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**REPORT ON MICROBIOLOGICAL TESTS CARRIED OUT ON THE
BEHALF OF PURE HYDRATION ON TWO MAD WATER
FILTRATION BOTTLES.**

Test Items

The MAD bottles manufactured by Pure Hydration. The bottles were delivered to the laboratory new and unused in sealed packaging.

One unit was designated as single membrane (MAD I) and the other as double membrane (MAD II). The bottles were delivered to the laboratory, new and in sealed packaging. Before testing each bottle was examined for mechanical defect or leaks and were primed using deionised water.

Before testing, each bottle was examined for mechanical defects and leaks. An integrity check of the bottle was carried out and the unit primed according to the manufacturer's instructions.

Test organisms

Escherichia coli ATCC 22952 at a concentration of 1.67×10^6 CFU (colony forming units) per millilitre.

Poliovirus type 1 (Sabin vaccine strain) 5.00×10^7 PFU (plaque forming units) 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) 50ml of *poliovirus* suspension was added to 1000ml of challenge water and mixed thoroughly. The seeded test water was pumped through the bottle and collected in sterile containers for assay. For the bacteriological challenge 50ml 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 particle and bacteria determined in parallel with the filtered samples.

Microbiological assay

- 1) For virus assay, 9ml volumes of water (treated and untreated) are added to 1ml of $\times 10$ cell culture medium and diluted 10-fold steps in single strength medium. Four replicates of each dilution are added to VERO cell monolayers and incubated for 3 days before examining for CPE (cytopathic effect). The amount of virus in the treated sample when compared to the untreated sample is measured and the log reduction calculated.
- 2) For bacteria, 1ml samples are assayed for *Escherichia coli* by spread plate and Miles & Misra techniques. The tests are preformed in parallel, in duplicate.
- 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 a ultra violet microscope.
- 4) Suitable controls, positive and negative were included in all assays.

Test results

Table 1- Summary of Assay results of all samples

unit	poliovirus	<i>Escherichia coli</i>	beads	Log reduction		
				virus	bacteria	beads
	5.00×10^7 PFU/ml	1.67×10^6 CFU/ml	1.40×10^4 /500ml			
MAD I	5.00×10^2	No bacteria found	No beads found	5.00 (99.999%)	≥ 6.52 ($\geq 99.99997\%$)	≥ 4.15 ($\geq 99.993\%$)
MAD II	5.00×10^2	No bacteria found	No beads found	5.00 (99.999%)	≥ 6.52 ($\geq 99.99997\%$)	≥ 4.15 ($\geq 99.993\%$)

The bottles were tested three times and each of the results averaged

Summary

The Pure Hydration bottles were tested using microorganisms in greater numbers than you would expect to find in natural water sources. This is the worse-case scenario approach recommended by the EPA and their guidelines have been used to draw up own protocols for testing of all the microbiological water purifiers.

The microbiological reduction figures showed that Pure hydration MAD water filtration bottles meet and exceeded the EPAs Microbiological requirements as shown in the US *National Primary Drinking Water Regulations* (<http://epa.gov/safewater/mcl.html>) under the Safe Drinking Water Act.

The requirements for bacteria are 6 log₁₀ reduction (99.9999% removal), for viruses a 4 log₁₀ reduction (99.99%) and for Cryptosporidium a 3 log₁₀ reduction. We can assume based upon size that fungi will be removed. These reduction requirements are also shown in the WHO guidelines for safe drinking water and are the basis for current UK and European legislation on drinking water standards.

Regulatory Compliances

The Pure hydration, MAD water filtration bottles meet and exceed the following:

UK compliance Water Supply (Water Quality) Regulations 2000

EU compliance European Drinking Water Directive Council

Directive 98/83/EC

US compliance	Environmental Protection Agency –EPA’s Microbiological Reduction requirements as shown in the US National Primary Drinking Water Regulations (http://epa.gov/safewater/mcl.html) under the Safe Drinking Water Act.
WHO compliance	World Health Organisation-Guidelines for Drinking-water Quality First Addendum 3 rd Edition

Conclusions

Signed on 6th September 2011



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