

## Removal of bacteria, protozoa and viruses through a multiple-barrier household water disinfection system

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### ABSTRACT

Municipal water disinfection systems in some areas are not always able to meet water consumer needs, such as ensuring distributed water quality, because household water management can be a contributing factor in water re-contamination. This fact is related to the storage options that are common in places where water is scarce or is distributed over limited time periods. The aim of this study is to assess the removal capacity of a multiple-barrier water disinfection device for protozoa, bacteria, and viruses. Water samples were taken from households in Mexico City and spiked with a known amount of protozoa (*Giardia* cyst, *Cryptosporidium* oocyst), bacteria (*Escherichia coli*), and viruses (rotavirus, adenovirus, F-specific ribonucleic acid (FRNA) coliphage). Each inoculated sample was processed through a multiple-barrier device. The efficiency of the multiple-barrier device to remove *E. coli* was close to 100%, and more than 87% of *Cryptosporidium* oocysts and more than 98% of *Giardia* cysts were removed. Close to 100% of coliphages were removed, 99.6% of the adenovirus was removed, and the rotavirus was almost totally removed. An effect of site by zone was detected; this observation is important because the water characteristics could indicate the efficiency of the multiple-barrier disinfection device.

**Key words** | disinfection, drinking water, household water disinfection, microbial removal, multiple barrier system

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### INTRODUCTION

Water for human use and consumption must comply with regulatory quality parameters according to public health standards.

The authorities that manage the water supply systems for human population centers have a responsibility to ensure the provision of sufficient amounts of quality water that is suitable for human use; however, the efficiency of water provision is not always adequate. Furthermore, water management in households is a key factor related to microbiological water quality. The latter is related to the various storage options that are common in places where water is scarce or distributed for limited

periods of time; in these cases, water can be supplied with an acceptable quality but due to indoor storage it can be contaminated prior to its consumption (Clasen & Bastable 2003; Wright *et al.* 2004; Nath *et al.* 2006; Eshcol *et al.* 2009).

Microbiological water assessment has been based on coliform bacteria, which have been considered as an indicator of microorganisms for more than 100 years (Ashbolt *et al.* 2001); however, it has been shown that the presence or absence of coliform bacteria is not related to viral agents and protozoa, both of which are relevant to public health (Lambertini *et al.* 2011; WHO 2011a).

Disinfection at the point of use is one option for improving the drinking water quality supplied through the formal distribution system. It has been shown that the disinfection of domestic water or the disinfection of water at the point of use improves drinking water quality and reduces the risk of disease associated with contaminated water (Clasen *et al.* 2006; WHO 2011b). The most relevant results have been obtained in systems with frequent water contamination due to pipe breaks or discontinuous water flow, or in situations in which the contamination of the water source is known (WHO 2011a).

The World Health Organization (WHO) highlights technologies such as the following: (i) chemical disinfection with chlorine, ozone, or another oxidant; (ii) filtration through membranes, ceramics, or composed filters; (iii) filtration through granular media; (iv) solar disinfection; (v) ultraviolet light; (vi) heat disinfection; (vii) coagulation-precipitation or sedimentation; and (viii) multiple barrier treatments. There is a wide range of efficiency levels among the different methods; however, it is noteworthy that the production of safe water in the household depends on the presence of microorganisms in the water.

Multi-barrier treatment refers to any combination of two or more technologies employed, simultaneously or sequentially (WHO 2011b). Some of these treatments comprise commercial devices that combine particle removal by physical methods and microbial inactivation using chemicals (i.e. chlorine). It is important to consider that water features are variable according to the various water sources, such as groundwater or surface water, and including rainwater. There are microbiological and chemical processes that take place at different locations, therefore, it is not advisable to generalize the capabilities of commercial purifying devices that have been evaluated under specific conditions, as these capabilities may be related to the water in which they have been tested.

In Mexico, there are different commercial device options that use multi-barrier treatments, for example, there are commercial devices that employ filtration and ultraviolet (UV) light or filtration and inverse osmosis. Limited information is available to the population, however, about the disinfection efficiency of these devices. The specific multi-barrier purifier water device utilized in this study is an integrated unit with a sequence of filtrations through a microfiber that retains large particles, a pre-

formed carbon filter, a chlorination unit, and finally, an activated carbon polisher to reduce excess chlorine.

The aim of this work was to evaluate the reduction of rotavirus, adenovirus, coliphages, *Giardia* cysts, and *Cryptosporidium* oocysts that had been added to drinking water samples by employing a popular commercial purifier device, Pureit® Classic, which uses a multi-barrier combination of disinfection technologies. The device was also tested using groundwater from different Mexico City areas that correspond to different aquifer subsystems.

## METHODS

### Study area

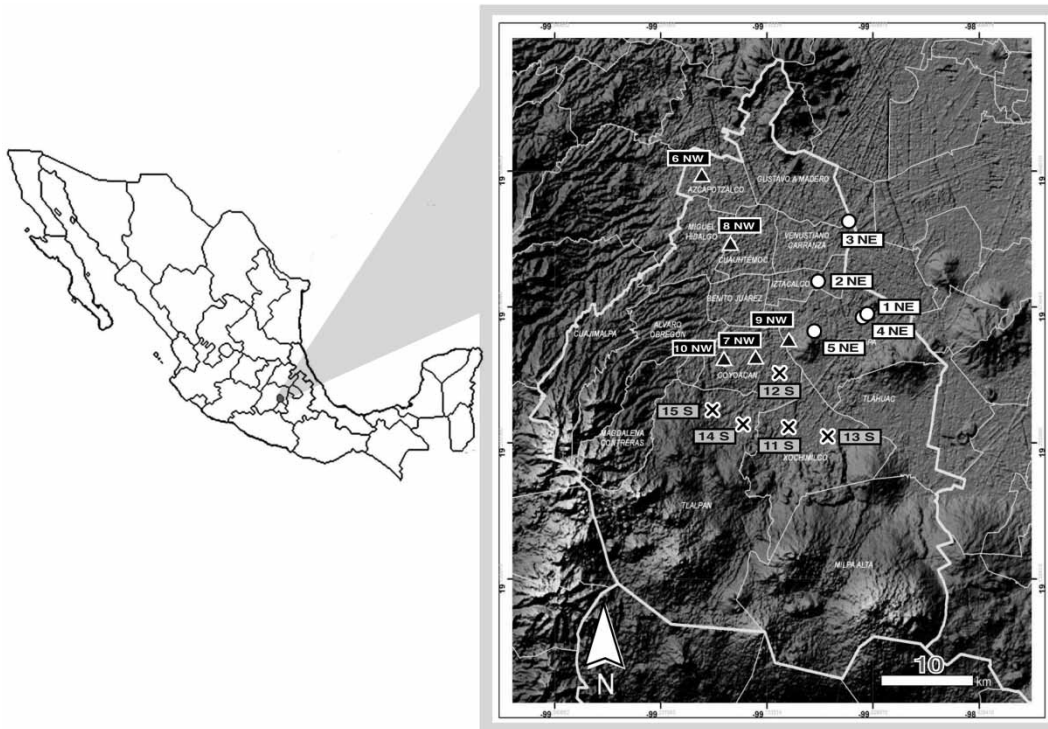
Seventy percent of the Mexico City Metropolitan Area (MCMA) water supply derives from groundwater, while 30% is supplied from surface sources from within its basin or other basins, as well as from a river and a few springs.

Groundwater for the MCMA is extracted from three aquifer subsystems that are denominated *Ciudad de México*, *Texcoco*, and *Chalco-Xochimilco* (Mazari & Mackay 1993). The aquifer subsystems present different hydrogeologic features that are constituted of diverse materials and that consequently present different permeability rates, causing varying water infiltration into each subsystem (DGCOH 1992). This groundwater was used as the experimental matrix for the spiked microorganisms, serving as a good approximation of real Mexico City groundwater source conditions (Figure 1).

To obtain water that represents at least the three main conditions of the water supply sources to Mexico City inhabitants, a directory of household addresses was integrated from people who had indicated their willingness to participate in this study. From the list of addresses, the final sites were randomly selected, obtaining five sites for each of the three Mexico City areas; all sites were sampled in triplicate.

### Sample preparation

Drinking water samples were collected using 10 L polypropylene bottles that had been previously washed and sterilized (APHA 2005). Physicochemical parameter measurements *in situ* included pH, temperature,



**Figure 1** | Sampling sites in Mexico City: Northwest, Northeast and South.

conductivity, turbidity, total dissolved solids and dissolved oxygen using a multiparameter sonde YSI 6600-M (Loveland, CO). Following the HACH manual (HACH 2002), residual chlorine was also measured, and if it was detected, sodium thiosulfate (J.T. Baker, Edo México) was added to neutralize the sample. To eliminate any microorganism that could be present in the water, samples were sterilized (at 120 °C for 20 min), leaving water samples to cool overnight.

Simultaneously, 1 L samples were collected in sterilized polypropylene bottles. These samples were processed to determine the presence of fecal coliform bacteria in household drinking water by means of the standard membrane filtration method (APHA 2005).

### Positive controls of experimental microorganisms

#### Coliform bacteria

Two strains of *Escherichia coli* (ATCC 23631 and 700891), acquired from ATCC (Manassas, VA), were cultivated and employed to spike the drinking water samples and as hosts

for recovering MS2 coliphages after the multi-barrier treatment.

The *E. coli* (ATCC 23631) was propagated in 50 mL of tryptone glucose yeast extract broth (TGYB) medium (Becton Dickinson, Cuautitlán, México) and incubated for 12 h at 37 °C (ISO 1995) in an aerobic incubator (Binder, Tuttlingen, Germany). The *E. coli* (ATCC 700891) was propagated in 50 mL of Trypticase soy broth medium (Becton Dickinson, Cuautitlán, México) and incubated for 3 h at 37 °C in a water bath (Daiki Science Co., Korea) under slow agitation (EPA 2001).

#### Enteric virus culture

Rotavirus SA-11 VR-1565 (ATCC, Manassas, VA) was propagated by infecting MA104 cells (kindly donated by Dr C. Árias, IBT-UNAM). Briefly, rotavirus was activated with 3 µg/mL of trypsin for 45 min at 37 °C in 5% CO<sub>2</sub> incubator NU-4750, (NUAIRE, US). Confluent MA104 cells in flasks were inoculated with rotavirus and incubated for 1 h at 37 °C and 5% CO<sub>2</sub> to allow for rotavirus adsorption. Non-supplemented DMEM was added to the infected

flasks (Huang *et al.* 1992), and these flasks were incubated for 18–20 h at 37 °C and 5% CO<sub>2</sub>.

Adenovirus 41 VR-930 (ATCC, Manassas, VA) and A549 CCL-185 cells (ATCC, Manassas, VA) were obtained from ATCC and used for adenovirus propagation. Confluent A549 cells (CCL-185 ATCC) were inoculated with adenovirus, allowing for 1 h of adsorption at 37 °C and 5% CO<sub>2</sub>. The non-supplemented Dulbecco's modified Eagle's medium (DMEM) was added to the flask with cells and viruses, which was incubated for 10 days at 37 °C and 5% CO<sub>2</sub>.

The lysates for both viruses were recuperated by freezing and thawing three times. The rotavirus titer was obtained by the TCID<sub>50</sub> method, and the adenovirus was quantified by quantitative real-time polymerase chain reaction (qPCR) (Xagorarakis *et al.* 2007). Both virus lysates comprised the stocks for spiking the sterilized drinking water samples.

MS2 coliphages (ATCC 15597-B1, Manassas, VA) were propagated in *E. coli* as host bacteria. The protocols followed for MS2 propagation were EPA 1602 (EPA 2001) and ISO 10705-1 (ISO 1995). This procedure allowed for the detection of 10 plaque-forming units (PFU)/mL; this limit of detection was determined by the MS2 stock serial dilutions assay.

## Protozoa

*Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts were obtained from residual water that tested positive for both pathogens. Their identification and quantification in the residual water was carried out directly using fluorescence microscopy and monoclonal antibodies for *Giardia lamblia* and *Cryptosporidium parvum*, as described in the recovery section.

## Spiked drinking water samples

The sterilized 10 L drinking water samples were inoculated, and the final concentrations of each microorganism were *E. coli* ( $9.49 \times 10^{13}$  colony-forming units (CFU)/10 L), rotavirus SA-11 ( $7 \times 10^5$  50% tissue culture infective dose (TCID<sub>50</sub>)/10 L), adenovirus 41 ( $7.8 \times 10^7$  PFU/10 L), coliphage MS2 ( $1.5 \times 10^{14}$  PFU/10 L), *Giardia lamblia* cysts (164.1 cysts/10 L) and *Cryptosporidium parvum* oocysts (22.5 oocysts/10 L).

To avoid interaction between *E. coli* and MS2 coliphages, the temperature of the spiked samples was maintained at 20 °C, considering that bacteria pili are present at 37 °C.

Because *G. lamblia* cysts and *C. parvum* oocysts were obtained from residual water, the entire inoculation and disinfection process by the multi-barrier device, as well as concentration sampling, recovery, and quantification, was performed separately, with simultaneous sample collection. These samples were prepared in the same way as those that were inoculated with rotavirus, adenovirus, coliphages, and *E. coli*.

## Multi-barrier water purifier device

The Pureit® Classic (Unilever, India) multi-barrier water purifier device utilized in this study is an integrated unit that allows for particle reduction through a microfiber filter and a carbon filter that retains protozoan (oo)cysts and chemical contaminants (i.e. pesticides, organic compounds). Afterward, the water flows through a chlorination chamber with a chlorine feeder cartridge with a contact time of approximately 20–30 min, and the water passes through a silver-impregnated granular activated carbon filter. This latter treatment removes the excess chlorine and chlorination byproducts, thus improving the taste of the water (Clasen *et al.* 2006; Verma & Arankalle 2009; Pureit® Manual 2011).

One part of the Pureit® device comprises a closed deposit to store finished drinking water, which is dispensed through a spigot from which the water sample was collected for the analyses (approximately 10 L).

## Concentration of drinking water samples

Once the samples were inoculated and homogenized, each was treated with the multi-barrier device following the manufacturer's instructions for use. During the test, it was important to work with the whole multi-barrier device, not with separate treatment stages, because we needed to relate the results with the whole system, in accordance with WHO recommendations (WHO 2011b). After the multi-barrier treatment, the drinking water samples were concentrated by ultrafiltration using the method developed by Polaczyk *et al.* (2008).



Sodium polyphosphate (NAPP) 0.1% (Sigma-Aldrich, MO) was added to each sample as a disaggregant after the sample had passed through a hollow fiber F80A polysulfone filter (Fresenius, Medical Care, MA) and a continuous flow peristaltic Masterflex (Cole-Parmer Instruments Co., USA) pump. Samples were concentrated at a rate of 1,700 mL/min. The ultrafiltration system had previously been disinfected using a circulating chlorine solution and was also treated with a 0.1% solution of NAPP (Figure 2). The sodium polyphosphate treatment of the filter produced an electronegative charge to avoid adherence to the hollow fiber filter surface. The final volume of each concentrated sample was approximately 50 mL. All concentrates were divided in aliquots and stored under refrigeration according to the requirements for the recovery of each microorganism.

### Recovery of rotavirus and adenovirus

An aliquot of the concentrated drinking water sample was used to quantify the rotavirus and adenovirus by MA104 and A549 cell infection, respectively, using the TCID<sub>50</sub> method. Because rotavirus and adenovirus were inoculated together in the drinking water samples, to control the possible co-infection of cells, rotavirus and adenovirus were neutralized alternately prior to infecting the confluent cells. The monoclonal anti-rotavirus antibody SC-58188 (Santa Cruz, TX) was employed to assess adenovirus infectivity, and the monoclonal anti-adenovirus antibody SC-58651 (Santa Cruz, TX) was utilized to evaluate rotavirus infectivity.

Trypsin was added to aliquots separately to measure rotavirus infectivity (Huang *et al.* 1992), as described

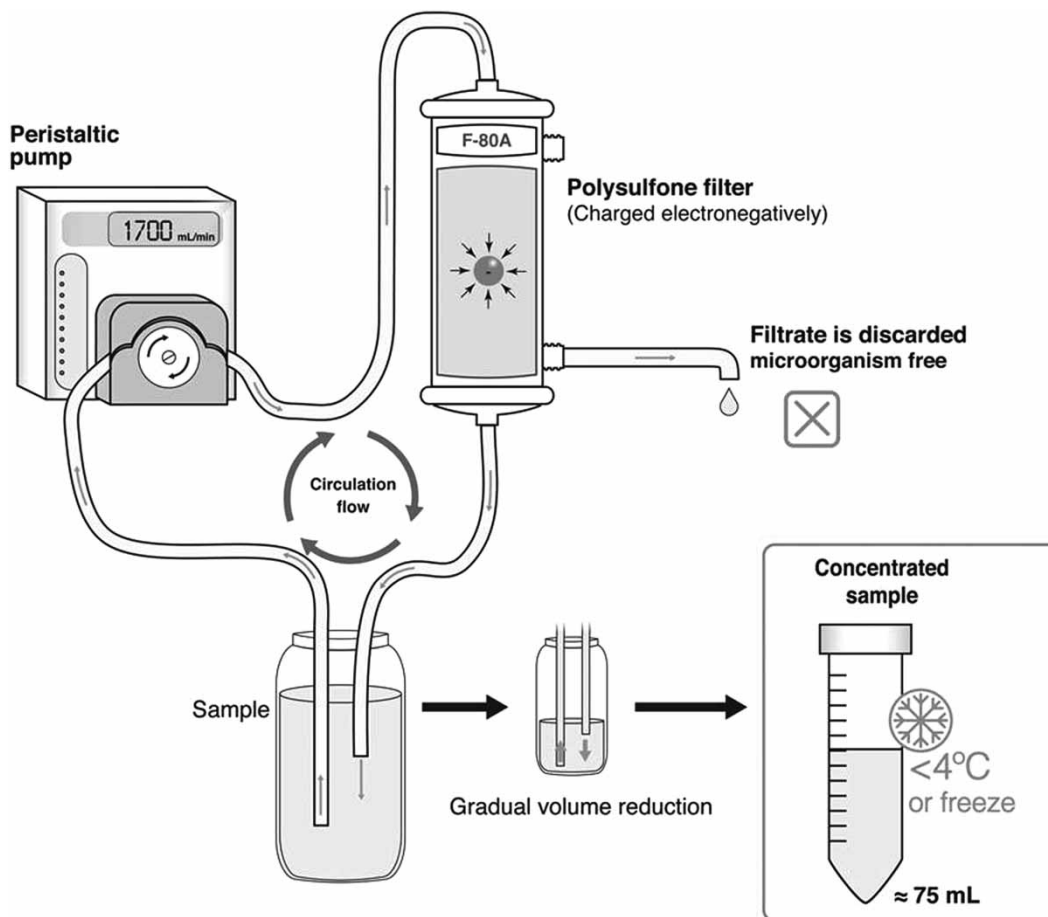


Figure 2 | Diagram of the ultrafiltration system used to concentrate drinking water samples.

previously, to activate the rotavirus present in the samples in which the adenovirus had been neutralized by a monoclonal antibody. Then, 96-well plates with confluent MA104 cells were infected with 100  $\mu$ L of 10-fold serial dilutions of the activated concentrate. The plates were incubated for 48 h at 37 °C and 5% CO<sub>2</sub> (Huang et al. 1992).

For adenovirus, 5-fold serial dilutions from samples in which the rotavirus had been neutralized by a monoclonal antibody were prepared and 96-well plates with confluent A549 cells were infected with 100  $\mu$ L of each dilution. The plates were incubated for 10 days at 37 °C and 5% CO<sub>2</sub> (Jiang et al. 2009). After incubation of the rotavirus and adenovirus, 96-well plates were fixed with 50  $\mu$ L/well of methanol (4 °C) for 5 min. The methanol was discarded, and the cells were dyed with crystal violet in ethanol (0.1%) for 20 min. Finally, the plates were washed with tap water to eliminate the excess dye; the results were reported as TCID<sub>50</sub>.

This protocol allowed for the quantification of rotavirus and adenovirus with a limit of detection of 7 TCID<sub>50</sub>/mL.

#### ***Giardia lamblia* and *Cryptosporidium parvum* recovery**

Fifty-milliliter aliquots from sample concentrates were processed by a second concentration through centrifugation at 3,000  $\times$  g for 15 min (Centra CL3R, Thermo-IEC, MA); each pellet was then re-suspended in 3 mL of phosphate-buffered saline (PBS) solution (Sigma-Aldrich, MO). When required, the solution was stored with glycerol (10%) at -20 °C (Rangel-Martínez 2010; Tapia-Palacios 2012).

For (oo)cyst detection, 1 mL of the solution concentrate was incubated with a mouse monoclonal IgG antibody, either the anti-*Giardia* antibody SC-57743 (Santa Cruz, TX) or the anti-*Cryptosporidium* antibody SC-58112 (Santa Cruz, TX), at 4 °C overnight. Afterward, a fluorescein isothiocyanate (FITC)-conjugated rabbit antibody was incubated at 37 °C for 1.5 h to react with the previously added anti-mouse IgG. Finally, a propidium iodide (Santa

Cruz, TX) (1:100 in PBS) and 4',6-diamidino-2-phenylindole (DAPI) (BioGenex, CA) solutions (1:100 in PBS) were added, and the samples were incubated at 37 °C for 30 min prior to visualization under fluorescence microscopy.

The parameters considered for detection and quantification included the size, shape, and wall integrity of the fluorescent (oo)cysts (Smith & Rose 1990).

The method described allowed for the detection of (oo)cysts when there were at least six (oo)cysts/10 L present, determined by serial dilutions of the stocks.

## **RESULTS AND DISCUSSION**

To record the main features of the drinking water supplied in three Mexico City areas *in situ*, the following parameters were measured: temperature, pH, conductivity, total dissolved solids (TDS), and dissolved oxygen. Table 1 depicts the results for each Mexico City area related to the three aquifer subsystems.

The NE drinking water area presented the highest conductivity and TDS of all of the areas around Mexico City; this result must be due to the concentrations of salts and particles typical of the water in the NE aquifer subsystem (Texcoco). Although NE conductivity and TDS are adequate for drinking water, there are parameters that must be taken into account because salts and particles can affect the performance of the multi-barrier device with regard to process time and the lifetime of consumables (microfiber and carbon filter). Measurements of the remaining parameters did not show significant differences. However, the drinking water samples collected, as previously described, represent a better approach to the real conditions of the drinking water distributed in Mexico City.

Analyses for fecal coliform bacteria in drinking water samples prior to sterilization were all negative. Therefore, sterilization ensures the elimination of all microorganisms

**Table 1** | Average values of measured parameters in the drinking water of houses of three Mexico City areas

Mexico City area	Temperature (°C)	pH	Conductivity (mS/cm)	TDS (mg/L)	Oxygen (mg/L)
Northeast (NE)	23.29	7.53	0.633	0.395	5.73
Northwest (NW)	20.53	7.27	0.238	0.169	4.39
South (S)	22.67	7.37	0.244	0.166	6.90

that could be present in drinking water samples and avoids microbial overestimation.

Free chlorine is a basic water quality indicator, and, in many countries, is the only monitoring parameter. In Mexico, drinking water regulations establish a free chlorine concentration of 0.2–1.5 mg/L (DOF 2000). The multi-barrier treatment of the Pureit® Classic device includes a final step to reduce chlorine. Free chlorine concentration measurements in triplicate for drinking water samples *in situ* (households) and at the end of treatment are presented in Table 2.

A minimal free chlorine concentration (or the absence of free chlorine) makes it necessary to recommend care to prevent recontamination. It is interesting to note that the free chlorine concentrations in household drinking water in this study, with the exception of two samples from the NE and two from the S, complied with the Mexican regulations (DOF 2000). This observation is interesting because normally it is assumed that drinking water in Mexico City very frequently does not meet the regulatory requirements.

**Table 2** | Average free chlorine concentrations in the household drinking water of three areas of Mexico City, and in drinking water after the multi-barrier household treatment

Sampler points in Mexico City	Households (mg/L)	After multi-barrier mg/L <sup>c</sup>	After multi-barrier mg/L <sup>d</sup>
1 NE	1.2	1.23	1.75
2 NE	>2.0 <sup>b</sup>	>2.0 <sup>b</sup>	>2.0 <sup>b</sup>
3 NE	Not measured	Not measured	Not measured
4 NE	1.98	0.23	<0.2 <sup>a</sup>
5 NE	1.25	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>
6 NW	0.78	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>
7 NW	0.81	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>
8 NW	0.28	0.22	0.21
9 NW	1.16	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>
10 NW	Not measured	Not measured	Not measured
11 S	0.36	0.23	<0.2 <sup>a</sup>
12 S	0.21	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>
13 S	0.38	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>
14 S	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>
15 S	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>	<0.2 <sup>a</sup>

<sup>a</sup>Free chlorine concentration below the detection limit (0.2 mg/L).

<sup>b</sup>Free chlorine concentration above the detection limit (2.0 mg/L).

<sup>c</sup>Samples spiked with rotavirus, adenovirus, bacteriophage MS2, and *E. coli*.

<sup>d</sup>Samples spiked with *Giardia lamblia* and *Cryptosporidium parvum* oocysts.

One stage of the multi-barrier Pureit® Classic device was not measured due to the integrity device, which was the chlorination chamber for evaluating the chlorine feeder operation, although 6 mg/L of free chlorine in the water within the chamber was reported (Clasen *et al.* 2006).

Recovery analyses for each microorganism obtained from the spiked drinking water samples showed a reduced number of positive samples. The spiked bacteria were eliminated to the level of non-detection in all samples, but there were positive samples noted for enteric viruses and protozoa. However, is important to consider the limit of detection of the quantitative methods used to evaluate the removal capacity of the Pureit® device. This feature is important because the capacity of the Pureit® device, and most likely of other commercial devices, depends on the quantities and types of microorganisms, as well as on the specific conditions of the processed water. These aspects, which have not been included in other studies, are considered in the present study. It has been previously noted that these factors should be taken into account (Clasen *et al.* 2006, 2009; Verma & Arankalle 2009; WHO 2011b).

The results suggest that it is necessary to analyze the presence of microorganisms with respect to the sampling sites, due to a possible drinking water source effect, or to simply discard the site effects and consider the microorganisms' tolerances to multi-barrier treatment.

To identify the possible effect of the Mexico City site or area (Figure 1) on the presence of microorganisms, our team applied Student's *t*-test and linear generalized models.

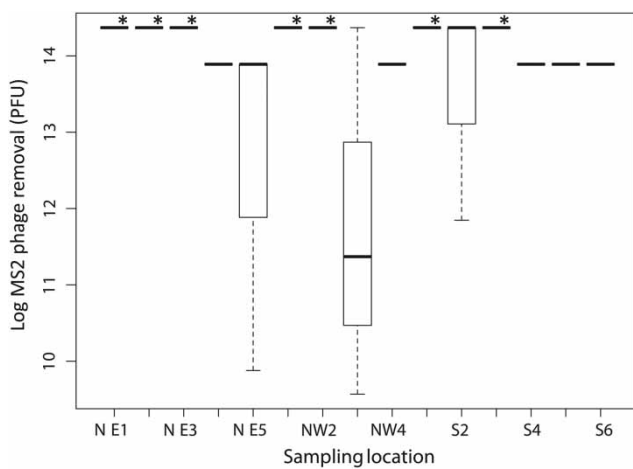
The *E. coli* that had been spiked into the drinking water samples was removed to a non-detectable level; this result represents a removal of >10 log units, independent of the site or the water source.

In Mexico, there are regulations that establish criteria, but these criteria only point out limits for bacteria reduction with which all water purifier devices must comply; including a reduction capacity of 4 log units of total coliform bacteria and 95% for mesophilic aerobic bacteria (DOF 2009). The results of the present study show that the Pureit® Classic device meets the Mexican regulatory requirements, however, the authors of this study believe that these water-purifying device requirements are incomplete if we focus on the necessity for safe water indoors.

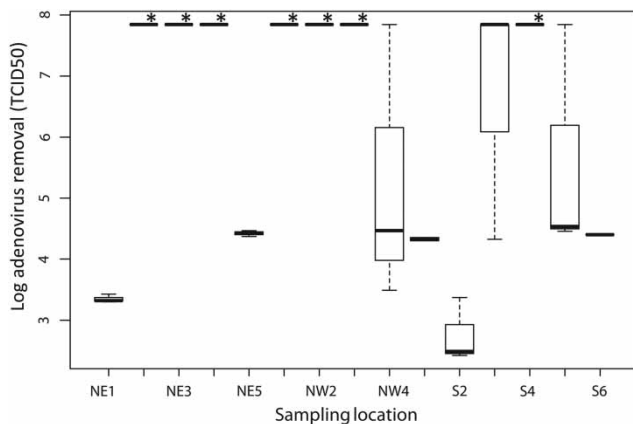
Removal of the MS2 is shown in Figure 3. Except for sites NE5, NW3, and S2, the removal of spiked MS2 was  $>10$  log units. The linear model shows significant differences at sites NW3 ( $p = 0.00206$ ) and NE5 ( $p = 0.02512$ ).

With respect to the removal of adenovirus, except for the S2 site, removal was at least from 3 log units to 7 log units (Figure 4). However, the results showed variability within area S, and the linear model suggests a site effect on adenovirus removal.

Rotavirus removal at the majority of sites was  $>6$  log units. However, the generalized linear model test results



**Figure 3** | Removal of MS2 spiked in drinking water samples (10 L) processed by the multi-barrier device. The boxes show the variability between replicates and dashed lines the data distribution. \*MS2 removal for these samples was below the method limit of detection.



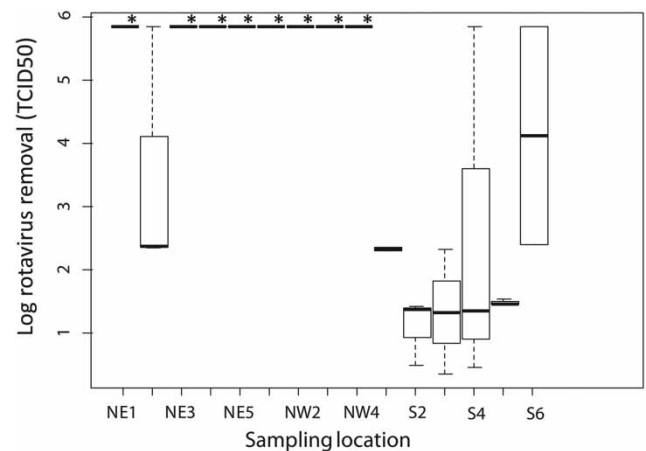
**Figure 4** | Removal of adenovirus spiked in drinking water samples (10 L) processed by the multi-barrier device. The boxes show the variability between replicates and dashed lines the data distribution. \*Adenovirus removal for these samples was below the method limit of detection.

were significant for samples of areas S and NE2. In area S (S2, S3, S4, and S5), rotavirus removal was lower than average (Figure 5). Both the rotavirus and adenovirus removal levels obtained in this study were similar (6.53 log units) to those reported for hepatitis E and hepatitis A viruses that had been spiked in the water and removed using the Pureit<sup>®</sup> device in India (Verma & Arankalle 2009).

The capacity of the Pureit<sup>®</sup> Classic device for removing *Cryptosporidium parvum* oocysts was 2 log units  $>87\%$  of oocysts spiked, but the method's limit of detection of six oocysts/10 L must be considered. It is important to emphasize that in this case, the stock was recovered from the residual water and the spiked oocysts were only 2 log units (Figure 6).

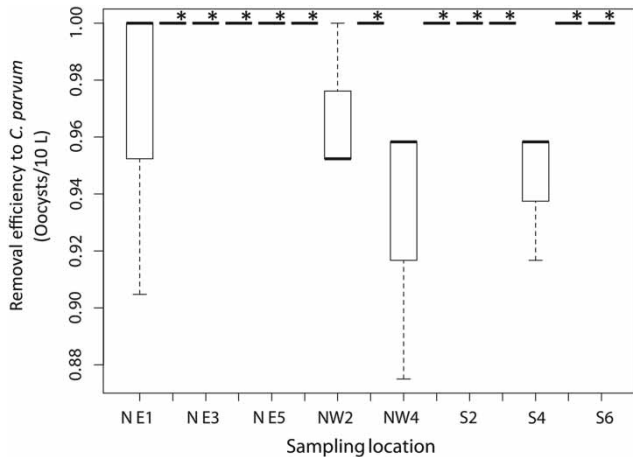
To verify whether *Cryptosporidium parvum* oocyst removal depends on an area or on the site, a generalized linear model was fitted with a Poisson-type error and logarithmic link. The results do not show a relationship between the site and the oocyst quantity present in the drinking water after multi-barrier treatment ( $p > 0.05$ ). Therefore, there is no site-based pattern that affects the removal of *Cryptosporidium parvum* oocysts.

*Giardia lamblia* cyst removal was 2 log units  $>98\%$  (Figure 7) because cysts were below the limit detection (six cyst/10 L) at the majority of sampling sites. The test to identify a relationship between removal and site was not significant ( $p > 0.05$ ); therefore, any site pattern was

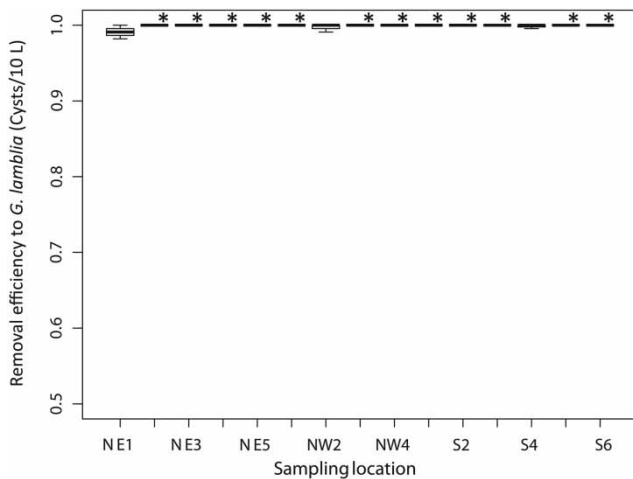


**Figure 5** | Removal of rotavirus spiked in drinking water samples (10 L) processed by the multi-barrier device. The boxes show the variability between replicates and dashed lines the data distribution. \*Rotavirus removal for these samples was below the method limit of detection.





**Figure 6** | Removal of *Cryptosporidium parvum* oocysts spiked in drinking water samples (10 L) processed by the multi-barrier device. The boxes show the variability between replicates and dashed lines the data distribution. \*Oocyst removal for these samples was below the method limit of detection.



**Figure 7** | Removal of *Giardia lamblia* cysts spiked in drinking water samples (10 L) processed by the multi-barrier device. The boxes show the variability between replicates and dash lines the data distribution. \*Cyst removal for these samples was below the method limit of detection.

discarded. For protozoan removal, Clasen et al. (2006) evaluated the Pureit® removal capacity using microspheres as a surrogate for the protozoan cysts. Their results showed a reduction of at least 3 log units after Pureit® device treatment. We could not attempt to replicate this result because the (oo)cyst stocks (*C. parvum* and *G. lamblia*) were inoculated at only 2 log units in the experimental water samples; our analysis did not allow us to determine whether, under our experimental conditions, the multi-barrier device is

able to remove >3 log units. For the latter, it is advisable to perform this type of evaluation with an excess of (oo)cysts.

This research has demonstrated the importance of efficiency assessment with a perspective concerning which differences in water sources and water characteristics (groundwater or surface) can have an effect on the microorganisms and on the water purifier performance.

In this case, a site effect was detected on the reduction of enteric viruses in drinking water from three aquifer subsystems; however, an experimental design is needed to identify the main factors or parameters that must be determined, an issue to be considered by purifier device manufacturers.

Point of use water disinfection today is a real option for a safe water supply in households, with the water quality control in the hands of the consumer. However, it is noteworthy that this must not eliminate the responsibility of the authorities to supply the population with safe water from the integrated water management perspective, that is, from the supply source to the consumer, as suggested by the WHO (2011a). The maintenance and operation of pumps, disinfection systems, pipes, and tanks, to mention only a few factors, always play a major role with the purpose of improving and protecting the health of the population as related to water quality.

## CONCLUSIONS

The multi-barrier water purification device showed a removal capacity of >10 log units of *E. coli* that had been inoculated into drinking water without differences among samples from three Mexico City areas. For MS2, phage inactivation was observed in the majority of drinking water samples. The removal capacity was >10 log units, but the inactivation process included a site effect for all three sites.

A site effect was also detected in the removal of adenovirus and rotavirus, demonstrating the importance of water source characteristics to be taken into account for evaluating the disinfection efficiency of any point of use household device.

The minimum and maximum removal capacities of the multi-barrier Pureit® Classic were 3 and >7 log units for adenovirus, respectively, and 2 and 6 log units for rotavirus, depending on the site. Rotavirus inactivation was less in the

southern area of Mexico City compared with the NE and NW areas.

*Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts of 2 log units were removed from drinking water without any site pattern detected.

Under Mexico City-specific conditions, the multi-barrier Pureit® Classic device showed to be efficient for the reduction of *E. coli*, MS2 coliphages, adenovirus, and rotavirus, as well as for the *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts, all of which were spiked in real drinking water samples collected from Mexico City households that had been supplied by three aquifer subsystems.

The Pureit® Classic device improved the microbial quality of the drinking water inside the house, but it is advisable that the capacity for principal chemical contaminant removal be assessed, as these contaminants have been scarcely studied in household water purifiers.

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