa showed that bacteria and fungi were isolated from 96% of the used banknotes collected in the study (Barro, Bello, & Savadogo, 2006). No microorganisms were isolated from new banknotes received directly from the bank. The source of contamination on the used notes must be from handling and use. Of particular concern was the isolation of Shigella and Salmonella from the currency, which

indicated fecal contamination. This finding supports the theory that individuals who prepare food after handling contaminated currency notes have a higher risk of infecting themselves and others with foodborne pathogens.

2.2.3 Developed Nations

Microbial contamination of paper money is not only confined to developing nations. Several studies from the United States reported contamination of coins and paper bills and revealed the presence of pathogenic microbes like *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella enterobacter* (Vriesekoop, Russell, & Alvarez-Mayorga, 2010). One such study of US currency isolated 93 types of bacteria (belonging to the species *Staphylococcus, Streptococcus, Enterobacter, Acinetobacter, Pseudomonas, Bacillus,* Diptheroids, *Klebsiella pneumoniae,* and *Escherichia vulneris)* (Ahmed, Parveen, Nasreen, & Feroza, 2010).

2.2.4 United States and Canadian Currency

The United States and Canadian type of banking systems revolve around a continuous cycle of printing new bills while destroying old "contaminated and mutilated" banknotes. This system is, to date, the most effective method of getting contaminated banknotes out of circulation before they can cause health problems to vulnerable individuals (Bank of Canada, 2012a) (Federal Reserve Bank, 2012).

A study of American coins and currency revealed the presence of pathogenic bacteria on 18% of the coins and 7% of the bills (Raloff, 2010). The study stated that the "cleanest" banknotes contained 20 CFUs (colony-forming units) and the "dirtiest" banknotes contained more than 25,000 CFUs (Raloff, 2010).

No published studies of Canadian currency contamination were found at the time of this writing. However, in 2007 a former British Columbia Institute of Technology (BCIT) student

(Tony Gill) conducted a research paper titled, “Is the Amount of Contamination on Money a Significant Threat to Public Health?” Although his research provided data regarding paper $20 bills, the data will not be used in this study because it met the definition of exclusion data listed in Section 4.6. Gill’s study did, however spark my interest in the association between contaminated banknotes and foodborne illness.

2.3. Pathogens of Concern

2.3.1 Common Pathogens Found on Currency

Potentially dangerous bacterial agents that have been isolated on paper currency include the following:

1. *Streptococcus* and *Staphylococcus* that have developed resistance to conventional antibiotics (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).
2. *E. coli* is usually nonpathogenic, but some strains can cause serious (potentially fatal) food-poisoning infections (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).
3. *Enterobacter cloacae* is associated with urinary tract and respiratory tract diseases (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).
4. *Staphylococcus epidermidis* is usually nonpathogenic but can cause infection in patients whose immune system is compromised (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).
5. *K. pneumoniae* is a virulent organism that can cause pneumonia, typically along with urinary tract and wound infections, particularly in immunocompromised individuals (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).

6. *Enterobacter aerogenes* is a nosocomial and pathogenic bacterium that causes opportunistic infections in skin and other tissues (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).
7. *Salmonella choleraesuis* can cause salmonellosis, an acute gastroenteritis with sudden onset of headache, abdominal pain, diarrhea, nausea, and sometimes vomiting (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).
8. *S. aureus* can cause a range of illnesses, from minor skin infections such as pimples, impetigo boils, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis endocarditis, toxic shock syndrome (TSS), and septicemia (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).

2.3.2 Vulnerable Populations

Most of the bacteria mentioned in Section 2.3.1 do not typically cause infections in healthy people. Rather they have been known to cause infections in young children and those with a suppressed immune system (including those with HIV, undergoing chemotherapy, or taking other medications that suppress the immune system).

2.4 Implications for Food Premises

2.4.1 Foodborne Illness and Currency

Data accumulated during the last 20 years indicate that pathogens on currency notes could represent a potential cause of foodborne illness (Micheals, 2002). Many food outlets rely heavily on the exchange of paper currency for their products. If the same person is handling both money and food products (especially ready-to-eat products), the risk of cross-contamination increases (Green, Selman, & Radke, 2006). These findings have resulted in several changes regarding how food is prepared and handled in the food service industry. In some instances, the

handling of food and money has been physically separated. In other instances, gloves are used to handle food and bare hands are used to handle the money, or vice versa. In both instances, employees of food service establishments are often observed handling money and food improperly (Green, Selman, & Radke, 2006).

2.4.2 Mobile Food Vending Operations

Vending operations are of particular concern (food carts, local markets, etc.), as operators often prepare, serve, and collect money from numerous patrons without properly washing their hands (Barro, Bello, & Savadogo, 2006). A significant association has been established between contamination and sources of currency (minibus drivers, butchers, food sellers, and banks were examined in this study), with the highest levels of contamination found among currency notes from minibus drivers (84.8%), followed by butchers (78.0%) and food sellers (62.1%). No bacterial contamination was found on new banknotes obtained from banks (Lamichhane, Adhikary, Guatam, & Maharjan, 2009).

2.5 Canadian Guidelines and Regulations

2.5.1 Bank of Canada

The Bank of Canada simply destroys all contaminated and mutilated currency notes. There is no definition for "contamination" listed, but their Website states, "Contaminated notes could be harmful to one's health or safety because they have come in contact with toxic substances (e.g., blood, mould, drugs, and unknown substances)" (Bank of Canada, 2012a). The Bank of Canada will reimburse patrons for submitted notes and may perform testing if it is deemed necessary (Bank of Canada, 2012a)

2.5.2 Food Premises Regulation

There are no specific regulations under the Food Premises Regulation that pertain to proper money-handling procedures. However, Section 12 states, "Every operator of food premises must ensure that all food on the premises is protected from contamination, and stored, handled, prepared, displayed and dispensed in a sanitary manner" (Food Premises Regulation, 2009).

2.6 Introduction of New Canadian Bills

2.6.1 New Canadian Notes Enter Circulation

Beginning in 2011, the Bank of Canada began circulating plastic Canadian currency modeled after the notes produced by the Reserve Bank of Australia (Bank of Canada, 2012b) (Reserve Bank of Australia). Because of the sensitive nature of this information, only a limited amount of information is available regarding the manufacturing process. Currently the plastic $100, $50, and $20 notes are being circulated, while the paper $100, $50, and $20 notes are being transitioned out of circulation. Remaining denominations ($10 and $5) are scheduled for circulation by the end of 2013. The plastic banknote is apparently a success, as 24 countries have adopted the plastic currency to date. Besides looking and feeling different, these bills have built-in features that should improve their durability and longevity, plus they are less prone to contamination.

2.6.2 Public Health Features

This polypropylene material is very durable and is basically waterproof. It does not absorb moisture and can be washed with household cleaners without issue. Thus, moisture and organic material cannot easily diffuse through the polypropylene matrix, making the plastic banknotes theoretically less prone to contamination than paper banknotes (Boaden, 2008).

3.0 Purpose of Study

The purpose of this experiment was to determine the number of aerobic and *E. coli* CFUs that can be isolated from both circulating plastic and paper currency. Collected data was statistically analyzed to test the hypothesis that the new plastic currency harbors fewer aerobic CFUs than traditional paper currency. If this hypothesis is correct, current food service money-handling procedures may become obsolete with the introduction of plastic bills.

4.0 Methods and Materials

4.1 Materials Used

The materials used in this experiment are outlined in Tables 4.1-1 and 4.1-2.

Table 4.1-1 Scientific Materials Required for This Experiment
Latex gloves
Sterile plastic bags
Coliform Count Petrifilm (3M)
Aerobic Count Petrifilm (3M)
Incubator set at 35°C
Sterile water
Thermometer

Table 4.1-2 Statistical Materials Required for This Experiment
Standard computer
NCSS 8 statistical software
Microsoft Excel 2010

4.2 Standard Methods

The study design is a straightforward microbial sampling experiment. A total of 60 banknotes (30 plastic and 30 paper bills) were tested using the sampling media discussed in Section 4.2.1. All sample banknotes consisted of randomly collected $20 bills, as described in

Section 4.6. The researcher statistically analyzed and compared the data (see Sections 5.0 and Tables 5.5-2 and 5.5-3, respectively) to determine whether plastic currency harbors fewer CFUs than comparable paper currency.

4.2.1 Sampling Media

As recommended by Kim Cummings and Ken Keilbert (Cummings & Keilbert, 2012), both paper and plastic currency notes were sampled using two types of 3M Petrifilm to ensure enough useable data was collected. These Petrifilm plates were prepared to function in similar fashion as a RODAC (Replicate Organism Detection and Counting) plate. By simply preparing a blank Petrifilm plate, we were able to sample the bills by direct contact (see Figure A-1 in Appendix A). This method was recommend to the researcher because the supplies were readily available and cost-efficient (one-third the cost of RODAC plates). Descriptions of the media are found in Section 4.2.2; see Appendix A for a detailed methodology of sampling techniques.

4.2.2 Description of Media

The 3M Petrifilm Aerobic Count (AC) plate is a sample-ready culture medium that contains standard methods nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. Petrifilm AC plates are commonly used for the inventory of aerobic bacteria in the food and beverage industries. Petrifilm AC plate components are decontaminated though not sterilized (3M Canada, 2012a).

The 3M™ Petrifilm *E. coli*/Coliform Count (EC) plate is a ready-made culture medium system that contains Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity (BCIG), and a tetrazolium indicator that facilitates colony enumeration. Petrifilm EC plates are useful for the enumeration of *E. coli* and coliform bacteria

in the food and dairy industries and are decontaminated though not sterilized (3M Canada, 2012b).

4.3 Alternative Methods

Three alternative methods were available for this study:

1. A bill could be aseptically cut into pieces and soaked in a letheen broth. The test tube containing the bill and broth would be vortexed for one minute. A 1-mL aliquot of broth would be plated on 3M Petrifilm. After being incubated at 35°C for 24 hours, the Petrifilm can be counted and a total sample count calculated. The drawback of this method is the destruction of Canadian currency.
2. The swab method could be performed using 3M's Quick Swab Kit. This method is quick and easy to perform but is only able to sample small surface spaces and lacks the agitation needed to accurately sample the entirety of the banknote (Cummings & Ken Keilbert, 2012).
3. RODAC plates are commonly used to enumerate CFUs on various environmental surfaces, including plastic and paper products (BD, 2012). This method was considered the primary contingency plan if the Petrifilm method of sampling failed. See Appendix B for a photographic representation of the sampling media considered for this experiment.

4.4 Justification of Methods

Conducting an aerobic plate count and a coliform plate count using 3M Petrifilm was determined to be the most efficient and economical way to conduct this experiment. All materials were available on the BCIT campus and experimentation could start immediately.

4.5 Reliability and Validity of Measures

To ensure that resulting colony counts were solely from currency contamination and not from external sources, the following precautions were taken:

- Positive controls: A thumbprint on Petrifilm plates was used to demonstrate that the media was working up to manufacturer specifications.
- Negative controls: An unopened Petrifilm plate was incubated at 35°C for 24 hours to ensure that the media did not contain inherent contamination.
- Control sample: New, uncirculated banknotes were received directly from the Bank of Canada via an interested employee at Bank of Montreal. The researcher was able to take three randomly selected samples from a sealed shipment of newly printed $20 plastic banknotes on November 8, 2012. These bills were aseptically placed in a sealed bag and immediately taken to the food-processing lab located at BCIT for testing.
- Only one researcher conducted the experiment (improved consistency).
- Instructions provided by 3M were followed closely to ensure accurate results.
- Aseptic technique was used throughout the experimental process.
- A pilot study was performed to eliminate any obscure errors.
- All media were ordered from the manufacturers to avoid preparation errors.
- The incubator temperature was verified with a thermometer during the entire process.
- Incubated media were retrieved and analyzed within manufacturers' specifications.

4.6 Inclusion and Exclusion Criteria

Included data consists of:

- $20 plastic currency denominations.
- $20 paper currency denominations printed during or after 2010.
- Imperfect bills (creases, tears, markings, etc.) that were accepted in this study.
- Bill randomly selected from local food establishments.

- Bills that were all sampled in the same location (center of bill on the front and back side). See Figure A-1 in Appendix A for a photographic representation.
- Petrifilm plate sample area ($15cm^2$) results that were multiplied by 14 to accurately represent the surface area of the entire bill ($210cm^2$).

Excluded data includes:

- Paper bills produced prior to 2010.
- Other forms of currency (coin, checks, credit cards, etc.).
- $100 bills (excluded because they are the least circulated and therefore presumed to be the least contaminated (Pradeep, Marulasiddaiah, & Chetana, 2012)).
- $10 and $5 bills (excluded for two reasons: their plastic counterpart has yet to enter circulation, and they are presumed to be the most contaminated and may create bias when analyzing the data (Pradeep, Marulasiddaiah, & Chetana, 2012)).

4.7 Pilot Study

On November 8, 2012, a pilot study was performed to verify that correct sampling methods and mathematical analyses were being utilized. First, a currency note was sampled, incubated, and enumerated using the Petrifilm method described in Appendix A. Those data were extrapolated upon to approximate the larger sample size expected after the experiment was complete. The extrapolated data were subjected to statistical analysis using NCSS 8 statistical software to determine whether the results were mathematically viable. After conducting the pilot study, it was determined that Petrifilm plates were an adequate sampling medium and that paired t-tests would provide accurate statistical results.

5.0 Statistical Analysis

5.1 Description of Data

The data collected in this study was quantitative, numerical, and directly measured by counting visible aerobic and *E. coli* coliform colonies on 3M Petrifilm plates. The recorded data can be found on the data collection sheets found in Appendixes C and D.

5.2 Statistical Package Used

The collected data were evaluated using NCSS 8. Descriptive statistics (mean, median, and standard deviation) and inferential statistics (paired t-tests) were examined using NCSS 8.

5.3 Descriptive Statistics

The mean, median, and standard deviation were calculated using NCSS 8 descriptive statistics function, and the summarized results can be found in Table 6.0-1. The descriptive statistics report produced by NCSS 8 for paper bills can be found in Appendix E, whereas the descriptive statistics report for plastic bills can be found in Appendix F.

5.4 Inferential Statistics

To gain a better understanding of the contamination rates of Canadian currency, the following null and alternative hypotheses were proposed:

- H_{o1}: Plastic Canadian currency harbors aerobic CFUs that are equal to or more than traditional paper currency.
- H_{a1}: Plastic Canadian currency harbors fewer aerobic CFUs than traditional paper currency.
- H_{o2}: Plastic Canadian currency harbors *E. coli*/Coliform CFUs that are equal to or more than traditional paper currency.

- H_{a2}: Plastic Canadian currency harbors fewer *E. coli*/Coliform CFUs than traditional paper currency.

A paired t-test was conducted with NCSS 8 to determine whether the rates of aerobic CFU contamination were lower on plastic (compared to paper) banknotes. See table 6.0-2 for the summarized results and Appendix G for the paired t-test report produced by NCSS 8. A paired t-test was not performed on the obtained *E. coli* data because of a lack of analyzable data (only four bills tested positive for *E. coli*).

6.0 Results

The statistical results are shown in Tables 6.0-1 and 6.0-2.

Table 6.0-1 Descriptive Statistical Results Using NCSS 8

	Plastic bills (CFUs)	**Paper bills (CFUs)**
Mean	73.26667	107.2667
Median	49	76
Standard deviation	74.05214	120.1843

Table 6.0-2 Inferential Statistical Results of the Paired t-Test Using NCSS 8

Test	**Parameter**	**p-Value**	**Power**	**Statistically significant**	**Accept or reject H_o**
Wilcoxon Signed–Ranked Test for Difference in Means	Plastic bills contain less aerobic CFUs than paper bills	0.090332	0.380125	No	Fail to Reject (H_o)

6.1 Interpretation of Results

6.1.1 Descriptive Statistics Results

The following can be interpreted from the highlighted descriptive data in Table 6.0-1:

1. The mean aerobic CFU count on paper bills is greater than the aerobic CFU count on the plastic bills sampled.
2. The median aerobic CFU count on paper bills is greater than the aerobic CFU count on the plastic bills sampled.
3. The standard deviation of aerobic CFUs is lower on plastic bills than the paper bills sampled, which indicates that the data points are spread over a smaller range of values (Heacock & Crozier, 2011).

6.1.2 Inferential Statistics Results

Table 6.0-2 highlights the important inferential statistics produced by NCSS 8. The Wilcoxon Signed–Ranked Test for Difference in Means was performed since normality was rejected (see Appendix G). The p-value is greater than 0.05 (0.090332); therefore, we fail to reject the null hypothesis that new plastic bills contain fewer CFUs than traditional paper bills, and thus concluding that this experiment could not prove that plastic currency contains less CFUs than paper currency. The statistical power was 0.380125, indicating a high probability that a type II or "beta" error has occurred. These statistical values signify that the data collected was not statistically significant.

6.2 Alpha and Beta Error Discussion

Type I or "alpha" errors occur when the test rejects the null hypothesis if it is true (Moore & McCabe, 2006). This analysis did not produce an alpha error because the p-value is above the critical value of 0.05 and therefore fails to reject the null hypothesis.

Type II or "beta" errors occur when the null hypothesis was not rejected despite being incorrect (Moore & McCabe, 2006). The results indicate a power equal to 0.380125, indicating a high probability that a beta error has occurred. Increasing the number of samples would help reduce the chance of a beta error and would likely produce statistically significant results.

7.0 Discussion

This study was conducted to determine whether plastic banknotes harbor fewer CFUs than traditional paper banknotes. The data collected during this study cannot confirm or deny this hypothesis due to an inadequate sample size. Although plastic currency notes did (on average) have significantly fewer aerobic CFUs than paper bills, the statistical analysis could not corroborate these results. However, the researcher believes that if an adequate sample size were to be tested, the results would statistically indicate that plastic bills harbor fewer aerobic CFUs.

Statistical information could not be produced for *E. coli* contamination on both paper and plastic bills because of the lack of positive results. Only four out of sixty bills (6.66%) tested positive for *E. coli,* and those that did had few CFUs present (1 or 2 CFUs per $15cm^2$). These results were discouraging from a study point of view, but encouraging from a public health perspective.

E. coli contamination of Canadian banknotes appears to be comparable to the rates found in the Unites States (6.6% compared to 7%, respectively). This comparison was made using *E. coli* data from both this experiment and a similar study on US currency, discussed in previously in Section 2.2 (Raloff, 2010). The researcher surmised that contamination rates of other pathogens might also be comparable for discussion purposes. With this rather large assumption, we can be somewhat reassured by the thought that Canadian (and US) currency has the lowest

contamination rates when compared to all other national currencies researched during the preparation of this report (see Section 2.0).

The Bank of Canada's system of continuously destroying old bills while continuously printing new bills is an effective system to reduce contamination. However, the new plastic bills are expected to stay in circulation longer because of the durability of the construction material. Although the plastic bills had a lower CFU count than paper bills, it should be noted that the $20 bills had only been in circulation for three months when the experiment was conducted. The lower rates we noticed could simply be due to lack of contact time with the general population. Since the plastic bills are expected to stay in circulation for a longer period of time, ultimately the contamination rates of plastic bills may become similar to paper bills over time.

As a side experiment, a single plastic bill with a high concentration of aerobic CFUs was identified; the researcher disinfected the bill with 70% isopropyl alcohol and tested the bill again. The initial test resulted in 20 aerobic CFUs (per $15cm^2$) and the subsequent test resulted in 1 aerobic CFU (per $15cm^2$). This unofficial test indicates the new plastic bills can easily and effectively be cleaned due to the nature of their fabrication materials.

8.0 Limitations

1. Sample size limitations resulted in a reduced statistical validity and potentially resulted in a type II (beta) error. These errors could be eliminated with a larger sample size.
2. Only $20 banknotes were tested because lower denomination plastic bills had yet to enter circulation. If we accept the conclusions from previous studies that lower bills are the most contaminated (Lamichhane, Adhikary, Guatam, & Maharjan, 2009), results from testing $5 or $10 bills may provide different results.

3. Plastic $20 banknotes had only been in circulation for three months when the testing was conducted; further circulation may increase the rate of contamination.
4. Time and budget restraints limited the amount of bills that could be tested.

9.0 Conclusions

The main conclusions resulting from this study include the following:

1. Contamination rates for plastic bills are statistically similar to those of paper bills.
2. On average, plastic bills had fewer aerobic CFUs than traditional paper bills.
3. That Canadian bills have a similar contamination rate as US bills. This study showed a 6.6% rate of *E. coli*-contaminated Canadian bills, compared to 7% in the United States (Raloff, 2010).
4. The Bank of Canada's system of continuously destroying old bills while continuously printing new bills is an effective system to reduce contamination.

10.0 Recommendations

Based on the results of this study, the following recommendations that pertain to the environmental health field are provided:

1. Money-handling procedures should remain the same for food establishments. Since there is no evidence that plastic bills have a reduced bacterial load, food establishments should continue to follow the money-handling procedures put in place for paper currency.
2. Mobile food carts and temporary markets should be extra vigilant with hand washing when money handling and food preparation are occurring at the same time.

11.0 Future Research Suggestions

The following topics could be considered for future research projects:

1. A similar study with increased sample size.
2. A similar study looking at different denominations of bills.
3. A similar study focused on coin contamination.
4. Determine how effectively plastic notes can be cleaned using a disinfectant.

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Appendix A: 3M Petrifilm Aerobic and *E. Coli* Plate Count Procedures.

Plating:

1. Place Petrifilm on a flat, level, and sanitized surface.
2. Label Petrifilm with sample number and pertinent information.
3. Lift the top film and pipette 1 mL of sterile water onto the center of the Petrifilm.
4. Shape sterile water into a 25cm^2 circle using a circular spreader.
5. Let the water settle on the Petrifilm plate for 2 minutes and allow gel to form.
6. As shown in Figure A-1, lift top film and take sample by firmly pressing the clear film onto the currency note.
7. Return top film so it covers the sample and place it in the incubator.

Figure A-1: A bank issued control bill being sampled using the Petrifilm method.

Incubation:

1. Incubate plates in a horizontal position with the clear side up in stacks of no more than 20 plates (3M Canada, 2012a).
2. Incubation times will be between 24 and 48 hours at 35°C.

Interpretation:

- Petrifilm AC plates can be counted using a standard colony counter or other illuminated magnifier.
- Count all red colonies regardless of size or intensity.
- The circular growth area is approximately 20 cm^2.
- Estimates can be made from averaging the colonies per 1 cm^2 and applying that figure to the overall size of the bill.
- If plates cannot be counted within 1 hour of removal from the incubator, they must be stored for later enumeration by freezing in a sealable container at temperatures of -15°C for no longer than one week (3M Canada, 2012a).

Appendix B: Sampling Techniques Tested.

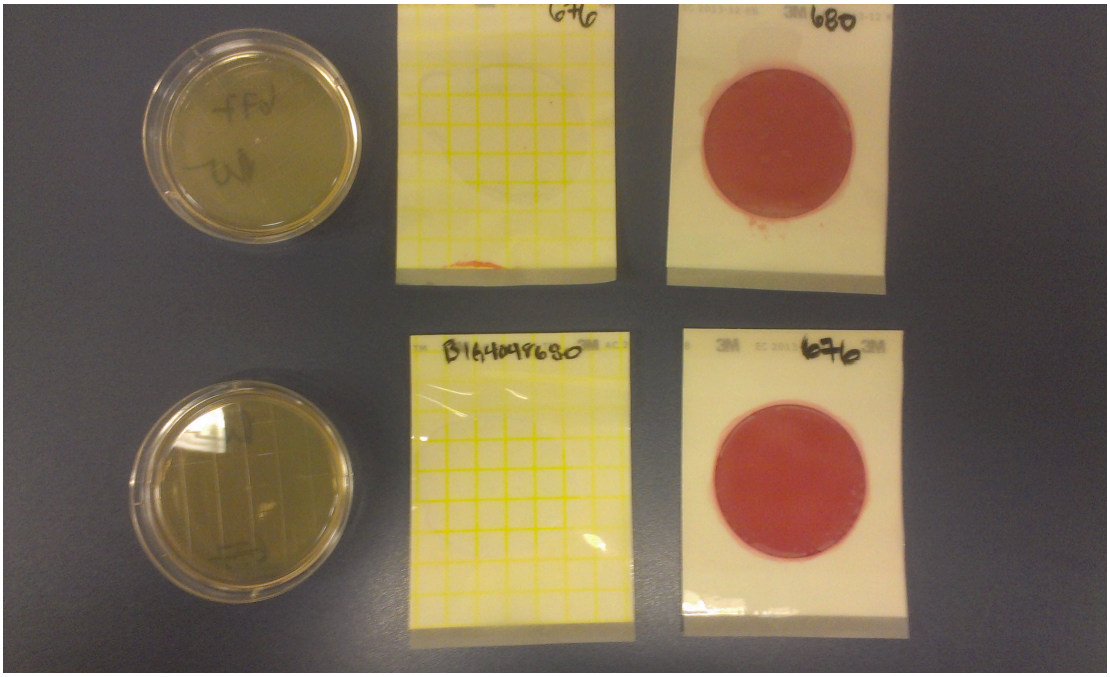

Figure B-1: (Left) RODAC plate, (center) Petrifilm AC plate; (right) Petrifilm *E. coli*/Coliform plate

Note the similar sample size of all three tests (the center samples are difficult to see, but they are of equivalent size as the red plates on the right).

Appendix C: Total Bacterial Count on Paper Bills.

Ryan Olivier's Total Bacterial Count of Paper Currency

Sample #	Sample date	Count date	Aerobic colonies	E. Coli colonies
1	1/31/13	2/1/13	56	0
2	1/31/13	2/1/13	42	0
3	1/31/13	2/1/13	14	0
4	1/31/13	2/1/13	616	0
5	1/31/13	2/1/13	168	0
6	1/31/13	2/1/13	42	0
7	1/31/13	2/1/13	0	0
8	1/31/13	2/1/13	28	28
9	1/31/13	2/1/13	168	0
10	1/31/13	2/1/13	168	0
11	1/31/13	2/1/13	28	0
12	1/31/13	2/1/13	42	28
13	1/31/13	2/1/13	140	0
14	1/31/13	2/1/13	14	0
15	1/31/13	2/1/13	28	0
16	2/7/13	2/8/13	224	0
17	2/7/13	2/8/13	140	0
18	2/7/13	2/8/13	0	0

Ryan Olivier's Total Bacterial Count of Paper Currency (continued)

Bill #	Sample date	Count date	Aerobic colonies	*E. coli* colonies
19	2/7/13	2/8/13	14	0
20	2/7/13	2/8/13	0	0
21	2/7/13	2/8/13	238	0
22	2/7/13	2/8/13	42	0
23	2/7/13	2/8/13	182	0
24	2/7/13	2/8/13	196	14
25	2/7/13	2/8/13	126	0
26	2/7/13	2/8/13	140	0
27	2/7/13	2/8/13	70	0
28	2/7/13	2/8/13	98	0
29	2/7/13	2/8/13	112	0
30	2/7/13	2/8/13	28	0

Appendix D: Total Bacterial Count on Plastic Bills.

Ryan Olivier's Total Bacterial Count of Plastic Currency

Sample #	Sample date	Count date	Aerobic colonies	*E. coli* colonies
1	2/14/13	2/15/13	56	0
2	2/14/13	2/15/13	0	0
3	2/14/13	2/15/13	0	0
4	2/14/13	2/15/13	168	0
5	2/14/13	2/15/13	0	0
6	2/14/13	2/15/13	42	0
7	2/14/13	2/15/13	182	0
8	2/14/13	2/15/13	98	0
9	2/14/13	2/15/13	280	0
10	2/14/13	2/15/13	154	0
11	2/14/13	2/15/13	126	28
12	2/14/13	2/15/13	14	0
13	2/14/13	2/15/13	0	0
14	2/14/13	2/15/13	14	0
15	2/14/13	2/15/13	168	0
16	2/21/13	2/22/13	28	0
17	2/21/13	2/22/13	0	0
18	2/21/13	2/22/13	196	0

Ryan Olivier's Total Bacterial Count of Plastic Currency (continued)

Sample #	Sample date	Count date	Aerobic colonies	E. Coli colonies
19	2/21/13	2/22/13	84	0
20	2/21/13	2/22/13	112	0
21	2/21/13	2/22/13	70	0
22	2/21/13	2/22/13	0	0
23	2/21/13	2/22/13	42	0
24	2/21/13	2/22/13	140	0
25	2/21/13	2/22/13	84	0
26	2/21/13	2/22/13	0	0
27	2/21/13	2/22/13	28	0
28	2/21/13	2/22/13	28	0
29	2/21/13	2/22/13	0	0
30	2/21/13	2/22/13	56	0

Appendix E: Descriptive Statistics Report for Paper Bills.

Summary Section of Aerobic Colonies Paper Bills

Count	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Range
30	107.2667	120.1843	21.94255	0	616	616

Counts Section of AC paper bills

Rows	Sum of Adjusted Frequencies	Missing Values	Distinct Values	Sum	Total Sum Squares	Sum Squares
30	30	0	17	3218	764068	418883.9

Means Section of AC paper bills

Parameter	Mean	Median	Geometric Mean	Harmonic Mean	Sum	Mode
Value	107.2667	76	78.1149	49.24802	3218	42
Std Error	21.94255				658.2767	
95% LCL	62.3891	42	52.97578	34.91588	1871.673	
95% UCL	152.1442	140	115.1835	83.53867	4564.327	
T-Value	4.888522					
Prob Level	3.457889E-05					
Count	30		27	27		4

The geometric mean confidence interval assumes that the ln(y) are normally distributed.
The harmonic mean confidence interval assumes that the 1/y are normally distributed.

Variation Section of AC paper bills

Parameter	Variance	Standard Deviation	Unbiased Std Dev	Std Error of Mean	Interquartile Range	Range
Value	14444.27	120.1843	121.2247	21.94255	140	616
Std Error	8625.752	50.74978		9.2656		
95% LCL	9161.481	95.71563		17.4752		
95% UCL	26103.45	161.5656		29.49771		

Appendix F: Descriptive Statistics Report for Plastic Bills.

Summary Section of Aerobic Colonies on Plastic Bills

Count	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Range
30	73.26667	74.05214	13.52001	0	280	280

Counts Section of AC on plastic bills

Rows	Sum of Frequencies	Missing Values	Distinct Values	Sum	Total Adjusted Sum Squares
30	30	0	16	2198	320068

Means Section of AC on plastic bills

Parameter	Mean	Median	Geometric Mean	Harmonic Mean	Sum	Mode
Value	73.26667	49	70.03317	48.53128	2198	0
Std Error	13.52001				405.6003	
95% LCL	45.61514	28	48.19202	34.48944	1368.454	
95% UCL	100.9182	98	101.7729	81.85885	3027.546	
T-Value	5.419128					
Prob Level	7.917163E-06					
Count	30		23	23		7

The geometric mean confidence interval assumes that the ln(y) are normally distributed.
The harmonic mean confidence interval assumes that the 1/y are normally distributed.

Variation Section of AC on plastic bills

Parameter	Variance	Standard Deviation	Unbiased Std Dev	Std Error of Mean	Interquartile Range	Range
Value	5483.72	74.05214	74.69315	13.52001	119	280
Std Error	1474.853	14.08303		2.571198		
95% LCL	3478.126	58.97564		10.76743		
95% UCL	9910.086	99.54942		18.17515		

Appendix G: Paired t-Test Report.

Variable X1 = AC on paper bills, X2 = AC on plastic bills

Tests of Assumptions about Differences Section

Assumption	Value	Probability	Decision(.050)
Skewness Normality	1.5911	0.111591	Cannot reject normality
Kurtosis Normality	2.2024	0.027637	Reject normality
Omnibus Normality	7.3821	0.024946	Reject normality
Correlation Coefficient	0.084138		

T-Test For Difference Between Means Section

Wilcoxon Signed-Rank Test for Difference in Medians

Alternative Hypothesis	T-Value	Prob Level	Reject H0 at .050	Power (Alpha=.05)	Power (Alpha=.01)
AC_old_bills-AC_new_bills<>0	1.3717	0.180664	No	0.263727	0.100158
AC_old_bills-AC_new_bills<0	1.3717	0.909668	No	0.001416	0.000138
AC_old_bills-AC_new_bills>0	1.3717	0.090332	No	0.380125	0.154397

W Sum Ranks	Mean of W	Std Dev of W	Number of Zeros	Number Sets of Ties	Multiplicity Factor
296.5	227.5	48.51289	4	7	132

	Exact Probability		Approximation Without Continuity Correction			Approximation With Continuity Correction		
Alternative Hypothesis	Prob Level	Reject H0 at .050	Z-Value	Prob Level	Reject H0 at .050	Z-Value	Prob Level	Reject H0 at .050
X1-X2<>0			1.4223	0.154938	No	1.4120	0.157951	No
X1-X2<0			1.4223	0.922531	No	1.4326	0.924015	No
X1-X2>0			1.4223	0.077469	No	1.4120	0.078976	No