LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE
(University of London)
Department of Infectious and Tropical Diseases
Keppel Street, London WC1E 7HT

Tel: (Direct) +44 020-7927 (Switchboard) +44 020-7636 8636
Fax: (Direct) +44 020-7927 E-mail: Peter.Donachie @lshtm.ac.uk

Peter Donachie BSc
Principal Scientific Officer (Medical Microbiology)
Faculty of Infectious and Tropical Diseases

8 May 2013

REPORT ON MICROBIOLOGICAL TESTS CARRIED OUT ON THE BEHALF OF WATER-TO-GO LTD. ON TW0 WATER FILTERATION BOTTLES BY THE LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE.

Test Items

The bottles manufactured by Water-to-go Ltd.

The bottles were delivered to the laboratory new and unused**.** Before testing each bottle was examined for mechanical defect or leaks and was primed using deionised water according to the manufacturer's instructions.

Test organisms

Poliovirus type 1 (Sabin vaccine strain) at a concentration of 24.50×10^{6} PFU (plaque forming units) per millilitre.
Escherichia coli ATCC 22952 at a concentration of 26.00×10^{6} CFU (colony forming units) per millilitre.
Fluorescent beads. The size of the beads was chosen to mimic Cryptosporidium oocysts at a concentration of 10.85×10^{3} beads per millilitre.

Test Water

Autoclaved Distilled Water.

Test procedure

1. Bottles were primed according to user instructions and then washed several times with deionised water before challenge.

2. 100ml of poliovirus suspension was added to 1500ml of challenge water and mixed thoroughly. The seeded test water was sucked through the bottle and collected in sterile containers for assay. For the bacteriological challenge 50ml of an overnight culture of Escherichia coli suspension was added to 1000ml of challenge water.

3. Prior to filtration, a sample of the seeded test water was taken and the number of virus particles and bacteria determined in parallel with the filtered samples.

Microbiological assay

1. For virus assay, 9ml volumes of water (filtered and unfiltered) were added to 1ml of ×10 cell culture medium and diluted 10-fold steps in single strength medium. Four replicates of each dilution were added to VERO cell monolayers and a plaque assay performed and incubated for 2 days before examination for plaque formation. The amount of virus in the filtered sample when compared to the unfiltered sample was measured and the log reduction calculated.

2. For bacteria, 1ml samples were assayed for Escherichia coli by spread plate and Miles & Misra techniques. The tests were performed in triplicate.

3. For fluorescent beads the water was filtered through filter paper membranes known to have pores smaller than the beads and the membrane viewed under an ultra violet microscope.

4. For the reduction of chlorine, 10ml water samples were treated with N,N,-diethyl-p-phenylenediamine which reacts which free chlorine and produces a red complex and the intensity of the colour was measured by eye compared to known standards using a Lovibond comparator.

5. Suitable controls, positive and negative were included in all assays.

Test results

Table 1- Summary of Assay results of all samples

bottle	Test organism	Inflowing (log_{10})	outflowing (log_{10})	% reduction (log_{10} reduction)
1	Poliovirus	2.48×10^{5} PFU/ml (5.39)	156.8 PFU/ml (2.20)	99.982% (3.73)
2			45.60 PFU/ml (1.66)	99.937% (3.20)
1	*Escherichia coli*	2.60×10^{7} CFU/ml (7.41)	2.10×10^{2} CFU/ml (2.32)	99.9992% (5.09)
2			4.25×10^{3} CFU/ml (3.63)	99.9837% (3.79)
1	Beads	1.09×10^{4}/ml (4.04)	≤168/ml (≤2.27)	≥99.982906% (≥3.77)
2			≤168/ml (≤2.27)	≥99.982906% (≥3.77)
1	Free Chlorine	60ppm	<0.4ppm	
2			<0.4ppm	

The reduced Chlorine reading was between 0 and 0.4ppm as 0.4ppm represented the lowest comparator disc.

Summary

Under the conditions of testing in the laboratory of the London School of Hygiene and Tropical Medicine as shown in this report, these results show that the Water-to-go Ltd bottle removed more than 99.9% of bacteria, viruses and *Cryptosporidium oocsyst* from contaminated water.
There was also a significant or total reduction in free chlorine by the filter.

Signed on 8th May 2013

Peter Donachie BSc (Hons.)
Principal Scientific Officer (Medical Microbiology)
London School of Hygiene & Tropical Medicine