Aquavalens Project

"Protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation."

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Deliverable D6.4

"An evaluation of the performance and limitations of microfluidic systems to perform sample separation in the analysis of waterborne pathogens"

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Executive Summary

In WP6 Task 6.4 the **potential of microfluidics to perform sample separation in the analysis of waterborne pathogens has been evaluated**. The work package has delivered a **review paper** published in Water Research **overviewing the state of the art in the literature** with regard to the use of microfluidics in waterborne pathogen monitoring. Building on this literature review and the gaps identified a series of different **experimental projects** have been undertaken, resulting in a **further four publications in high impact factor journals and two conference papers**.

In summary, a **high-throughput method for the concentration and sorting of waterborne pathogens has been demonstrated**. This builds on a passive method of microfluidic operation in which spiral channel geometries and appropriate flow rate selection controls the behaviour of differently sized particles within the channel such that they adopt particular locations perpendicular to the channel flow direction. Therefore, particles (or pathogens) can be collected into different outlets, the location of which can be designed to correspond to the appropriate channel locations. **High recovery rates of pathogens (up to 95%)** have been demonstrated and the systems have also been **run successfully using tap water**. By stacking together several of these systems (a 10 layer stack was produced by Epigem) **50mL of sample was concentrated in approximately 10 mins** with performance comparable to the single layer system. This demonstrates that microfluidics offers the **potential to replace centrifugation** since sufficient volumes can be processed in a reasonable time frame.

Another component of the WP was **targeting species and viability based separations** using microfluidic devices, with active forces incorporated. In this case microfluidic systems were manufactured (again by Epigem) with electrodes included to generate electric fields inside the flow channel. By appropriate choice of electric field **species based and viability based separations can be successfully performed** (e.g. **95% of viable oocysts** extracted to one channel outlet).

The evaluation of the potential of microfluidics to perform sample processing of waterborne pathogens concludes that there are **significant opportunities for microfluidics to add value to traditional methods of waterborne pathogen processing**. Increased sample throughput has been demonstrated showing that microfluidics has the **potential to replace centrifugation**, which is particularly advantageous as centrifugation is challenging to automate whereas integration and automation of microfluidic systems is relatively easier to undertake. Additionally, by incorporating electric fields into microfluidics highly **successful species and viability based sorting** is possible thus enabling **improved characterisation** of identified pathogens.

Remaining challenges include creating **easy-to-use automated systems** capable of use by relatively inexperienced (in terms of microfluidics) end-users such as water utilities. Further demonstration and **performance validation with a wider range of pathogens and a variety of source waters** would also strengthen the evidence for wider adoption of this type of system.

Summary of task and outputs

Task 6.4. Microfluidic techniques for the separation of pathogens in filter concentrates (UEDIN, Epigem, DTU-FOOD, UB, NFA, GPS, Ceeram, Moredun Scientific)

This task will design and manufacture a variety of different microfluidic devices to deal with the selected pathogens (virus, bacteria, protozoa). The first set of systems will rely on hydrodynamic forces to achieve size based separation, further concentration and/or inhibitor removal. The second set of systems will incorporate active mechanisms, e.g. electric fields, with the aim of delivering species and viability based separation. Design will be aided by computer simulation of the fluid flow in the above devices to predict performance. The microfluidic systems will be characterized with fluorescent beads, via fluorescent microscopy imaging of flow pathways through the device, and the selected pathogens, using flow cytometry to determine recovery rates of spiked samples. PCR kits will also be used to assess recovery rates and inhibitor removal. The advantage of this approach is a simple one-step process enabling sample separation without the need for a variety of reagents. Tasks 1-3 will provide filter processed samples (size 10 ml volumes; containing either viruses or bacteria and protozoa) for microfluidic testing.

(Further discussions with Karin Jacobson in WP6 suggested that replacing the centrifugation step with microfluidics would be a useful addition to the detection protocol – requirements are thus processing 500mL of sample within 2hrs at a cost of less than €5000 – equipment cost included)

Outputs

1. Chapter 10 in Waterborne Pathogens: Detection Methods and Applications published by Elsevier (https://www.elsevier.com/books/waterborne-pathogens/bridle/978-0-444-59543-0; http://www.sciencedirect.com/science/article/pii/B9780444595430000104)
2. Review paper in Water Research - Application of microfluidics in waterborne pathogen monitoring: A review (http://www.sciencedirect.com/science/article/pii/S0043135414001018)
3. Review paper in Lab on a Chip – Deterministic lateral displacement: a review (http://pubs.rsc.org/en/content/articlelanding/2014/lc/c4lc00939h#!divAbstract)
4. Conference paper at Microfluidics 2014 – How could microfluidics be applied for waterborne pathogen detection?
5. Journal article in Chemical Engineering Science – Efficient separation of small micro particles at high flowrates using spiral channels: application to waterborne pathogens (http://www.sciencedirect.com/science/article/pii/S0009250915006089)
6. Submitted journal article to Scientific Reports – Concentration of small microparticles in stacked spiral channels: understanding the challenges using particle tracking
7. Conference paper at Microfluidics 2014 – Continuous sorting and concentration of viable and non-viable *Cryptosporidium parvum* oocysts using dielectrophoresis.
8. Outreach activities – Fountainbridge Canal Festival 2013, Chemistry Conversations 2013, Bang goes the Borders 2014, European Researchers' Night 2014, HWU Interactive Workshop competition 2014 (Runner's Up Prize, voted the most fun activity by the participating children), Edinburgh Doors Open Day 2015
9. Journal article in Lab on a Chip summarising outreach activities - Angry pathogens, how to get rid of them: introducing microfluidics for waterborne pathogen separation to children

(http://pubs.rsc.org/en/Content/ArticleLanding/2015/LC/C4LC00944D#!divAbstract) which was also selected to feature in their blog
Overview of Considerations when Utilising Microfluidics in Waterborne Pathogen Monitoring

For a review of existing microfluidic approaches to waterborne pathogen monitoring see outputs 1 and 2 (Appendix 1 for output 2, the review paper).

This section summarises the considerations and design choices made for the work within this task:

LARGE SCALE FILTRATION/CENTRIFUGATION REPLACEMENT

Two options can be considered when thinking about large scale microfluidic processing of water samples for monitoring purposes.

1) entirely replace filtration with microfluidics (processing 1000L in 24 hours e.g. to replace the *Cryptosporidium* protocol)
2) replace the centrifugation step after an initial off-chip filtration (processing 500mL in 2 hours)

Both are highly challenging for microfluidics and represent a large step up in volumes processed. Microfluidics systems typically operate at low flow rates (µL/hour –mL/hour) and therefore would struggle to process millilitres to litres of sample within a reasonable time frame (hours). Parallelisation of devices offers one approach to increase throughput but the limit of processing within one system depends upon how many devices can feasibly be stacked. However, even for stacked/parallelised systems it is ideal that a single device should operate in at least the mL/hour range (e.g. 1mL/hr would require 1736 devices to process 1000L over 24 hours or 250 to process 500mL in 2 hours) and preferably considerably higher.

Another consideration is that it is best to try to use systems which do not involve any other flows, e.g. focussing channels which act to the dilute the original sample. The aim is to concentrate the sample so diluting buffer flows would increase the volume to be processed further and additionally any other flow would need to be pathogen and inhibitor free which might difficult to implement for certain applications, e.g. out at treatment works.

High recovery rates are essential as pathogens are present at very low concentrations so techniques offering high recoveries are to be preferred.

The size range of pathogens is from up to 10µm for the protozoa, around 1µm for the bacteria and down to 20nm for the viruses: to entirely replace filtration then all pathogens need to be concentrated; however, the existing centrifugation steps leaves viruses in the supernatant so in this case one could utilise a design which separates and then concentrates the bacteria and protozoa together and the viruses independently).

Additionally, it is considered best to avoid the use of any “filtration” elements within the device, e.g. weirs, capture wells etc as there is a risk of clogging. Additionally, if these types of element are used

in a device to concentrate then subsequent wash and elution steps will also be required increasing complexity.

Particulates within finished drinking water samples are up to 300µm so therefore channel dimensions should possibly not be smaller than this to avoid clogging for processing of the largest sample volumes (note: this work targets 500mL as a maximum volume so some pre-processing would be performed which negates this as a strict requirement). Processing of raw water samples is likely to be even more challenging as a higher number of particles of potentially larger sizes could be present.

Following these considerations two passive hydrodynamic techniques were selected to be investigated: deterministic lateral displacement and inertial focussing. The initial task required processing of 10mL samples though this was extended to consider the possibility of replacing centrifugation. This part of the work addressed this segment of the task: "The first set of systems will rely on hydrodynamic forces to achieve size based separation, further concentration and/or inhibitor removal."

DOWNSTREAM PROCESSING – TARGETING SEPARATIONS

When using molecular methods of detection there are four main challenges:

- Removal of interferents which reduce the efficiency of the molecular method
- Separation of pathogens into viruses, bacteria and protozoa as optimal lysis methods differ between the kingdoms
- Determining viability
- Efficient lysis protocols, especially for protozoa

Detection using molecular methods could be performed on microfluidic chips but this is outwith the scope of WP6.

Microfluidics could address the 4 main challenges via:

- Creation of systems to shift pathogens into a clean flow for subsequent molecular detection
- Size based separation systems to sort into kingdoms such that optimal lysis techniques can be applied to each kingdom
- Use of active forces integrated on-chip to sort pathogens by viability before detection and speciation
- On-chip lysis systems to improve lysis performance and avoid/reduce reagent use

Following discussion with WP6 partners, the focus was selected to be on size based separation systems to sort into different kingdoms and the use of electrical forces with the aim of sorting pathogens based on species and viability. This part of the work addressed this segment of the task:

"The second set of systems will incorporate active mechanisms, e.g. electric fields, with the aim of delivering species and viability based separation."

Performance of Microfluidic Systems for Waterborne Pathogen Monitoring

LARGE SCALE FILTRATION/CENTRIFUGATION REPLACEMENT

Deterministic lateral displacement (DLD) devices were designed and manufactured. Testing with pathogens proved problematic due to issues relating to clogging, bubble formation and leaking. The flow rates achieved were also very low. For more information about the DLD technique please see output 3 (Appendix 2).

Therefore, the decision was made to concentrate on inertial focussing (IF) systems. A device was designed and manufactured by Epigem. Testing of this device with fluorescent beads as well as protozoan pathogens has been undertaken. Initial tests with bacteria indicated that not all bacteria were able to be focussed and concentrated within this set-up and a smaller scale device targeting this size range has been manufactured for future testing. Viruses could potentially be concentrated in this set-up by the use of capture agents to increase their size or perhaps via incorporation of the mesh material Epigem have developed.

The results have been presented at Microfluidics 2014 in Limerick in Ireland (showing both DLD and IF data) and at in New York in 2015 (concentrating on a detailed characterisation of IF performance). The latter presentation was judged and a peer-reviewed paper has been published in Chemical Engineering Science. Please see outputs 4 and 5 (Appendices 3 and 4) for details. A poster was also shown at the Microfluidics Congress in London in 2015.

Finally a scaled-up system incorporating a stack of 10 devices was produced by Epigem; this system was tested with fluorescent beads and shown to perform as well as a single layer and was capable of concentrating a 50mL sample in approximately 10mins. A journal article summarising these results has been submitted to Scientific Reports – for the submitted version please see output 6 (Appendix 5).

This work has demonstrated that microfluidics can process sufficient volumes in a reasonable time frame to target the replacement of centrifugation. High recovery rates of protozoan pathogens (up to 95%) have also been demonstrated. The systems have been successfully run with drinking water samples, and eluates from previous filtration stages, without clogging further demonstrating the feasibility of the approach.

The remaining challenges with this IF approach are minimising the dead volume and creating an automated system capable of recirculation and concentration of the water sample in a manner that is easy to use for unskilled operators. This is outwith the scope of this task and further funding is being sought.

DOWNSTREAM PROCESSING – TARGETING SEPARATIONS

Devices were designed utilising electric forces, specifically dielectrophoresis, for species and viability based separation. The devices were manufactured by Epigem and tested with protozoan pathogens. The results were summarised in a conference paper at Microfluidics 2014 (output 7; Appendix 6) attracting commercial interest from Dow. Successful viability separation was demonstrated and subsequent processing of the data confirmed highly efficient separation – 93% of the viable oocyst sample was displaced and thus separated (Figure 1). The sample utilised contained around 7% non-viable oocysts (as confirmed by an excystation assay) and thus the device effectively extracts all of the viable oocysts.

This work has demonstrated that microfluidics is capable of species and viability based separations of waterborne pathogens, using either size based IF devices for species/kingdom separations or dielectrophoresis (DEP) set-ups for either species or viability separations. Samples would be resuspended thus ensuring conductivity could be controlled for optimum operation of the DEP device.

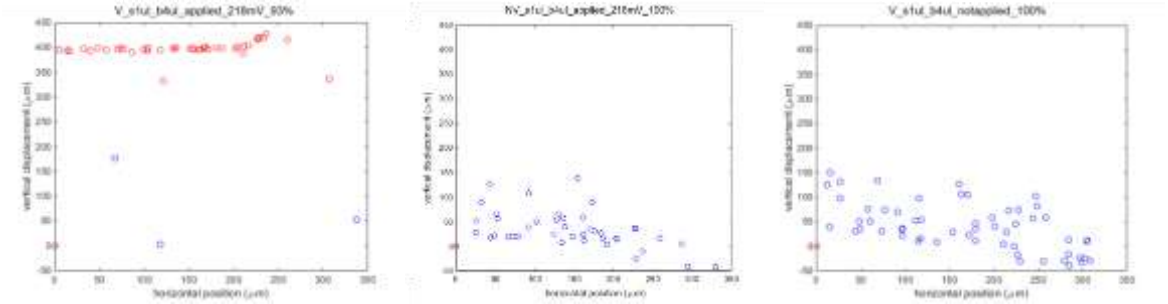

Figure 1: the left image indicates the behaviour of a sample of viable *Cryptosporidium* under an applied electric field whereas as the middle image shows the behaviour of heat-treated non-viable *Cryptosporidium* under an identical applied electric field; the right image shows the behaviour of the viable oocysts without the electric field. Flow direction in the device is along the x axis. The red dots indicate those oocysts which are displaced and will be collected into a different outlet channel compared to the blue dots (see Appendix 6 for device design and further explanation). This demonstrates that application of an appropriate electric field can selectively displace viable pathogens.

Public Engagement Activities

Additionally, several outreach activities were undertaken (output 8) reaching a couple of thousand people in the Edinburgh area and winning the Runner's Up Prize in the HWU Interactive Workshop Competition – these activities also led to Dr Bridle being nominated for the Royal Society of Edinburgh Prize for Public Engagement. Additionally, the results were published in Lab on a Chip (output 9; Appendix 7). The below photo (Figure 2) shows a young girl enjoying the outreach event at the Fountainbridge Canal Festival:

Figure 2: Dr Bridle explaining the microfluidics design using a scaled up Lego model and FIMO beads. The young girl has successfully used the model system to separate "pathogens" from other particles in the sample.

Implications of the results of Deliverable Report 6.4

Implications of the results for the Work Package (WP 6) (max. 350 characters)

Offers an alternative to centrifugation for secondary concentration of pathogens
Highly innovative and non-filter based technology
Adds value to the rest of the WP6 work by offering a means of species and viability based sorting

Implications of the results for this Cluster 2 and 3 (max. 400 characters)

Cluster 2: by offering microfluidics alternatives simplified integration and automation should be possible
Cluster 3: an alternative to existing secondary concentration steps and a method of species and/or viability sorting before molecular methods detection (the viability sorting could be particularly important for use in conjunction with detection methods that do not offer this capability)

Implications of the results for the whole project (max. 400 characters, specify Cluster & WP)

Highly novel method of sample processing and sorting
Numerous outputs in terms of journal publications and conference papers raising the profile of the project
Significant outreach activities undertaken ensuring public engagement with the project

Indicate key external stakeholders interested in the results of Deliverable Report 6.4

End-users: water utilities and other water testing laboratories along with food quality monitoring labs

Water and food regulators

Wider audience interested in the concentration and sorting of pathogens (e.g. Dow expressed an interest in the viability sorting)

Microfluidics and automation companies

Which internal partners should your deliverable be sent to?
All partners