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PII: S0167-7012(16)30068-9
DOI: doi: 10.1016/j.mimet.2016.04.008
Reference: MIMET 4875

To appear in: *Journal of Microbiological Methods*

Received date: 29 March 2016
Revised date: 7 April 2016
Accepted date: 7 April 2016

Please cite this article as: Jimenez, M., Bridle, H., Microfluidics for effective concentration and sorting of waterborne protozoan pathogens, *Journal of Microbiological Methods* (2016), doi: 10.1016/j.mimet.2016.04.008

**REVISION**

# Microfluidics for Effective Concentration and Sorting of Waterborne Protozoan Pathogens

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Abstract: We report on an *inertial focussing based* microfluidics technology for concentrating waterborne protozoa, achieving a 96% recovery rate of *Cryptosporidium parvum* and 86% for *Giardia lamblia* at a throughput (mL/min) capable of replacing centrifugation. The approach can easily be extended to other parasites and also bacteria.

Microfluidics has been proposed for the sample processing and monitoring of waterborne pathogens, with a greater emphasis in previous work being placed on the detection stages [1]. Some microfluidics waterborne pathogen sample processing has been demonstrated, e.g. on-chip immunomagnetic separation [2], electrical methods of pathogen isolation [3, 4] and on-chip filtration [5-7], although sample volumes have remained relatively small [8, 9]. By appropriate design of channel geometries high-throughput particle concentration and sorting can be achieved in microfluidics without the use of any labels, electrical fields or in-channel constrictions [10]. One such technique is known as inertial focussing [11-13] (Figure 1a) and here we report on the use of spiral channel inertial focussing microfluidics for the effective concentration of waterborne protozoa.

Devices were designed using AutoCAD and manufactured by Epigem in Epoxy and PMMA with a channel width of 170µm and a height of 30µm with four sample outlets (Figure 1b). The system was operated at flow rates between 200 and 1500µL/min using a mid-pressure syringe pump (neMESIS, Cetoni, GmbH). We have previously characterised the behaviour of a similar system with just two outlets using polystyrene beads (Magsphere Inc, USA) and shown that this system could also work successfully at 400µL/min with *Cryptosporidium parvum* (Waterborne Inc, USA) without notable impact on the viability of this pathogen [14].

Here we report on the use of a four outlet system with both *Cryptosporidium parvum* and *Giardia lamblia* (Waterborne Inc; spiked at a concentration of 1million (oo)cysts/mL) in deionised water (tap water samples have also been tested proving that the system is capable of handling these without clogging) at high flow rates (up to 1500µL/min), achieving a four-fold concentration with just one passage through the system. The behaviour of the pathogens within the flow channel has been analysed using a high-speed camera (CCD ProgRes, Jenoptik, GmbH on a Nikon, x 10 or x 25 magnification, inverted microscope) to image particle trajectories within the channel. Additionally, the recovery rates have been determined by counting the number of pathogens in each outlet; *from a few hundred image per outlets, taken during system operation, pathogens are counted, to a total of at least 1000, using thresholds based on intensity differences between the background and pathogens with a MATLAB script*.

Figure 1A illustrates the principle of inertial focussing in a spiral channel showing how the forces acting on particles within the flow result in them adopting laterally focussed positions, in general closer to the inner wall of the spiral. Figure 1B shows the particular device utilised within this work. By focussing pathogens into a particular location, with appropriate design of the channel geometry, pathogens can be concentrated and separated by direction to a particular outlet channel. A four outlet device achieves a concentration factor of four which can easily be increased by recirculation of the output of the appropriate outlet through the device until the desired concentration is reached *(with a stacked system we have concentrated 40mL to 0.5mL in less than 10 mins)*. An alternative would be to increase the number of outlets. Here, however, we have focussed on the behaviour of waterborne protozoa within a spiral channel, which has not been previously studied. Evidently, understanding the pathogen behaviour is essential information to inform accurate and appropriate outlet positioning. The focussing behaviour of pathogens is expected to vary from that of rigid spherical particles (on which the inertial focussing theory is developed) due to their non-uniform shape and their deformability. *Cryptosporidium parvum is approximately 4.5 by 5.5µm [15] and the deformability has recently been analysed with FluidFM [16]. Giardia lamblia cyst is approximately 10 to 20µm in length, 7-10µm in width and 0.3-0.5µm thickness [17, 18].*

Figure 2 shows how the channel focussing location of protozoa varies with flow rate in comparison to polystyrene beads of similar sizes. It is clear that the focussing positions of *Cryptosporidium* and *Giardia* are similar to that of 5.2 and 10.3µm rigid particles, respectively as might be expected from their sizes. However, particularly for *Giardia* the distribution of the focussed pathogens is wider than that of the respective beads, probably due to a greater size distribution in the pathogen population, or an impact of being non-spherical and deformable, compared to the particles: for a flowrate of 500µL/min, 10.3um beads present a standard deviation in their averaged lateral position of 8 µm while *Giardia* is at 24 µm (15µm for *Cryptosporidium*). *This difference in distribution might be explained by greater non-uniformity in the Giardia shape compared to Cryptosporidium oocysts, or deformability differences between the pathogens although data is not available to easily compare the relative deformability.*

In addition to effective high-throughput processing, recovery rate of protozoa is a critical factor for waterborne pathogen monitoring applications. Figure 3 illustrates the recovery rates of pathogens into the different outlets at different flow rates. 95% of *Cryptosporidium*, a rate comparable to that achieved in traditional IMS [5], is collected in outlet 4 at a flow rate of 1000µL/min demonstrating an higher recovery rate than that demonstrated for *Giardia* (86% to outlet 4 at 1500µL/min) probably due to the narrower size distribution observed for this pathogen.

It can also be noted that the focussing position of the *Giardia* is closer to the centreline (Figure 2), as would be expected for a biological deformable particle [19]. However, *Cryptosporidium* does not display this trend perhaps suggesting it is less deformable or that the results for *Giardia* are actually due to the highly irregular shape of this pathogen. The *Cryptosporidium* data indicates that the pathogen focussing position initially moves towards the inner wall as flow rates increase until a limit of 700 µL/min and further flow rate increases push the pathogen further and further back towards the centreline (Figure 2). This phenomenon has been previously observed for small particles [20], and indicates the importance of flow rate optimisation for this method to effectively concentrate pathogens. The flow rate must be selected to successfully focus all pathogens within the collection zone of a particular outlet as opposed to spreading them across two or more outlets. Additionally,

the flow rate can be manipulated to control whether simultaneous collection of both pathogens or separation of them can be achieved, e.g. as flow rates increase *Giardia* is increasingly found in outlet D whereas *Cryptosporidium* moves to being mainly collected in outlet C (Figure 3).

In conclusion, we have shown how inertial focussing microfluidics can be applied for the high-throughput concentration and separation of waterborne protozoa. By imaging the behaviour of pathogens within the channel at mL/min flow rates we have shown how this system concentrates protozoa into particular collection outlets. Therefore, the microfluidic system is capable of incorporation into existing protozoan monitoring processes: further scale-up by stacking of devices can enable processing of 50mL in less than 10 mins, and recirculation can increase the extent of concentration achieved; thus the use of microfluidics instead of centrifugation is now a feasible proposition. This could enable automation of the final stages of waterborne protozoa monitoring into an on-chip technology incorporating this concentration method with on-chip detection technologies. Furthermore, the approach can easily be adapted for other parasites or bacteria through simple modification of channel geometries.

Acknowledgements

M.J. and H.B. would like to would like to acknowledge EU funding for the project "AQUAVALENS: protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation" and the EPSRC Impact Acceleration Award. H.B. would like to acknowledge The Royal Academy of Engineering/EPSRC for her research fellowship. M.J. and H.B. would like to acknowledge Epigem for the manufacturing part and Brian Miller for design considerations of inertial focussing devices in this type of application.

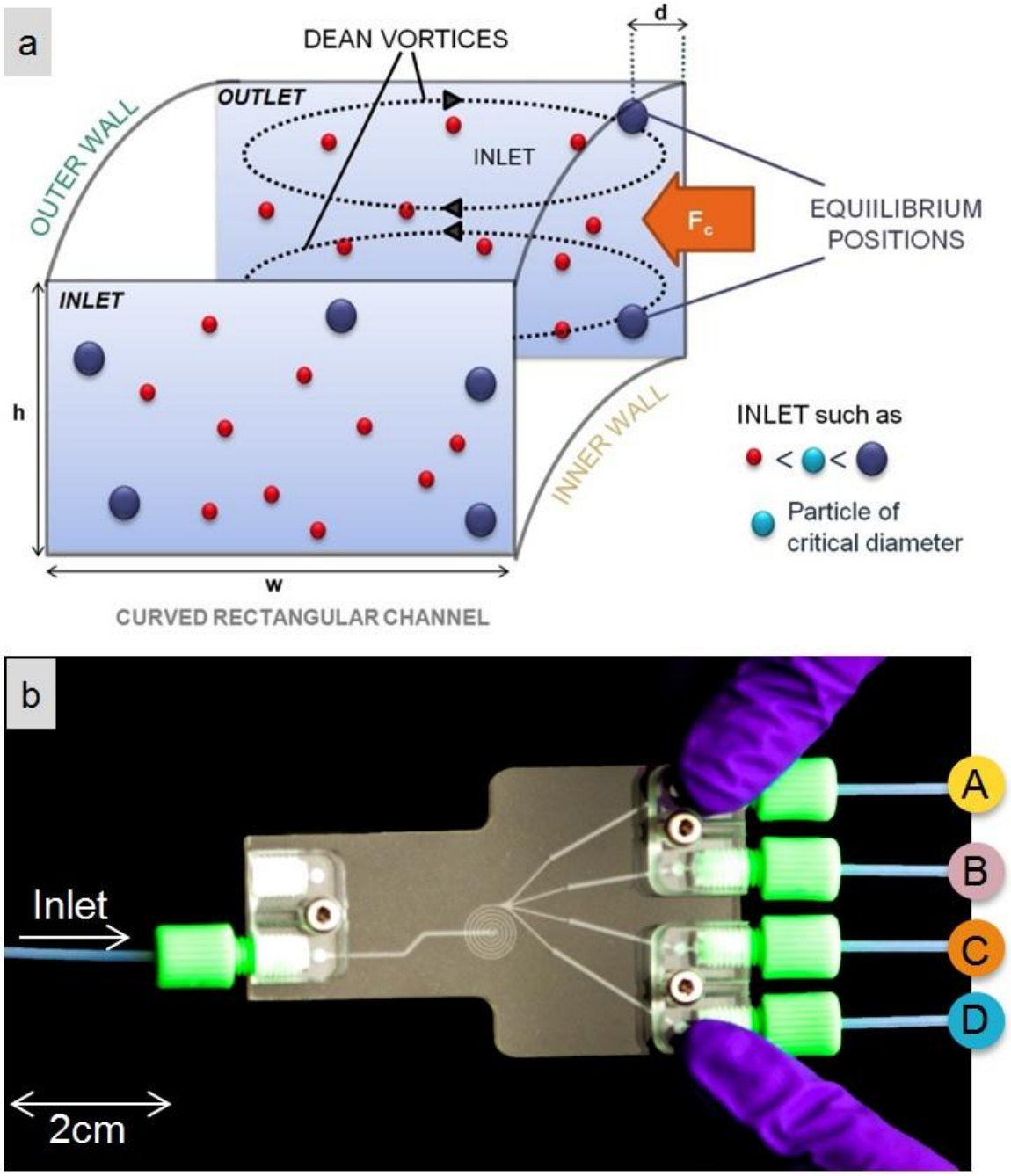


Figure 1: a) The schematic illustrates the principle of inertial focussing whereby the interplay of different forces within the flow channel act to locate particles at a particular channel location perpendicular to the flow direction (this is known as the focussing position and is typically closer to the inner wall of a spiral channel). The effect is dependent upon flow rate and particle size and deformability and Fc denotes the centrifugal action acting on the liquid towards the outer wall. Further details can be found in [11-13] ; b) The image illustrates the set-up and the size of the microfluidic device. This system can focus particles ≤ 2 µm. More details about the design of the spiral are available in [14].

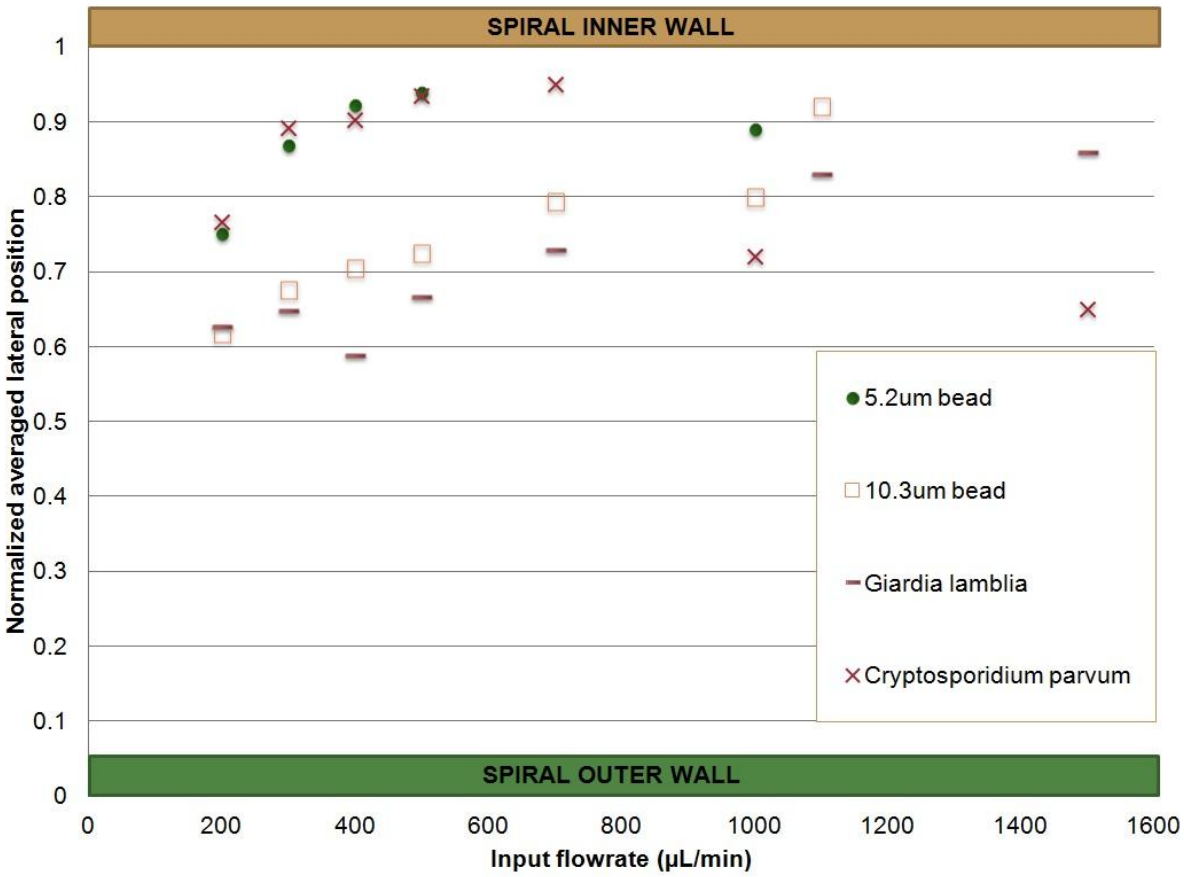


Figure 2: Focussing positions of the protozoan pathogens as compared to rigid polystyrene beads at different flow rates. The normalized averaged lateral position corresponds to d/w (distance from *outer* wall/channel width), notation as shown in Figure 1.

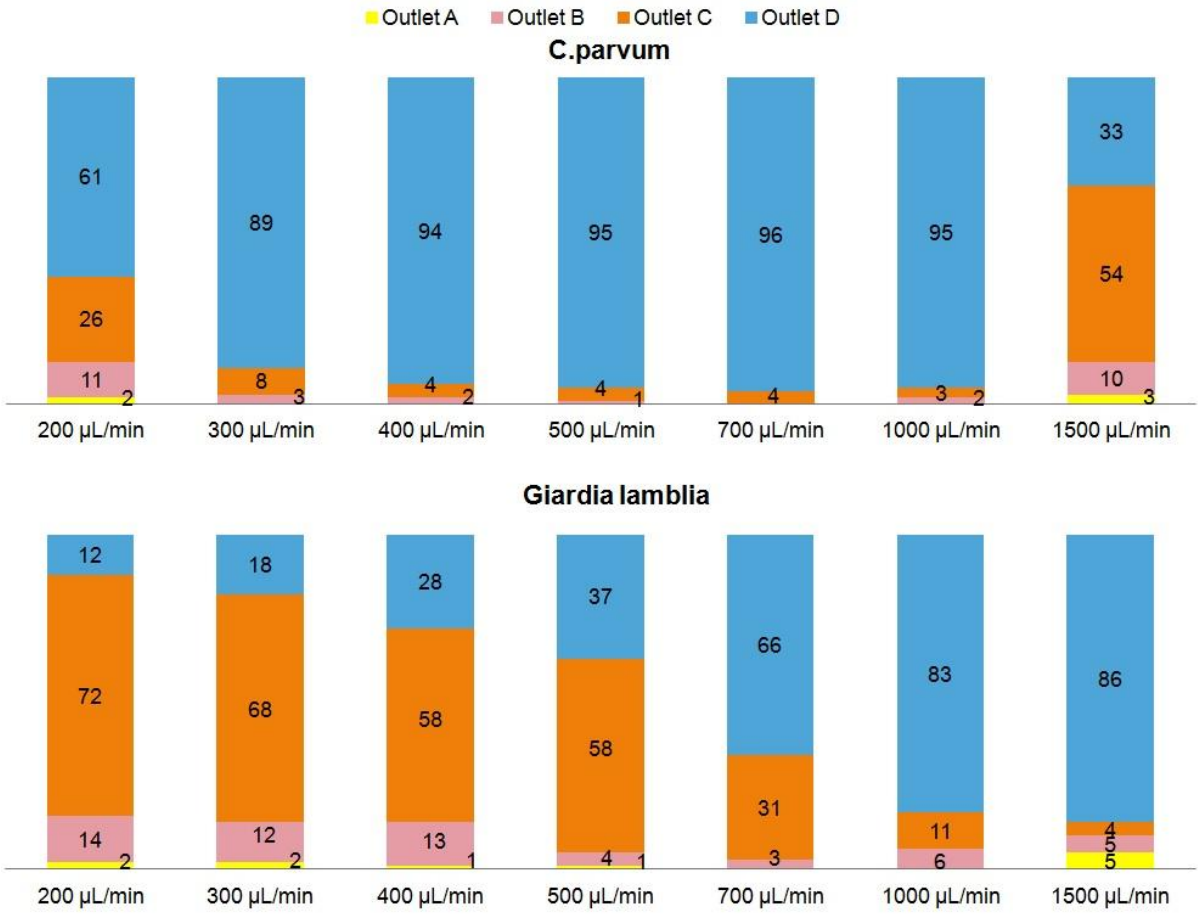


Figure 3: Recovery rates into the different outlets achieved for *Cryptosporidium parvum* and *Giardia lamblia* at different flow rates. The colour coding in the images corresponds to the different outlet colour-coding as shown in Figure 1. The percentage of pathogens found in each outlet at the different flow rates is shown, indicating the flow rate at which maximal recovery rates into one outlet can be achieved, i.e. into outlet D 0.7mL/min for *Cryptosporidium* at 96% and 1.5mL/min for *Giardia* at 86%.

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**HIGHLIGHTS**

Microfluidics for Effective Concentration and Sorting of Waterborne Protozoan Pathogens

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- Concentration of parasites with a 96% recovery rate at 1.5mL/min using microfluidics
- Cryptosporidium and Giardia can be separated or jointly concentrated, depending on device operation
- Inertial focussing microfluidics could process 50mL in less than 10 mins