London School of Hygiene & Tropical Medicine
(University of London)
Keppel Street, London WC1E 7HT

Tel: +44 (0)20 7636 8636

Department of Infectious and Tropical Diseases

Peter Donachie BSc
Chief Scientific Officer (Microbiology)
John Krahn BSc
External Consultant, Scientist (Environmental Microbiology)

17th December 2007

REPORT ON MICROBIOLOGICAL TESTS CARRIED OUT FOR

LIFESAVER SYSTEMS

Test Item

The 'Lifesaver Bottle' manufactured by Lifesaver Systems. The bottles were delivered to the laboratory new and unused in sealed packaging. A user operating manual was enclosed.

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 manufacturers instructions. The teat on the end of the bottle was removed for ease of testing and to reduce the chances of accidental contamination with unfiltered water during sampling procedures.

Test organisms

Escherichia coli NCTC 10418 at a concentration of 5×10^{10} CFU (colony forming units) per Litre

Poliovirus Type 1 (Sabin vaccine strain) at a concentration of 10^8 $TCID_{50}$ per Litre

Test water

Two types of challenge water were used:

i) London tap water treated according to our standard Testing Protocol for Microbiological Water Purifiers which is in accordance with US Environmental Protection Agency (EPA) guidelines.
ii) Unmodified pond water collected on the day of testing (High Turbidity).

London School of Hygiene & Tropical Medicine
(University of London)
Keppel Street, London WC1E 7HT

Tel: +44 (0)20 7636 8636

Department of Infectious and Tropical Diseases

Test Procedure

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

2) 10 ml of *poliovirus* suspension was added to 700 ml of challenge test water and mixed thoroughly. The seeded test water was pumped through the bottle and collected in sterile containers for assay. For the bacteriological challenge, 10 ml of *Esch. coli* suspension was added to 700 ml of challenge test water and samples collected as for *poliovirus.*

3) Tests were repeated on all bottles with the activated carbon filter removed.

4) The tests were also repeated on bottles without the activated carbon using spiked high turbidity pond water (as per EPA guidance) in place of modified tap water.

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

Microbiological Assay

1) For virus assay, 9ml volumes of water (treated and untreated) are added to 1 ml of x10 cell culture medium and diluted in 10-fold steps in single strength medium. Four replicates of each dilution are added to VERO cell monolayers and incubated for 3 – 4 days before examining for CPE. The amount of virus in the treated sample when compared to the untreated sample is measured and the log reduction calculated.

2) For bacteria, 1 ml samples are assayed for *Esch. Coli* by the spread plate and Miles & Misra techniques. The tests are performed in parallel, in triplicate.

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

London School of Hygiene & Tropical Medicine
(University of London)
Keppel Street, London WC1E 7HT

Tel: +44 (0)20 7636 8636

Department of Infectious and Tropical Diseases

Test Results

Table 1 – Summary of Assay results of all samples.

Bottle Number	With Carbon	Test Water	Poliovirus 10^8 $TCID_{50}$ / L	Escherichia coli 5×10^{10} CFU / L	Log Reduction	
					Virus	Bacteria
1	No	Tap (modified)	No virus found	No bacteria found	>5 (>99.999%)	>7.5 (>99.999995%)
1	No	Spiked Pond Water	No virus found	No bacteria found	>5 (>99.999%)	>7.5 (>99.999995%)
1	Yes	Tap (modified)	No virus found	No bacteria found	>5 (>99.999%)	>7.5 (>99.999995%)
2	No	Tap (modified)	No virus found	No bacteria found	>5 (>99.999%)	>7.5 (>99.999995%)
2	No	Spiked Pond Water	No virus found	No bacteria found	>5 (>99.999%)	>7.5 (>99.999995%)
2	Yes	Tap (modified)	No virus found	No bacteria found	>5 (>99.999%)	>7.5 (>99.999995%)
3	Yes	Tap (modified)	No virus found	No bacteria found	>5 (>99.999%)	>7.5 (>99.999995%)
3	No	Tap (modified)	No virus found	No bacteria found	>5 (>99.999%)	>7.5 (>99.999995%)

Summary

The 'Lifesaver bottles' were tested using micro organisms in far greater numbers than could ever be found in natural water sources. This is the worse-case scenario approach recommended by the EPA and their guidelines have been used to draw up our own protocols for testing of all Microbiological Water Purifiers. As well as using modified tap water for our tests we use samples taken from a pond or stream as our challenge test water to simulate 'real-life' situations as per EPA guidelines.

The table of results for the bottles tested shows that all viruses and bacteria were removed after being pumped through regardless of the type of water used. The Microbiological Reduction figures shown by the 'Lifesaver Bottle' meet and exceed the EPAs 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.*

London School of Hygiene & Tropical Medicine
(University of London)
Keppel Street, London WC1E 7HT

Tel: +44 (0)20 7636 8636

Department of Infectious and Tropical Diseases

These requirements for bacteria are a 6 log reduction (99.9999% removal) and for viruses a 4 log reduction (99.99%). Since both types of organisms are considerably smaller than Giardia or Cryptosporidium (both protozoa) we can safely assume that these organisms would also be removed. We can also safely assume based upon size that fungi will also 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 LIFESAVER bottle meets and exceeds 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 3rd Edition

Conclusions
Under the conditions of testing in our laboratory as shown in this report, our results show that the 'Lifesaver Bottle' removed all bacteria and viruses from a contaminated water source in excess of legal requirements and as such, complies with all British, US and European Drinking Water Regulations for Microbiological Reduction.

Signed on 17th December 2007

Peter Donachie BSc
Chief Scientific Officer (Microbiology)
London School of Hygiene & Tropical Medicine

John Krahn BSc
External Consultant, Scientist (Environmental Microbiology)
London School of Hygiene & Tropical Medicine

All data will be held at LSHTM for a minimum of 10 years. During that time it shall remain available solely at the request of M. Pritchard or other nominated executive of Lifesaver Systems, and under no circumstances released to a third party.

This report is designed for internal developmental use, product registration or as evidence of full independent efficacy testing in defence of any official regulatory or legal enquiry. It must not be used for advertising purposes. In no way does any comment made in this report constitute an endorsement of any product by LSHTM, and no such claims, directly or by inference, made by any company or individual will be permitted to this effect.