

Schlieren, 22 June 2012
ar/SISVestergaard Frandsen SA
Chemin de Messidor 5-7
1006 Lausanne

Analytical report

Object: Reduction tests on LifeStraw (hollow-fiber)

Bachema AG
Rütistrasse 22
Postfach
CH-8952 SchlierenTelefon
+41 44 738 39 00
Telefax
+41 44 738 39 90
info@bachema.ch
www.bachema.chChemisches und
mikrobiologisches
Labor für
die Prüfung von
Umweltproben
(Wasser,
Böden, Abfall)
Akreditiert nach
ISO 17025/STS
Nr 064

| | |
|-----------------------------------|---------------------------------|
| Bachema order number | 20123646 |
| Sample number | 19334-19337, 19340, 19345-19348 |
| Date of sampling | 06 June 2012 |
| Date of arrival at Bachema | 06 June 2012 |
| Place of sampling | Schlieren |
| Samples taken by | Dr. A. Rust, Bachema AG |

| | |
|---------------------------------|---|
| Customer | Vestergaard Frandsen SA, Chemin de Messidor 5-7, 1006 Lausanne |
| Invoice address | Vestergaard Frandsen SA, Chemin de Messidor 5-7, 1006 Lausanne |
| Report to | Vestergaard Frandsen SA, D. Frauchiger, Chemin de Messidor 5-7, 1006 Lausanne |
| Report copy by e-mail to | Vestergaard Frandsen SA, D. Frauchiger, df@vestergaard-frandsen.com |

Yours sincerely,
BACHEMA AGAnnette Rust
Dr. sc. nat. / dipl. Natw. ETH

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Customer: Vestergaard Frandsen SA
Bachema order number: 20123646



Overview over the samples

| Bachema number | Sample description | Sampling / Arrival |
|----------------|--|---------------------|
| 19334 W | aging water (before test start) | 06.06.12 / 06.06.12 |
| 19335 W | aging water after filtration of 10 L filter 1 | 06.06.12 / 06.06.12 |
| 19336 W | aging water after filtration of 10 L filter 2 | 06.06.12 / 06.06.12 |
| 19337 W | aging water after filtration of 10 L filter 3 | 06.06.12 / 06.06.12 |
| 19340 W | challenge water before filtration | 06.06.12 / 06.06.12 |
| 19345 W | challenge water before filtration (microbio) | 06.06.12 / 06.06.12 |
| 19346 W | challenge test after filtration of 10 L filter 1 | 06.06.12 / 06.06.12 |
| 19347 W | challenge test after filtration of 10 L filter 2 | 06.06.12 / 06.06.12 |
| 19348 W | challenge test after filtration of 10 L filter 3 | 06.06.12 / 06.06.12 |

Abbreviation

| | |
|------|--|
| n.d. | Not detected |
| cfu | Colony forming units |
| W | Water sample |
| F | Solid sample |
| dw | dry weight |
| < | For the results, the value after the sign < (smaller than) indicates the limit of quantification for the corresponding method. |
| * | Parameters marked with a * are not within of the accredited area of Bachema AG or are external analysis. |

Accreditation

| | |
|---|--|
|  | Partially copying of the analytical report is only allowed with permission from Bachema AG. |
|  | Detailed information on analytical methods, precision of the measurement and further data are available on request (see also www.bachema.ch). |

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Sample description

Sample number
 Date of sampling
 Time of sampling

| | | | | | |
|---------------------------------------|--|--|--|--|--|
| aging water (before test start) | | | | | |
| 19334 | | | | | |
| 06.06.12 | | | | | |
| 15:30 | | | | | |

Physicochemical parameters

| | | | | | | | |
|---|-------|------|--|--|--|--|--|
| Turbidity nephelometric (after homogenization) | TE/F | 13 | | | | | |
| Conductivity (25°C) | µS/cm | 452 | | | | | |
| pH-value (lab) | pH | 7.64 | | | | | |

Sample description

Sample number
 Date of sampling
 Time of sampling

| | | | | | |
|--|--|--|--|--|--|
| aging water after filtration of 10 L filter 1 | aging water after filtration of 10 L filter 2 | aging water after filtration of 10 L filter 3 | | | |
| 19335 | 19336 | 19337 | | | |
| 06.06.12 | 06.06.12 | 06.06.12 | | | |
| 15:30 | 15:30 | 15:30 | | | |

Field parameters

| | | | | | | | |
|-----------------------|-------|-------|-------|-------|--|--|--|
| Flow rate | L/min | 0.560 | 0.600 | 0.680 | | | |
| Temperature (on-site) | °C | 20.8 | 21.0 | 20.8 | | | |

Physicochemical parameters

| | | | | | | | |
|---|-------|------|------|------|--|--|--|
| Turbidity nephelometric (after homogenization) | TE/F | 0.23 | 0.40 | 0.25 | | | |
| Conductivity (25°C) | µS/cm | 453 | 451 | 454 | | | |
| pH-value (lab) | pH | 7.62 | 7.57 | 7.55 | | | |

Sample description

Sample number
 Date of sampling
 Time of sampling

| | | | | | |
|--|--|--|--|--|--|
| challenge water before filtration | challenge water before filtration (microbio) | | | | |
| 19340 | 19345 | | | | |
| 06.06.12 | 06.06.12 | | | | |
| 17:30 | 17:30 | | | | |

Physicochemical parameters

| | | | | | | | |
|---|-------|------|--|--|--|--|--|
| Turbidity nephelometric (after homogenization) | TE/F | 81 | | | | | |
| Conductivity (25°C) | µS/cm | 3040 | | | | | |
| pH-value (lab) | pH | 7.36 | | | | | |

Microbiological parameters

| | | | | | | | |
|-----------------------------|------------|--|-----------|--|--|--|--|
| <i>Escherichia coli</i> | cfu/100 mL | | 145000000 | | | | |
| Enterococci | cfu/100 mL | | 85000000 | | | | |
| Microscopic investigation * | Counts/mL | | 13000 | | | | |

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Sample description

Sample number
 Date of sampling
 Time of sampling

| challenge test after filtration of 10 L filter 1 | challenge test after filtration of 10 L filter 2 | challenge test after filtration of 10 L filter 3 | | | |
|---|---|---|--|--|--|
| 19346 | 19347 | 19348 | | | |
| 06.06.12 | 06.06.12 | 06.06.12 | | | |
| 18:30 | 18:30 | 18:30 | | | |

Field parameters

| | | | | | | | |
|-----------------------|-------|------|------|------|--|--|--|
| Flow rate | L/min | 340 | 520 | 230 | | | |
| Temperature (on-site) | °C | 20.2 | 20.2 | 20.8 | | | |

Physicochemical parameters

| | | | | | | | |
|---|-------|------|------|------|--|--|--|
| Turbidity nephelometric (after homogenization) | TE/F | 0.26 | 0.07 | 0.25 | | | |
| Conductivity (25°C) | µS/cm | 3030 | 3030 | 3030 | | | |
| pH-value (lab) | pH | 7.42 | 7.42 | 7.51 | | | |

Microbiological parameters

| | | | | | | | |
|-----------------------------|------------|------|------|------|--|--|--|
| <i>Escherichia coli</i> | cfu/100 mL | n.d. | n.d. | n.d. | | | |
| Enterococci | cfu/100 mL | n.d. | n.d. | n.d. | | | |
| Microscopic investigation * | Counts/mL | n.d. | n.d. | n.d. | | | |

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Assessment

Material and Methods

Model types "HF-LS" of LIFESTRAW were tested, widely according to the protocol of SOP.HFLS.1.3.2, Vestergaard Frandsen SA, "Determination of Hollow Fiber LifeStraw performance in microbes' elimination" from where the terminology can be derived. An aging with 10 L of aging water per test straw followed by a challenge test with also 10 L of challenge water per test straw was performed.

Strains and Medium

The HF-LS test filters were challenged with the following bacterial species:

Escherichia coli (ATCC 25922)

Enterococcus faecalis (ATCC 19433)

Microspheres: Polybead Polystyrene Microsphere 3.00 µm (Polysciences Inc.) that were used as surrogates for protozoan oocysts or cysts (e.g. *Cryptosporidium parvum*, *Giardia lamblia*, respectively).

The bacterial strains were grown in Tryptic Soy Broth (TSB) (BioMérieux) at 37°C over night before use.

The aging water was prepared as follows:

- 5.5 g of MgSO₄ x 7 H₂O (VWR International article no. 5886)
- 5.81 g of CaCl₂ x 2 H₂O (VWR International article no. 2382)
- 4 g of NaHCO₃ (VWR International article no. 1.06329.0500)
- 2.5 g of KHCO₃ (VWR International article no. 26731.295)
- 0.25 g humic acid (Fluka Chemie GmbH article no. 53680)
- 1.125 g ISO 12103-A2 fine test dust (Ellis Components)

was added to 50 L deionised water.

The challenge water was prepared as follows:

- 5.5 g of MgSO₄ x 7 H₂O (VWR International article no. 5886)
- 5.81 g of CaCl₂ x 2 H₂O (VWR International article no. 2382)
- 4 g of NaHCO₃ (VWR International article no. 1.06329.0500)
- 2.5 g of KHCO₃ (VWR International article no. 26731.295)
- 0.5 g humic acid (Fluka Chemie GmbH article no. 53680)
- 7.5 g ISO 12103-A2 fine test dust (Ellis Components)
- 75 g Sea salt (Sigma article. no. S9883)

was added to 50 L deionised water.

The aging water and the challenge water were kept under continuous stirring for optimal homogenisation during the tests.

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Analysis methods

The concentration of the challenge bacteria are measured by standard methods for bacterial drinking water analysis according to the Swiss Manual of Food Testing. (The methods correspond and are equal to international standard ISO 9308 for *E. coli* and ISO 7899 for Enterococci.)

The microspheres were analysed and counted under the microscope after necessary concentration.

In the sample of the challenge water, before filtration, the microspheres were concentrated 10 times by centrifugation at $16.1 \times g$ for 10 minutes. The concentration of microspheres was determined by counting in the Thoma chamber under the microscope.

In the samples after filtration the microspheres were concentrated by filtration and centrifugation: 100 mL of sample volume was filtered through filters with pore size $0.2 \mu\text{m}$ (Millipore, cat. no. GTBP01300). The microspheres (and other particles taller than $0.2 \mu\text{m}$) were trapped on the filter and then resuspended in 1 mL nanopure water (double filtrated). The particles in these suspensions were concentrated by centrifugation in several steps to a volume of about $2 \mu\text{L}$ that was entirely examined under the microscope. The microspheres with their defined size and shape could be distinguished and counted. The recovery rate of this concentration method by filtration and subsequent centrifugation was estimated in side experiments and is at least 25%. The detection limit can therefore be theoretically estimated to be <0.1 counts/mL. To stay on the safe side, 10 times above the theoretical detection limit, in the result table the detection limit is indicated with "not detected" ("n.d.") in 1 mL.

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Flow-through system with different flow rates and different directions of the LIFESTRAWs

A flow-through system was used where the test filters could be inserted after flow regulation valves (Key Instruments, USA). The flow was forced by gravitation. Therefore, a reservoir for the aging water and the challenge water was installed with the upper water level about 2 m above the test-LIFESTRAWs. After installation of the flow-through system, it was slightly disinfected with hydrogenperoxide to remove contamination from building the system. The system was thoroughly rinsed with deionised water. Then, the LIFESTRAW-units to test were inserted into the flow through system.

The aging water was added to the reservoir and filtered through the test filters. After 5 L that had passed each filter, a backwash was performed according to SOP HFLS1.3.2. After 10 L samples were taken at the outflow for chemical measurements. Again, a backwash was performed.

The challenge water was added to the reservoir and filtered through the test filters. During the first 5 L that passed through the filters samples were taken from the challenge water for chemical and microbiological characterisation. After 5 L a backwash was performed. After 10 L samples were taken at the outflow of the test filters for microbiological and chemical characterisation.

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Results and discussion

During aging, the flow rates of the 3 tested HF-LS units remained constant at 495 mL/min (minimum) to 700 mL/min (maximum). The temperature of the test water was 20.0 to 20.8°C.

During challenge, the observed flow rates were slightly reduced compared to the aging, but were still acceptable at 220 mL/min (minimum) to 440 mL/min. The temperature was 19.8 to 20.8°C.

After 10 L of challenge water the following reductions rates (expressed as logarithmic reduction with the base of 10) in average of the 3 test straws in parallel could be shown as follows:

Turbidity: -2.6
Escherichia coli: -8.2
Enterococci: -7.9
Microspheres: -4.1

All examined reduction rates and parameters fulfill the set requirements.



Annette Rust, Dr. sc. nat. / dipl. Natw. ETH

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