Molecular methods for pathogen detection in drinking water treatment

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Molecular methods for pathogen detection in drinking water treatment

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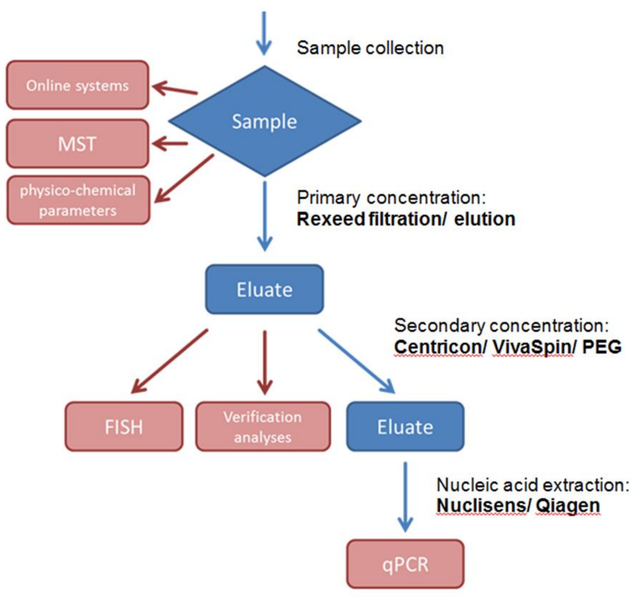
Motivation

Within the EU-project Aquavalens new molecular methods for a fast detection of microbiological risks have been tested. These methods for pathogen and indicator detection were tested in large drinking water systems under the conditions of routine laboratories. A monitoring program over 13 months was performed in 4 large water systems in Great Britain, Germany, Denmark and Spain, that included monitoring of the pathogenic microorganisms Norovirus, Campylobacter, Giardia and Cryptosporidium and the indicators *E. coli* and coliform bacteria.

Methods

Outline



For the detection of the parameters the newly developed molecular methods (qPCR, FISH) were compared to the classical cultural methods. The monitoring included monthly sampling and analyses of different water types in different treatment steps (raw water, process water, treated water).
For the detection, large volumes of 100 l to 1000 l of the different water types were concentrated. As primary concentration step, a dead-end-ultrafiltration-system (Rexeed® filters) was used, followed by a secondary concentration by centrifugation and nucleic acid extraction.

Investigated parameters and pathogens

PCR

- **Viruses**
  - Ceeram kits: **Norovirus GI, GII** and Hepatitis A virus
- **Bacteria**
  - GPS kits: ***E. coli, Campylobacter spp, C. jejuni***, *P. aeruginosa, Salmonella spp, L. pneumophila*
- **Protozoa**
  - Ceeram kits: ***Giardia spp, Cryptosporidium spp***
  - GPS kits: ***G. intestinalis, Cryptosporidium spp***, *T. gondii*

FISH and microscopy techniques

- **Total cell count (DAPI staining)**
- **Viable cell count (FISH)**
- ***E. coli* (FISH)**
- **thermophilic Campylobacter (FISH)**

Verification analyses

- ***E. coli***
- **Coliform bacteria**
- *Campylobacter spp*
- *C. perfringens*
- *Somatic coliphages*
- *Enterococci*
- *P.aeruginosa*
- *L.pneumophila*
- ***Giardia spp***
- ***Cryptosporidium spp***

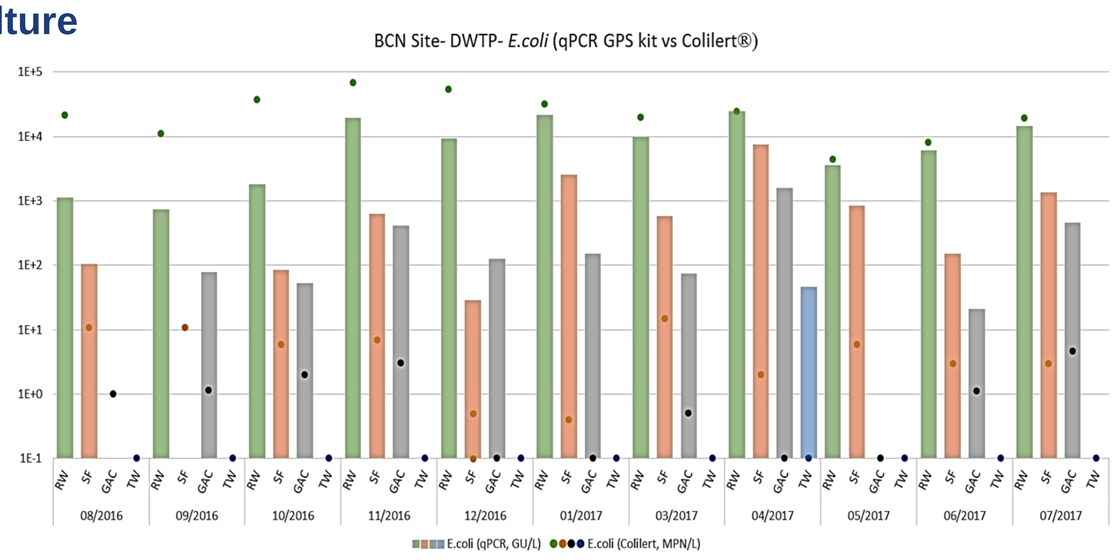
Results

The concentration steps showed to be very effective in decreasing the detection limits.
For qPCR-methods, such a concentration step is necessary for clean waters (treated water) in order to decrease the detection limit.
The qPCR-methods were sensitive to inhibitory effects, especially in the raw waters. Application of quantitative FISH-methods was also problematic in raw waters, as the enriched particles disturbed the microscopic evaluation.
In contrast, the use of the new methods was not disturbed by these effects in treated water samples.

E. coli: detection by qPCR and culture

Concentrate after secondary concentration (from left to right: RW, FS1, AKF1, DN)

Increased levels of particles:
Inhibition in qPCR-detection.



Raw water: Culture > qPCR, treated water: Culture < qPCR

		Aquavalens method	
Number of comparisons	213	Positive	Negative
Verification method	Positive	33%	6%
	Negative	8%	52%

Good correlation of positive/negative results between qPCR and culture method

Conclusions

1st concentration step

- Rexeed™ filtration has proved to be a **good tool** in terms of recovery and concentration **for viruses and bacteria in process and drinking water with large water volumes**.
- Concentrating with Rexeed™ is **not useful for very loaded** water sources for bacteria (*i.e.* Spanish site raw water). In addition, **humic substances** in water sources may cause excessive **foam formation during elution**.
- Concentrating with Rexeed™ has **enabled verification analyses, such as Colilert®, to be more sensitive**, thus lowering the detection limits significantly.
- Rexeed™ did not present **any clogging problems** in any of the sampling campaign, even when filtering 1000 L (drinking water)

Use of the new detection methods from Aquavalens combined with concentration steps:

- **Very effective in special cases for trouble shooting.**
- **Not yet possible to replace classical methods for routine monitoring in drinking water: Not robust enough, not standardized yet.**
- **Concentration step combined with cultural detection: Higher sensitivity can be reached.**

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