A METHOD FOR THE CONCENTRATION OF MICROBES IN LARGE VOLUMES OF WATER

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A METHOD FOR THE CONCENTRATION OF MICROBES IN LARGE VOLUMES OF WATER

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Summary

This report describes a method for concentration of micro-organisms in large volumes of water. The analysis of large volumes of water is necessary for a reliable assessment of the elimination of micro-organisms during treatment, and for an estimate of the infection risk by pathogenic organisms in drinking water.

The study was divided into phases. The first phase was designed to assess the recovery rate of bacteriophages at concentrating 2000 litres to 500 ml.
In the second phase, the recovery rates of several other micro-organisms were measured at concentrating 2000 litres to 500 ml.
The third phase evaluated post-concentration processes for further reduction of the sample volume.

The findings of the study are:

- The concentration of maximum volumes of 2000 litres produces high recovery rates ($\geq$ 65%) for all organisms except *Campylobacter*,
- The results of the several experiments have a standard deviation between 7-33%,
- The efficiency of the detection of *Cryptosporidium* and *Giardia* is much higher and more reproducible than with the existing Envirochek concentration method,
- Concentrates obtained with the Hemoflow-installation can be post-concentrated without a significant reduction of the recovery rate,
- Post-concentration of phages must take place by centrifugation with Centricon®,
- Post-concentration of bacteria must take place by centrifugation (5-10 min, 900 g) with complete examination of the pellet,
- For the assessment of F-specific and somatic phages, the examinable volume has been increased from 10 ml to 2000 litres,
- The Hemoflow concentration method makes it possible to simultaneously concentrate the organisms that are to be examined.

Recommendations are:

- For every unknown water type to produce at least one recovery rate assessment for one of the micro-organisms, since water types may produce a smaller recovery rate than usual,
- To use the Hemoflow-method in assessment programs since this method comprises a profound improvement relative to the concentration methods currently used.

Contents

1 Introduction

Since the amount of pathogenic micro-organisms in surface water is low, particularly after one or more purification processes, concentration methods are necessary to detect and quantify these organisms.
For this purpose, in 1996 the MF-sampler was designed, an adequate method or installation for the concentration of indicator bacteria for fecal contamination [BTO 92.200].

For the sampling of *Cryptosporidium* and *Giardia*, a different concentration method is available. Water is filtered over an Envirochek filter. After sampling, at the laboratory this filter is shaken with a buffer in order to loosen the *Cryptosporidium* and *Giardia* from the filter. Among the disadvantages of this method are the usually low output and the variable results. For these reasons, quantification is a problem and the assessment of viability as well as genotyping of the isolated organisms is not very useful.
In addition, the demand is rising for the detection of F-specific RNA-bacteriophages and somatic coliphages in large volumes. These organisms can not be concentrated with the two methods mentioned. Research of phages is conducted in order to gain insight into the elimination of viruses. With the available method of analysis, the volume to be examined for phage research can not be larger than 10 ml.

Simmons [2001] describes a cross flow ultrafilter (Hemoflow-filter) that is used for renal dialysis, but can also be used for the concentration of micro-organisms in water. An important advantage of this filter is that it concentrates parasitic protozoa, bacteria, spores and viruses/phages.

This report describes the validation of concentration of micro-organisms with the Hemoflow-filter in several types of water samples. We studied the attainable recovery rate at filtration of different volumes and the question whether the recovery rate is the same for different micro-organisms. Also, we examined the reproducibility of the findings.
In addition, we investigated to what extent the Hemoflow-concentrate can be concentrated further to as little as some millilitres. Two techniques were used for this purpose: centrifugation and Centricon®-concentration.

2 Recovery experiments

2.1 Design

For the assessment of the recovery rate, several micro-organisms were studied (Table 1).

Table 1: The micro-organisms used in this study

Micro-organisms	Code	size (µ)	origin
Escherichia coli	WR1	1-2	strain cultured in drinking water
Streptococcus faecium	WR63	1-2	strain cultured in drinking water
Clostridium bifermentans	CP1	1	Spores harvested from a solid culture medium/from a soil sample
RNA bacteriophage	MS2	0,03	GAP, Canada
Somatic coliphage	ΦX174	0,03	GAP, Canada
Campylobacter jejuni	ATCC 29428	05,-2	cultured in Preston-medium
Cryptosporidium parvum	-	4	Waterborne, VS
Giardia lamblia	-	10-12	Waterborne, VS

During the first phase of this study, the recovery rate of the Hemoflow-concentrate was measured. The concentrate volume was around 500 ml and in this concentrate, the various parameters were examined without further post-concentration.
In the second phase, the concentrate was concentrated further through centrifugation or by Centricon®-concentration, in order to reduce the concentrate volume as much as possible - oftentimes, a volume of approx. 3 ml is attainable. The resulting pellet (centrifugation) or end-concentrate (Centricon®-concentration) was used for the output measurement of the various organisms.

2.1.1 Materials

The materials used in this study are:

- Hemoflow HF80S, Fresenius Polysylfone® High-Flux,
- Hose pump, Masterflex IP,
- Water meter,
- Stainless steel tank of 600 l,
- Synthetic tanks of 20 litres,
- Vacuum hoses, Masterflex Norprene®,
- Centricon® Plus-70 Centrifugal Filter Devices (Millipore).

These parts were arranged on a mobile installation.

- Laboratory centrifuge appropriate for a goal of 900 g.

2.1.2 *Water types*

The study was performed on drinking water from the drinking water plant *Tull en 't Waal*. In addition, water was used from the Water transport company *Rijn-Kennemerland (WRK)* (product water); from drinking water plant *Leiduin* (before and after ozone); and from surface water (collection point *WRK Lek*-canal).

2.1.3 *Methods*

Method of Hemoflow-concentration

For Hemoflow-concentration, a tank is filled with a certain sample volume. From this tank, the water is pumped through the Hemoflow filter with a minimum speed of 4 litres per minute. The overpressure over the filter is increased (0.4 - max. 0.7 bar) until a portion of the water (filtrate) is slowly pressed through the walls of the straws (cross-flow) with a speed of 0.9 l/min. The constant flow lengthwise over the filter results in little or no residue on the filter. The concentrate is brought back into the tank, where the concentration of micro-organisms therefore keeps rising. The concentration process is stopped when the concentrate volume is small enough only to fill the hoses, and hardly/no water is left in the tank. The concentrate then present in the hose and filter is pumped into a sterile bottle and the tank is rinsed out with 0.5 l. of filtrate (with no pressure over the filter), which is pumped through the filter once and then collected in a second sterile bottle.

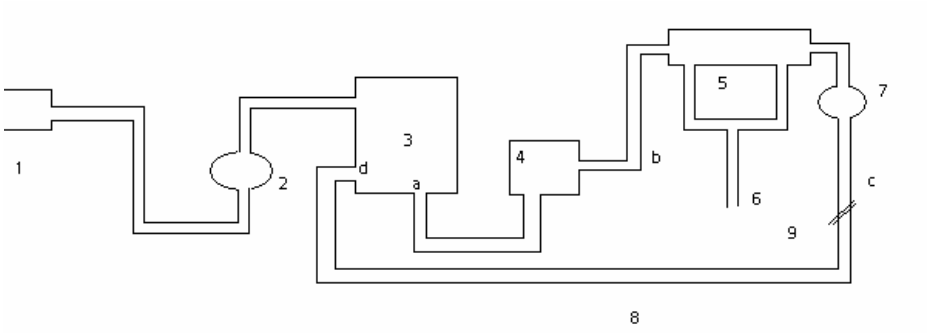

Figure 1: Diagram of a Hemoflow-installation
1 sample point; 2 water meter; 3 tank with floater; 4 pump; 5 Hemoflow-filter; 6 filtrate; 7 pressure meter; 8 return hose; 9 tube clamp; a, b, c, and d connecting points for tubes

In a tank (600 l) with drinking water from drinking water plant *Tull en 't Waal*, the micro-organisms to be examined were inoculated into a known amount of at least $6.78 * 10^4$ cells (appendix 1). Then the content of the tank was mixed. Next, the inoculation was assessed threefold in the tank. Subsequently the sample was concentrated in the above described manner. The total end volume of the filtrate and the rinse out volume were approx. 500 ml each. In

these volumes, the examined parameters were directly assessed in the manners described in the various Standards. The experiments were performed three times. First, a preliminary study was conducted with a pure culture of MS-2 phages; then all parameters were inoculated in one tank en simultaneously concentrated with one Hemoflow filter.

Methods for further concentration
Post-concentration was done by the use of two different techniques: (i) centrifugation or (ii) Centricon®-concentration. The purpose was to determine which post-concentration method was the most appropriate for the various organisms.
Dependant on whether the concentrate and the filtrate were estimated to be large enough for filtration over a membrane filter, a choice was made between a membrane filtration step or a centrifugation step.

Membrane filtration
For the detection of the bacteria the concentrate and the rinse out volume were filtered separately over a membrane filter of 0.45 µ. For Campylobacter, a membrane filter of 0.20 µ was used.

Centrifugation
Centrifugation was conducted during 10 minutes at 4,000 rpm (900 g) after which the centrifuge was allowed to roll out with no brakes applied. In order to examine the obtained pellet entirely, it was plated on specific culture mediums directly.
The pellet for the detection of *Cryptosporidium* and *Giardia* was processed further by Immuno Magnetic Separation (IMS) and then coloured and microscopically evaluated in the usual manner. The supernatant liquid obtained after centrifugation was not further examined for the presence of *Cryptosporidium* and *Giardia* since earlier research has shown that after centrifugation these organisms are found entirely in the pellet (unpublished). Phages were not examined with this concentration method since it is known that these organisms can not or hardly be found in the pellet.

Centricon®-concentration
Concentration by Centricon® was conducted by centrifugation of the concentrate and the rinse out volume through a special filter (5-10 min., dependant on the sediment in the sample, at 900 g) [Millipore, 1998]. The end volume amounted between 3 and 50 ml. Of this volume, as much as possible was examined. For the detection/determination of *Cryptosporidium* and *Giardia*, the examined volume of the filtrate was directly transferred to a membrane filter by means of a pipette, and then coloured with immunofluorescence.

Several times, the concentrate and the rinse out water were examined separately (appendix 1). The recovery rate for these samples were calculated as follows:

$$\text{recovery rate} = \frac{(\text{concentration}*\text{volume})_{\text{concentrate}} + (\text{concentration}*\text{volume})_{\text{rinse out}}}{\text{inoculated number}} * 100\%$$

If the concentrate and the rinse out water were examined as one sample, the recovery rate for this method was calculated as follows:

$$\text{recovery rate} = \frac{\text{concentration}_{\text{total volume}} * \text{volume}_{\text{total}}\,\text{volume}}{\text{inoculated number}} * 100\%$$

2.2 Results and discussion

2.2.1 *phase 1: recovery rate in the concentrate of the Hemoflow*

For every experiment, the recovery rates for the various micro-organisms per water type were calculated (table 1; separate data in appendix 1). From these results, the total recovery rates for all water types were calculated (table 1). The findings show that the mean recovery rate for drinking water (20-1146 litres) was always more than 80%, except for the recovery rate for somatic phages (65%) and for *Campylobacter* (52%). The mean recovery rate in water from the treatment plant (600 litres) was at the least 74%. This water type showed a strikingly higher mean recovery rate for somatic phages (93%) and for *Campylobacter* (96%) than was seen in drinking water, although the standard deviation for *Campylobacter* was very high. This high standard deviation was the result of one extremely high observation (142%). If this observation is left out in the calculation, then the recovery rate is 43% with a standard deviation of 11%. The mean recovery rate in the surface water samples (50 litres) was comparable to those in drinking water and in drinking water from the treatment plant. The standard deviations of the findings vary between 9% and 30% (excl. of *Campylobacter*: up to 66%).

Table 1: Mean recovery rate (in % ± SD) for various micro-organisms in different water types, determined directly in the Hemoflow-concentrate. Figures in brackets show the numbers of experiments.

Organism	Drinking water (20-1146 l)	Water from purification plant (600 l)	Surface water (50 l)
E.coli	81 ± 33 (3)	93 ± 8 (3)	93 ± 22 (3)
Enterococci	82 ± 25 (3)	74 ± 23 (3)	81 ± 9 (3)
Clostridium	90 ± 24 (3)	89 ± 18 (3)	91 ± 22 (3)
F-specific phages	82 ± 25 (8)	115 ± 7 (3)	79 ± 21 (3)
Somatic phages	65 ± 28 (3)	93 ± 21 (3)	111 ± 13 (3)
Campylobacter	52 ± 9 (3)	96 ± 66 (3)	35 ± 7 (3)
Cryptosporidium	87 ± 14 (8)	101 ± 29 (3)	67 ± 29 (7)
Giardia	93 ± 14 (8)	85 ± 13 (3)	86 ± 13 (7)

2.2.2 *phase 2: Recovery rate with Hemoflow combined with post-concentration of the concentrate*

For each experiment, the recovery rate of the various micro-organisms per water type was calculated (appendix 2). From these results, the mean

recovery rate for both water types (drinking water and water from purification plant) was calculated (table 2).

Table 2: Mean recovery rate (in % + SD) with Hemoflow followed by post-concentration through centrifugation and through Centricon®-concentration for various micro-organisms.

Organism	Centricon®-concentrate	pellet
E.coli	21 ± 1	78 ± 36
Enterococci	26 ± 23	50 ± 10
Clostridium	38 ± 1	113 ± 19
F-specific phages	60 ± 27	-
Somatic phages	74 ± 31	-
Campylobacter	70	3,5
Cryptosporidium	25 ± 30	84 ± 11
Giardia	42 ± 35	68 ± 8

-; not conducted

The results show that the recovery rate after centrifugation in every case produces the highest mean recovery rate of all bacteria, except for *Campylobacter*. This result was based on one measurement only.
In each case, the result of the mean recovery rate after post-concentration was lower than the recovery rate measured directly in the concentrate before post-concentration (table 1). The differences are only slight. After post-concentration by centrifugation, where the entire pellet was examined, a higher recovery was found for bacteria (except for *Campylobacter*) and for the protozoa than after post-concentration through Centricon®-concentration. Possibly, this is the result of damage of organisms when they are pressed against the membrane.

2.3 Conclusions

From this study, the following conclusions can be drawn:

- The recovery rate with Hemoflow-concentration is high and sufficiently reproducible for all organisms (except *Campylobacter*) and in all water types,
- The recovery rate of *Cryptosporidium* and *Giardia* in the concentrate of the Hemoflow is considerably higher and better reproducible than with the Envirochek-filter,
- Centricon®-concentration of the Hemoflow-concentrate produced high recovery rates of F-specific coliphages and somatic phages,
- The examinable volume for the detection of F-specific coliphages and somatic phages is enlarged by the use of Hemoflow from 10 ml to 100-1000 litres,
- Post-concentration through centrifugation of bacteria and of *Cryptosporidium* and *Giardia* followed by examination of the pellet, led to higher recovery rates than with Centricon®-concentration,
- Recovery rates show a standard deviation of some dozens of percents.

3 The influence of increased sample volume

3.1 Design

Phase 1 showed that concentration of samples with the Hemoflow-filter and post-concentration through centrifugation or Centricon®-concentration produces satisfactory and reproducible recovery rates. It was now necessary to determine the maximum sample volume at which the recovery rate is still acceptable.
In general, concentration of large volumes of surface water is not very useful in view of the expected concentrations of micro-organisms to be examined. Therefore, the experiments were carried out with drinking water.

In the experiments described in chapter 2, large sample tanks (approx. 600 l) were used. In this study, a small barrel (approx. 20 l) was used. As soon as the volume in this barrel had descended under a certain level, the barrel was topped up with fresh sample water by use of a floater. The advantage of this method is that the concentration process can take place unsupervised (e.g. overnight).

At first, the experiments were conducted with MS2-phages. In a full barrel (filled with drinking water from *Nieuwegein,* drinking water plant *Tull en 't Waal*, 61 °F), 5 * 10^4 MS2-phages were dosed. The barrel was connected to the water supply and disconnected when the desired sample volume was attained. Sample volumes of 50, 100, 500, 1000, 1500, 2000, 3000, and 7500 litres were examined. The concentration of a volume of 2000 litres took approx. 28 hours.
The filtrate was used to rinse out the barrel. Post-concentration was carried out through Centricon®-concentration.

After the maximum possible volume to be concentrated for MS2-phages was determined, the same procedure was followed for other organisms. These organisms were solely studied at the volume at which formerly, for the MS2-phages, recovery had not drastically decreased. These organisms were inoculated simultaneously in the barrel. A sample volume of 2200 litres was concentrated. Phages were post-concentrated through Centricon®-concentration. *Cryptosporidium* and *Giardia* were post-concentrated through centrifugation and IMS. All the other organisms were post-concentrated through centrifugation (10 min, 900 g) and then the pellet was spread on a specific culture medium. *Campylobacter* was not included in these experiments.

3.2 Results and discussion

The results showed that the recovery rate of MS2-phages remained constant up to sample volumes of approx. 2000 litres (table 3). At sample volumes of

approx. 3000 litres, the recovery rate had declined to around 10% and at a sample volume of 7500 litres it was less than 1%.

Table 3: Recovery rates of MS2-phages at different sample volumes after concentration with Hemoflow and Centricon®.

Volume (litres)	recovery (%)
20	96 ±17
50	119
100	106
500	83
881	99
1,500	100
1,907	100
3,161	13
7,549	0.2

The recovery rates of the other organisms, attained after concentration of 2200 litres, were similar (table 4) to the results attained at smaller volumes (table 2).

Table 4: Recovery rates of various micro-organisms after concentration of 2,200 litres of drinking water to which these organisms had been added.

Organism	recovery (%)
E.coli	70
Enterococci	61
Clostridium	72
F-specific phages	86
Somatic phages	65
Cryptosporidium	92
Giardia	79

3.3 Conclusions

From the study on increased sample volume, the following conclusions can be drawn:

- Increase of the sample volume to approx. 2000 litres bears little consequence for the recovery rates of most micro-organisms examined,
- Increase of the sample volume to 3000 litres or more leads to a recovery rate for MS2-phages of 10%.

4 Conclusions and recommendations

4.1 Conclusions

The findings of the study are:

- The concentration of maximum volumes of 2000 litres produces high recovery rates ($\geq$ 65%) for all organisms except *Campylobacter*,
- The results of the several experiments have a standard deviation between 7-33%,
- The efficiency of the detection of *Cryptosporidium* and *Giardia* is much higher and more reproducible than with the existing Envirochek concentration method,
- Concentrates obtained with the Hemoflow-installation can be post-concentrated without a significant reduction of the recovery rate,
- Post-concentration of phages must take place by centrifugation with Centricon®,
- Post-concentration of bacteria must take place by centrifugation (5-10 min, 900 g) with complete examination of the pellet,
- For the assessment of F-specific and somatic phages, the examinable volume has been increased from 10 ml to 2000 litres,
- The Hemoflow concentration method makes it possible to simultaneously concentrate the organisms that are to be examined.

4.2 Recommendations

Recommendations are:

- For every unknown water type to produce at least one recovery rate experiment for one of the micro-organisms, since there have been water types shown to produce a smaller recovery rate than usual,
- To use the Hemoflow-method in assessment programs since this method comprises a profound improvement relative to the concentration methods currently used.

5 Literature

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I Results of the recovery experiments of the concentrate

Drinking water *Nieuwegein*
Volume: 20 litres

Parameter: MS2

Experiment date	inoculation concentration	concentrate	rinse out	recovery
02-17-04	$2.44*10^7$	$2.42*10^7$	$2.28*10^6$	109 %
05-13-04	$1.85*10^7$	$1.12*10^7$	$2.60*10^6$	71 %
05-13-04	$1.13*10^7$	$8.37*10^6$	$4.06*10^6$	110 %
05-27-04	$1.95*10^7$	$1.13*10^7$	$5.64*10^6$	88 %
05-27-04	$1.18*10^7$	$1.04*10^7$	$1.51*10^6$	103 %

Parameter: *Cryptosporidium*

Experiment date	inoculation concentration	concentrate	rinse out	recovery
09-21-04	$8.58*10^4$	$5.22*10^4$	$9.97*10^3$	72%
09-21-04	$2.98*10^5$	$1.37*10^5$	$4.94*10^3$	62 %
09-21-04	$1.83*10^5$	$9.92*10^4$	6.79*104	91 %
09-28-04	$2.84*10^5$	$2.07*10^5$	$7.99*10^4$	101 %
09-28-04	$6.28*10^5$	$4.37*10^5$	$1.43*10^5$	98 %

Parameter: *Giardia*

Experiment date	inoculation concentration	concentrate	rinse out	recovery
09-21-04	$1.03*10^5$	$8.46*10^4$	$8.53*10^3$	90%
09-21-04	$1.06*10^5$	$8.15*10^4$	$1.02*10^4$	86%
09-21-04	$9.17*10^4$	$6.26*10^4$	$2.51*10^4$	96 %
09-28-04	$6.78*10^4$	$5.54*10^4$	$2.07*10^4$	112 %
09-28-04	$1.11*10^5$	$8.51*10^4$	$2.80*10^4$	102 %

Experiments were carried out in drinking water *Nieuwegein*
In experiment 2 and 3 the barrel with floater was used.

Experiment 1, volume 600 litres

Strain	inoculation	concentrate	rinse out	recovery
E. coli	$8.93*10^3$	$1.06*10^4$	-	119 %
Streptococci	$2.70*10^4$	$1.77*10^4$	$9.49*10^3$	100 %
Clostridia	$3.97*10^4$	$3.29*10^4$	$6.30*10^3$	99 %
Bacteriophages	$3.70*10^7$	$1*84*10^7$	$9.49*10^6$	75 %
Somatic phages	$2.70*10^6$	$2.09*10^6$	$5.31*10^5$	97 %
Campylobacter	$2.00*10^9$	$7.95*10^8$	$9.9*10^7$	45 %
Cryptosporidium	$7.38*10^5$	$5.54*10^5$	$3.13*10^4$	79 %
Giardia	$1.50*10^5$	$9.24*10^4$	$3.13*10^4$	73 %

Experiment 2, volume 1004 litres

Strain	inoculation	concentrate	rinse out	recovery
E. coli	$9.90*10^4$	$2.04*10^4$	$3.78*10^4$	59 %
Streptococci	$4.80*10^6$	$8.44*10^5$	$3.21*10^6$	93 %
Clostridia	$3.24*10^8$	$1.41*10^8$	$6.14*10^7$	63 %
Bacteriophages	$6.03*10^7$	$2.34*10^6$	$2.74*10^7$	49 %
Somatic phages	$1.26*10^9$	$3.91*10^8$	$2.69*10^8$	52 %
Cryptosporidium	$1.35*10^4$	$6.46*10^3$	$6.61*10^3$	97 %
Giardia	$7.56*10^3$	$3.06*10^3$	$2.83*10^3$	78 %

Experiment 3, volume 1146 litres

Strain	inoculation	concentrate	rinse out	recovery
E. coli	$4.30*10^6$	$1.27*10^6$	$1.58*10^6$	66 %
Streptococci	$6.53*10^5$	$1.07*10^5$	$2.41*10^5$	53 %
Clostridia	$2.20*10^7$	$1.77*10^7$	$6.18*10^6$	109 %
Bacteriophages	$3.90*10^9$	$7.12*10^8$	$1.25*10^9$	50 %
Somatic phages	$1.38*10^9$	$3.85*10^8$	$2.70*10^8$	47 %
Campylobacter	$2.0*10^5$	$1.16*10^5$	-	58 %
Cryptosporidium	$3.0*10^3$	$1.54*10^3$	$1.28*10^3$	94 %
Giardia	$4.1*10^3$	$2.31*10^3$	$2.20*10^3$	110 %

Experiment 1, 600 litres
WRK Nieuwegein, product water

strain	inoculation	concentrate	rinse out	recovery rate
E. coli	$6.47*10^5$	$3.60*10^5$	$1.90*10^5$	85 %
Streptococci	$7.83*10^6$	$3.28*10^6$	$1.36*10^6$	59 %
Clostridia	$4.80*10^7$	$1.22*10^7$	$2.03*10^7$	68 %
Bacteriophages	$2.42*10^7$	$2.08*10^7$	$8.70*10^6$	122 %
Somatic phages	$7.4*10^6$	$3.93*10^6$	$1.45*10^6$	73 %
Campylobacter	$9.00*10^7$	$3.93*10^7$	$4.84*10^6$	49 %
Cryptosporidium	$1.13*10^5$	$8.74*10^4$	$6.29*10^4$	133 %
Giardia	$7.92*10^4$	$4.27*10^4$	$2.42*10^4$	84 %

Experiment 2, 600 litres
Leiduin, before Ozone

strain	inoculation	Concentrate + rinse out	recovery rate
E. coli	$5.0*10^6$	$4.58*10^6$	92 %
Streptococci	$7.5*10^5$	$4.76*10^5$	63 %
Clostridia	$1.28*10^7$	$1.21*10^7$	95 %
Bacteriophages	$1.29*10^{10}$	$1.48*10^{10}$	115 %
Somatic phages	$2.4*10^9$	$2.75*10^9$	115%
Cryptosporidium	$7.36*10^4$	$6.74*10^4$	92 %
Giardia	$5.16*10^4$	$5.04*10^4$	98 %

Experiment 3, 600 litre
Leiduin, after Ozone

strain	inoculation	Concentrate + rinse out	recovery rate
E. coli	$5.64*10^5$	$5.71*10^5$	101 %
Streptococci	$5.93*10^5$	$5.90*10^5$	100 %
Clostridia	$3.20*10^8$	$3*29*10^8$	103 %
Bacteriophages	$2.16*10^{10}$	$2.35*10^{10}$	109 %
Somatic phages	$5.0*10^9$	$4.61*10^9$	92 %
Campylobacter	$4.60*10^6$	$6.53*10^6$	142 %
Cryptosporidium	$7.11*10^4$	$5.52*10^4$	78 %
Giardia	$1.76*10^4$	$1.28*10^4$	73 %

Surface water
Lek-canal. Collection point WRK.
Experiment 1, 50 litres

strain	inoculation	concentrate	rinse out	recovery rate
E. coli	$2.49*10^5$	$1.35*10^5$	$3.60*10^4$	69 %
Streptococci	$6.0*10^3$	$2.95*10^3$	$2.07*10^3$	84 %
Clostridia	$8.50*10^4$	$3.74*10^4$	$2.16*10^4$	69 %
Bacteriophages	$2.17*10^7$	$1.03*10^7$	$5.4*10^6$	75 %
Somatic phages	$2.04*10^6$	$1.22*10^6$	$1.08*10^6$	113 %
Campylobacter	$5.5*10^8$	$1.71*10^8$	270	31 %
Cryptosporidium	$4.40*10^5$	$2.10*10^5$	$2.00*10^5$	93 %
Giardia	$8.5*10^4$	$5.64*10^4$	$2.34*10^4$	94 %

Experiment 2, 50 litres

strain	inoculation	concentrate	rinse out	recovery rate
E. coli	$4*26*10^6$	$6.71*10^5$	$4.72*10^6$	127 %
Streptococci	$5.1*10^4$	$3.56*10^4$	$8.29*10^3$	71 %
Clostridia	$2.15*10^6$	$2.60*10^6$	$5.16*10^6$	92 %
Bacteriophages	$1.35*10^5$	$7.53*10^4$	$6.16*10^3$	60 %
Somatic phages	$4.3*10^5$	$1.09*10^5$	$3.08*10^5$	97 %
Campylobacter	$1.11*10^9$	$2.74*10^8$	$2.02*10^8$	43 %
Cryptosporidium	$3.58*10^4$	$8.22*10^3$	$1.74*10^4$	72 %
Giardia	$1.46*10^4$	$1.15*10^4$	$1.93*10^3$	92 %

Experiment 3, 50 litres Date 11-26-04

strain	inoculation	concentrate	rinse out	recovery rate
E. coli	$4.26*10^6$	$6.64*10^5$	$3.91*10^6$	107 %
Streptococci	$5.10*10^4$	$1.37*10^4$	$3.16*10^4$	89 %
Clostridia	$6.00*10^6$	$4.21*10^6$	$2.46*10^6$	112 %
Bacteriophages	$1.35*10^5$	$1.23*10^5$	$1.42*10^4$	102 %
Somatic phages	$4.30*10^5$	$3.61*10^5$	$1.67*10^5$	123 %
Campylobacter	$1.11*10^9$	$2.64*10^8$	$8.52*10^7$	31 %
Cryptosporidium	$3.58*10^4$ #	$1.41*10^3$	$1.14*10^4$	35 %
Giardia	$1.46*10^4$	$2.82*10^3$	$7.58*10^3$	71 %

The direct sample was counted again threefold on December 15. The separate values were $4.14*10^4$, $3.09*10^4$, and $3.51*10^4$. The mean value of these was used for calculation of the recovery rate.
Study of recovery rate in different water types.

sample	studied volume (l)	Recovery rate (%)	
		Giardia	*Cryptosporidium*
Lek-canal	42.1	76	68
PLV SB 001 (*Leiduin*)	58.4	14	45
Lateral canal	39	46	42
Maas	38	36	42

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II Results of the recovery experiments with the residue

Drinking water *Nieuwegein*
Concentrate after Hemoflow post-concentrated with centricon tubes

Experiment 1, 1004 litres

strain	inoculation	concentrate	rinse out	recovery rate
E. coli	$9.90*10^4$	586	$1.92*10^4$	20 %
Streptococci	$4.80*10^6$	$1.32*10^6$	$5.37*10^5$	42 %
Clostridia	$3.24*10^8$	$9.01*10^7$	$3.64*10^7$	39 %
Bacteriophages	$6.03*10^7$	$1.76*10^6$	$2.55*10^7$	48 %
Somatic phages	$1.26*10^9$	$3*93*10^7$	$3.60*10^8$	32 %
Cryptosporidium	$1.35*10^4$	455	68	3.9 %
Giardia	$7.56*10^3$	718	544	17 %

Experiment 2, 1146 litres

strain	inoculation	concentrate	rinse out	recovery rate
E. coli	$4.30*10^6$	$5.07*10^5$	$4.14*10^5$	21 %
Streptococci	$6.53*10^5$	$3.08*10^4$	$2.92*10^4$	9.2 %
Clostridia	$2.20*10^7$	$3.72*10^6$	$4.31*10^6$	37 %
Bacteriophages	$3.90*10^9$	$5.96*10^8$	$1.72*10^9$	59 %
Somatic phages	$1.38*10^9$	$2.34*10^8$	$1.03*10^9$	92 %
Campylobacter	$2.0*10^5$	$1.40*10^5$	132	70 %
Cryptosporidium	$3.0*10^3$	504	880	46 %
Giardia	$4.1*10^3$	1400	1320	66 %

Water from treatment plant

Part of the concentrate, for the parameters bacteriophages en somatic phages, was centrifuged with the centricon tubes. The rest of the sample was centrifuged at 4000 rpm and the pellet was processed for the other parameters.

Experiment 1, 600 litres
Water from *Leiduin* before Ozone

strain	inoculation	Concentrate + rinse out	recovery rate
E. coli	$5.0*10^{6}$	$2.59*10^{6}$	52 %
Streptococci	$7.5*10^{5}$	$3.24*10^{5}$	43 %
Clostridia	$1.28*10^{7}$	$1.61*10^{7}$	126 %
Bacteriophages	$1.29*10^{10}$	$4.36*10^{9}$	34 %
Somatic phages	$2.4*10^{9}$	$2.43*10^{9}$	101 %
Cryptosporidium	$7.36*10^{4}$	$6.74*10^{4}$	92 %
Giardia	$5.16*10^{4}$	$3.20*10^{4}$	62 %

Experiment 3, 600 litres
Water from *Leiduin* after Ozone

strain	inoculation	Concentrate + rinse out	recovery rate
E. coli	$5.64*10^{5}$	$5.87*10^{5}$	103 %
Streptococci	$5.93*10^{5}$	$3.38*10^{5}$	57 %
Clostridia	$3.20*10^{8}$	$3.27*10^{8}$	99 %
Bacteriophages	$2.16*10^{10}$	$2.11*10^{10}$	98 %
Somatic phages	$5.0*10^{9}$	$3.61*10^{9}$	72 %
Campylobacter	$4.60*10^{6}$	$1.63*10^{5}$	3.5 %
Cryptosporidium	$7.11*10^{4}$	$5.39*10^{4}$	76 %
Giardia	$1.76*10^{4}$	$1.28*10^{4}$	73 %

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III Results influence of increased sample volume

Determination of maximum volume at which the recovery rate of the MS2-phages was still 90% or more.

Volume	inoculation	Concentrate + rinse out	recovery rate
50 litres	$4.95*10^4$	$5.89*10^4$	119 %
100 litres	$4.95*10^4$	$5.24*10^4$	106 %
500 litres	$4.95*10^4$	$4.10*10^4$	83 %
881 litres	$4.95*10^4$	$4.90*10^4$	99 %
1500 litres	$2.76*10^5$	$2.75*10^5$	100 %
1907 litres	$2.76*10^5$	$2.75*10^5$	100 %
3161 litres	$3.50*10^5$	$4.20*10^4$	13 %
7549 litres	$3.50*10^5$	801	0.2 %

Determination of the recovery rate of various micro-organisms in the concentrate of 2200 litre

Strain	inoculation	Concentrate + rinse out	recovery rate
E. coli	$1.40*10^4$	$9.85*10^3$	70 %
Streptococci	$1.25*10^4$	$7.67*10^3$	61 %
Clostridia	$1.30*10^4$	$9.32*10^3$	72 %
Bacteriophages	$2.10*10^5$	$1.80*10^5$	86 %
Somatic phages	$2.2*10^3$	$1.43*10^3$	65 %
Campylobacter	$1.10*10^9$	$> 8.27*10^5$	-
Cryptosporidium	$2.80*10^4$	$2.59*10^4$	92 %
Giardia	$3.40*10^4$	$2.68*10^4$	79 %