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Note

# 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 (Bridle, 2014). Some microfluidics waterborne pathogen sample processing has been demonstrated, e.g. on-chip immunomagnetic separation (Ramadan et al.), electrical methods of pathogen isolation (Goater et al., 1997; Simmons et al., 2010) and on-chip filtration (Taguchi et al., 2005; Taguchi et al., 2007; Lay et al., 2008), although sample volumes have remained relatively small (Bridle et al., 2012, 2014). 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 (Wyatt Shields Iv et al., 2015). One such technique is known as inertial focussing (Zhang et al., 2016; Martel and Toner, 2014; Di Carlo, 2009) (Fig. 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 170 μm and a height of 30 μm with four sample outlets (Fig. 1b). The system was operated at flow rates between 200 and 1500 μL/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 400 μL/min with *Cryptosporidium parvum* (Waterborne Inc., USA) without notable impact on the viability of this pathogen (Jimenez et al.).

Here we report on the use of a four outlet system with both *C. parvum* and *Giardia lamblia* (Waterborne Inc.; spiked at a concentration of 1 million (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 1500 μL/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, ×10 or ×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.

Fig. 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. Fig. 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

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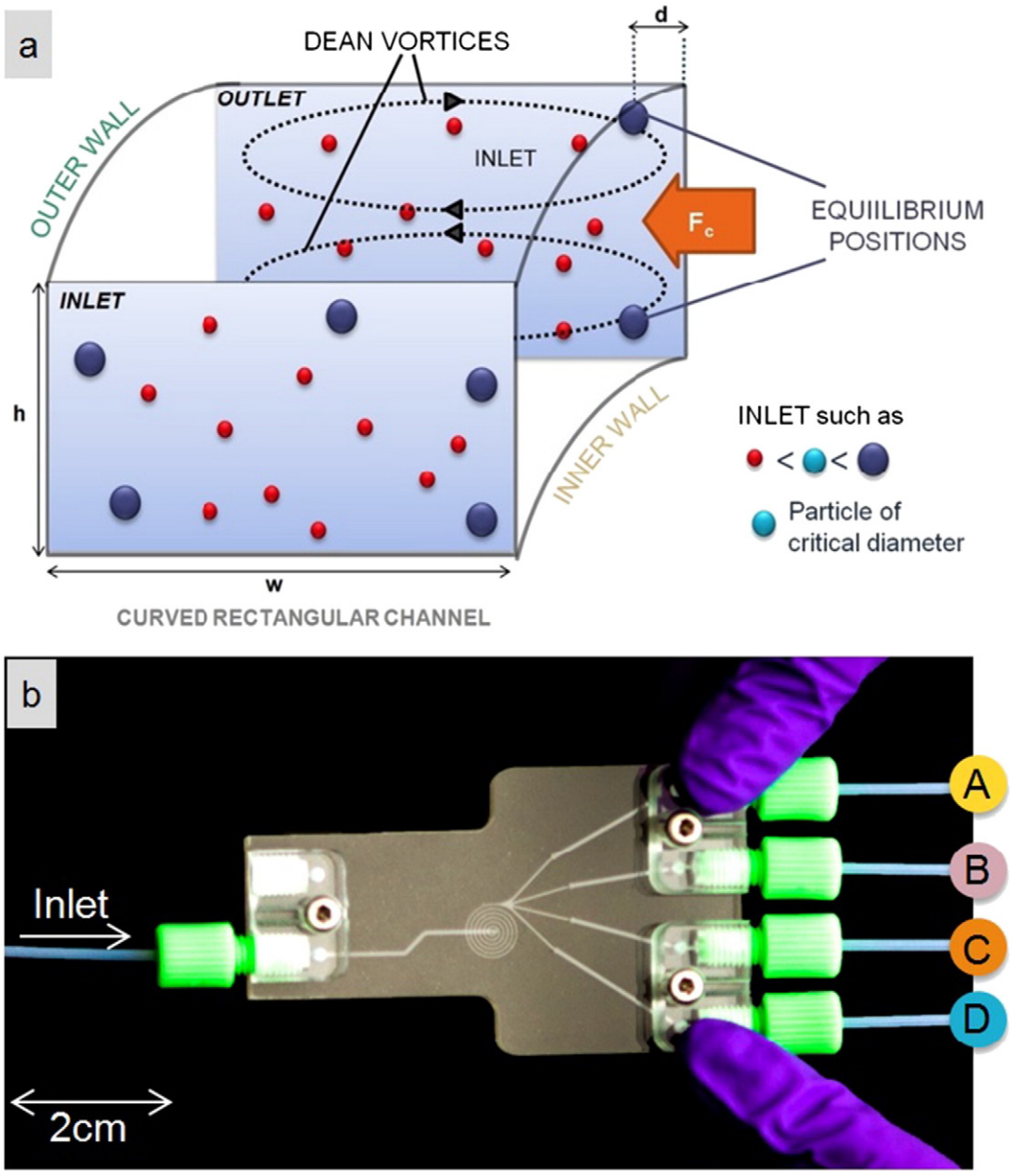


**Fig. 1.** a) The schematic illustrates the principle of inertial focussing whereby the interplay of different forces within the flow channel acts to locate particles at a particular channel location perpendicular to the flow direction (this is known as the focussing position and is typically closer 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 Zhang et al. (2016), Martel and Toner (2014) and Di Carlo (2009); 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 (Jimenez et al.,).

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 40 mL to 0.5 mL in less than 10 min). 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. *C. parvum* is approximately 4.5 by 5.5 μm (Smith and Nichols, 2010) and the deformability has recently been analysed with FluidFM (McGrath et al., 2016). *G. lamblia* cyst is approximately 10 to 20 μm in length, 7–10 μm in width and 0.3–0.5 μm thickness (Huang and White, 2006; Krauss et al., 2003).

Fig. 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 10.3 μm 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 500 μL/min, 10.3 μm beads present a standard deviation in their averaged lateral position of 8 μm while *Giardia* is at 24 μm (15 μm for *Cryptosporidium*). This difference in distribution might be explained by greater non-uniformity in the Giardia shape