

## BACTERIOLOGICAL ANALYSIS OF RANDOMLY SELECTED REFILLING STATIONS IN THE CITY OF MANILA

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### ABSTRACT

**Objectives:** To test the bacterial growth in randomly selected refilling stations in the City of Manila and to determine other characteristics of the refilling stations which could influence positive or negative results.

**Methods:** Pre-sterilized containers were used to collect samples from the refilling stations. 0.1mL of each sample was inoculated on MacConkey agar and incubated at 37°C for 48 hours. Microbial growth was noted at the end of the incubation period. A questionnaire was also used to note down the characteristics of the refilling stations. The station personnel and the medical technicians were blinded regarding the study.

**Results:** The study showed no microbial growth after 48 hours for all the refilling stations tested.

**Conclusion:** Freshly refilled water from randomly selected water refilling stations in the City of Manila is free from microorganisms. However, characteristics of the stations such as the disinfection method, frequency of monitoring, personnel hygiene, attire and practices all affect the safety and potability of the product water.

### INTRODUCTION

Water is necessary for life. The importance of water can never be over-emphasized. Safety is the primary consideration in choosing a source for potable water. However, modern technology has introduced various means of processing water in order to disinfect it. The following are the more common methods of processing water, namely: reverse osmosis, ultraviolet sterilization, deionization and ion-exchange filtration. Processed water is available as bottled or refilled water. Bottled water means water obtained from an approved source that has undergone minimum treatment consisting of filtration and ozonation or an equivalent disinfection process. Refilled water is defined as source water that has undergone additional processing and the product of multi-stage purification technology. Purchase of readily available processed water is less time consuming than boiling water. As a result, more and more families have come to rely on the convenience of these products. Thus, the safety of both bottled and refilled water is of utmost importance.

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Water used for drinking must be free from pathogenic organisms responsible for waterborne diseases. *Salmonella*, *Shigella*, *Enterotoxigenic Escherichia coli*, *Vibrio cholera*, *Yersinia enterocolitica* and *Campylobacter fetus* are the bacterial pathogens known to be transmitted in drinking water. The Bureau of Food and Drug (BFAD) is the agency responsible for monitoring the safety of the bottled water. The local government oversees the quality of the water from the refilling stations.

Despite being monitored by these agencies, there were reported cases of diarrhea in children who use bottled water. A local study done by Vitug (1999) showed that water purified via distillation and reverse osmosis was free from coliform but was positive for *Acinetobacter*, *Enterobacter* and *Pseudomonas*.<sup>1</sup> The problem of contamination does not only exist in the Philippines. A study done by Anderson et al in 1998 (Fu J of Public Health) showed that three different types of bottled water had counts of psychrophilic bacteria.<sup>2</sup> Another study in Germany done in 1997 (Schwartz, et al), stated that opportunistic species were found in bottled water.<sup>3</sup> *Pseudomonas*, *Flavobacterium*, *Pasteurella*, *Xanthomonas* and *Staphylococcus* were isolated in 51% of local bottled encarbonated mineral water and 49% of imported ones. In order to determine whether the water supplied is safe or acceptable for human consumption, samples should be collected from the water supply system for the bacteriological examination of the water (total coliform, thermotolerant coliform and *E. coli*).

The city or municipal health officer is deputized by the Secretary of Health to issue the Certificate of Potability of Drinking Water. Monitoring must be done on refilling stations at least monthly. However, based on records examined in the local government of Manila, no regular reports were available that showed that water was being examined regularly. It is therefore possible that the consumers are paying for water that is actually substandard and contaminated.

### OBJECTIVES

1. To test for bacterial growth using the spread-plate method on MacConkey agar on randomly selected refilling stations in the city of Manila.

- To determine if there were any other characteristics that the stations have such as disinfection method, personnel hygiene, practices and attire that will influence a positive or negative result.

### METHODOLOGY

A list of registered refilling stations in the City of Manila was obtained from the City Hall of Manila. To achieve statistical significance, fifteen percent (15%) from the list was randomly selected to be included in the study. A questionnaire was used to interview the personnel manning the refilling stations (Appendix C). A single water sample was obtained from each selected refilling station to represent each station. Autoclaved

glass containers with rubber stoppers were used to collect the samples. Containers were placed directly under the water jet and filled. Samples of 0.1 mL each were inoculated on MacConkey agar using the spread-plate method. Samples were incubated at 37 °C for the period of 48 hours. The samples were then inspected for microbial growth after the incubation period.

To avoid bias, it was not made known to the refilling stations that the water purchased was being analyzed. The medical technicians who did the cultures were likewise blinded on the identity of the refilling stations.

### RESULTS

All the samples taken from the refilling stations showed no bacterial growth after 48 hours of incubation.

**Table 1. Method of processing water and results of Microbial Analysis**

	Sample1	Sample2	Sample 3	Sample4	Sample5	Sample6	Sample7	Sample8
Purification Technique	UV Light	Multi-Stage Process <sup>*</sup>	Reverse Osmosis	Reverse Osmosis	UV Light	Reverse Osmosis	Multi Stage Process <sup>*</sup>	Multi Stage Process <sup>#</sup>
Growth After 48 Hours	+	-	+	+	+	+	-	-

\* multi-stage microfiltration, ultraviolet disinfection, ion-exchange, reverse osmosis, post-carbon filtration and ozone disinfection

† sediment filtration, ion exchange, activated carbon filtration, reverse osmosis, carbon block polishing, UV sterilization, ozonization and mixed bed ionization

# carbon filtration, reverse osmosis, UV ozonization, carbon polishing

Monthly monitoring is not being done on half of the refilling stations tested. Only two refilling stations required the personnel's medical certificate and none of the eight stations complied with the prescribed attires for the stations' staff.

**Table 2. Profile of the refilling stations where the samples were obtained.**

	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8
Monthly Monitoring	+	-	+	+	+	-	-	-
Years of operation	2	2	2	1	1	1	1	1
Interval from last Analysis	1 month	1 year	1 month	1 month	1 month	1 year	1 year	1 year
Personnel's Medical Certificate	+	-	-	-	-	+	-	-
Prescribed Attire	-	-	-	-	-	-	-	-

## DISCUSSION

Water is essential for survival. It can sustain life but it can harm life as well. Disease causing organisms can contaminate this seemingly harmless entity. Time and again, emphasis has been given on the importance of clean and safe water. In this day and age when everything is commercialized, water is not spared from becoming a lucrative business venture. Refilled water is now a famous source of drinking water for all ages. Many mothers depend on readily available refilled water in milk preparation for newborns and infants. In 1999 a study was done by Tolentino which showed that consumption of bottled water in children aged 0-6 years is associated with an increase in the rate of diarrhea.<sup>8</sup> The result of the study further strengthens the observation that processed water is not totally free from potentially harmful disease-causing microorganisms. It was recommended in the said study that regular potability testing be done.

The city or municipal health officer issues the Certificate of Potability of Drinking Water based on the following:

- Result of the water sampling and testing conducted by the DOH-accredited laboratory
- Report and recommendation of the Local Drinking Water Quality Monitoring Committee

According to the 1993 National Standards for Drinking Water, bacteriologic quality must be monitored at least monthly and that it must conform to its standards.<sup>9</sup> It is stated that all drinking supplies including bottled and refilled water must not contain *E. coli* and thermotolerant bacteria. Likewise, treated water entering within the distribution system must be free from bacteria. As the number of the population served increases, the frequency of monitoring also increases. The approach is to use normal enteric organisms such as *E. coli* as the essential indicator of fecal pollution. These organisms are easy to detect. The presence of *E. coli* in the sample indicates that water may be contaminated with organisms that can cause diseases. Several methods have been developed and are being used by refilling stations to ensure that water is free from contamination.

Reverse osmosis, originally invented to make sea water potable, is a process for the removal of dissolved ions from water in which pressure is forced through a semi-permeable membrane which will transmit the water but reject most of the suspended and dissolved minerals. However, it also removes the salts and

minerals the body needs. It is a wasteful process since it discards around four-fifths of the water and is a very slow process. Its benefits depend on the integrity of the filtering membrane's capacity to filter contaminants and microorganisms. Through time and frequent use, the membrane's capacity to filter contaminants and microorganisms may deteriorate. Microorganisms may then be able to pass through the membrane and proliferate such that there is an increase in gastrointestinal diseases. In this study, samples processed using reverse osmosis showed no growth after 48 hours.

Ultraviolet filtration is the process of killing bacteria and spores in water using ultraviolet ray. Susceptible microorganisms are deactivated upon exposure to the UV ray. There is adequate disinfection if water is maximally exposed to the UV light. UV transmittance of inlet water determines how well UV light penetrates and disinfects water. Transmittance decreases with increasing water turbidity and dissolved salts. The UV lamp should be replaced every year for units used 20 hours/day and every 2 years for units used 10 hours/day. Routine cleaning and inspection should be done every 6 months. UV light is dangerous when used at close range. Samples tested in this study yielded negative results after 48 hours.

Three of the refilling stations analyzed utilize a multi-stage process. It combines reverse osmosis and UV light with other methods such as deionization, carbon filtration, microfiltration, ion-exchange and ozonization. Deionization is the removal of ionized minerals and salts from a solution in a two-phase ion-exchange procedure. Ion-exchange is a reversible process in which ions are released from an insoluble permanent material in exchange for the other ions in the surrounding solution. These two methods are primarily concerned with purification and not disinfection. Filtration is the process of separating organic and inorganic solids from a liquid by means of a porous material such as a permeable fabric or membrane or layer of inert material. Most use activated charcoal filters. These filters are capable of removing micro-organisms without screening minerals. Some filters are treated with bacteriostatic. The filters have to be changed regularly to avoid proliferation of bacteria. Samples in the study which underwent the multi-stage process showed no bacterial growth after 48 hours.

In this study, the containers were pre-sterilized to prevent contamination of the refilled water. Freshly refilled water samples were immediately sent to the

laboratory after collection. All of the samples were placed in ice during transport to the laboratory. After 48 hours of incubation, all of the samples tested showed no growth of microorganisms.

The absence of microbial growth may be due to the fact that the samples had just gone through the disinfecting system just prior to being cultured. According to the Code on Sanitation of the Philippines (1999), holding time for stored refilled water must not go beyond 24 hours to prevent bacterial proliferation. Contrary to the findings of Vitug in 1999, water treated by reverse osmosis showed no growth of microorganisms in this study. Despite the absence of regular monthly bacteriologic monitoring in half of the stations tested, cultures remained negative. The stations tested have been in operation for two years or less. It is possible that the filters or membranes used in the refilling stations are still capable of blocking contaminants and impurities during the time of analysis. Since filters and membranes can deteriorate with prolonged use, regular cleaning and changing of filters is needed to maintain the cleanliness and safety of the water.

The water source and the treatment method are not the only sources of possible contamination of product water. Containers and caps provided by the establishment or customers should all be washed prior to sanitizing. Sanitizing solution and/or process must be

applied to all water containers and caps prior to refilling. Filling containers shall be in a manner that will not come in contact with the hands or other surfaces that may contaminate the water. Improperly sanitized containers and personnel hygiene can therefore be sources of contamination. Requirements for the personnel include a health certificate which will have to be renewed at least yearly. Street clothes and hair restraint is needed. Persons with communicable diseases are not allowed to handle water processing activities. It is unfortunate to note that many of the above guidelines are being violated as observed by the consumers. Therefore, the threat of contamination is a possibility from the moment water enters the treatment system until the container is handed to the consumer. Regular monitoring is a must for all refilling stations for the protection of the consumers.

## CONCLUSION

Freshly refilled water from randomly selected refilling stations in the city of Manila is free from microorganisms. However, the purification technique and the frequency of bacteriologic monitoring are not the only determinants for the possible contamination. Properly sanitized containers and caps, personnel hygiene and practices all play a role in the maintenance of the safety and potability of the water from the refilling stations.

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