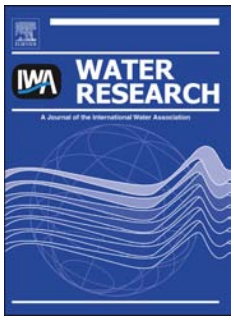
Application of microfluidics in waterborne pathogen monitoring

Accepted Manuscript



Application of Microfluidics in Waterborne Pathogen Monitoring: A Review

Helen Bridle, Brian Miller, Marc P.Y. Desmulliez

PII: S0043-1354(14)00101-8

DOI: 10.1016/j.watres.2014.01.061

Reference: WR 10464

To appear in: *Water Research*

Received Date: 31 October 2013

Revised Date: 23 January 2014

Accepted Date: 24 January 2014

Please cite this article as: Bridle, H., Miller, B., Desmulliez, M.P.Y., Application of Microfluidics in Waterborne Pathogen Monitoring: A Review, *Water Research* (2014), doi: 10.1016/j.watres.2014.01.061.

Highlights:

- First review of the application of microfluidics to waterborne pathogen monitoring
- Sample processing is divided into immunological, electrical and filtration methods
- Optical methods have had most attention but molecular methods maybe best
- Ultimate aim is multiplexed systems with integrated parts for automated operation
- Many challenges in applying microfluidics to this application: great future potential

Application of Microfluidics in Waterborne Pathogen Monitoring: A Review

Authors: Helen Bridle[1], Brian Miller[2], Marc P.Y. Desmulliez[3]

1. Heriot-Watt University, Institute of Biological Chemistry, Biophysics and Bioengineering (IB3), Riccarton, Edinburgh, United Kingdom. Phone: 0131 451 3355; E-mail: h.l.bridle@hw.ac.uk

2. University of Edinburgh, King's Buildings, Edinburgh, United Kingdom.E-mail: b.miller@ed.ac.uk

3. Heriot-Watt University, MicroSystems Engineering Centre (MISEC), Riccarton, Edinburgh, United Kingdom. Fax: 0131 451 4155; Tel: 0131 451 3340; E-mail: m.desmulliez@hw.ac.uk

Abstract:

A review of the recent advances in microfluidics based systems for the monitoring of waterborne pathogens is provided in this article. Emphasis has been made on existing, commercial and state-of-the-art systems and research activities in laboratories worldwide. The review separates sample processing systems and monitoring systems, highlighting the slow progress made in automated sample processing for monitoring of pathogens in waterworks and in the field. Future potential directions of research are also highlighted in the conclusions.

Keywords: microfluidics, waterborne pathogens, monitoring techniques, sample processing, detection methods

1. Introduction

Inadequate access to clean water is hugely detrimental both to economic development and human health. In the developing world 2,200 children die daily from diseases transmitted through unsafe water and, despite the Millennium Development Goal of halving the proportion of people without access to an improved water source being met, 780 million people still lack access to safe water (www.cdc.gov/healthywater/global/wash_statistics.html). Far from being a solely developing world problem, waterborne disease is a threat to citizens in the developed world. For example, one of the largest recent outbreaks affected Milwaukee in 1993 in which approximately 400,000 people were infected by cryptosporidiosis (Corso et al.

2003). Moreover, it has been estimated that 10% of total hospital patients in the USA contract diseases due to poor water sanitation, significantly increasing morbidity, mortality and financial burden. Overall, lost productivity in the USA due to waterborne diseases is estimated at $20 billion per year (Straub and Chandler 2003). According to the World Health Organisation (WHO), microbial hazards remain the primary concern in both developing and developed countries (WHO 2011).

Waterborne pathogens include viruses, bacteria and parasites, several of which are highly infectious, robust and long-lived in the environment as well as being resistant to standard methods of water treatment. Viruses are the smallest of these pathogens, typically around 20 – 300nm in diameter, which makes them difficult both to detect and to remove. Additionally, viruses are highly infectious and often long-lived in the aqueous environment, with norovirus for example being shown to remain infectious after over 2 months in groundwater (Seitz et al. 2011). Furthermore, many viruses are resistant to disinfection, particularly norovirus, which has demonstrated resistance to chlorination, and adenovirus, which has remained viable even after UV treatment (WHO 2011). Bacteria, with sizes on the order of a few micrometers, are often less infectious, with some notable exceptions (e.g. *E. coli O157:H7* and *Shigella*). Bacteria are more susceptible to chlorine disinfection. Parasites are the largest of the waterborne pathogens, of around 5µm and larger, and comprise protozoa and helminths. Helminth infections have decreased significantly over recent years as the causative agents (e.g. the host within the water environment) are easily removed by filtration. Protozoa however, remain a problem due to a low infectious dose, longevity in the environment and resistance to water treatment methods (Baldersson and Karanis 2011, Chen et al. 2007).

In recent years there have been numerous research advances in methods for monitoring waterborne pathogens (Bridle 2013). Monitoring plays several key roles in the design and implementation of water safety plans and can be applied for surveillance, operational or investigative means (WHO 2011). This review focuses on the role that miniaturisation, in particular using microfluidic systems, can play in the delivery of “lab-on-a-chip” devices to perform monitoring procedures.

Microfluidic systems, *i.e.* fluid handling systems with channel dimensions on the micrometer scale, have developed rapidly during the past decade and have found many applications, especially within chemical analysis and biological assays. This is unsurprising considering their numerous advantages which include reduced sample consumption, increased speed of analysis, improved efficiency and process parallelisation as well as access to phenomena and mechanisms that are not accessible on the macroscopic scale (Beebe et al. 2002). For example, there have been developments in using microfluidics to obtain better environmental control over cells or bacteria during culture, even now to the level of creating organs on-chip (Huh et al. 2011), which could lead to improvement in traditional culture based pathogen monitoring approaches. Microfluidics might also allow the design of environments to promote culture of microorganisms, which have not yet been cultured in the lab. Yoon and Kim, however, are not positive about this approach for foodborne (or waterborne) pathogens with the justification that lab-on-a-chip has focussed on rapid methods of detection while culturing is time-consuming (Yoon and Kim 2012). Their review from 2012 is an excellent overview of microfluidic detection methods and the latest application to foodborne pathogens (Yoon and Kim 2012), many of which are identical to waterborne pathogens. Another review of microfluidics for pathogens in general is that by Mairhofer in 2009 (Mairhofer et al. 2009). The Nature review by Yager discusses application of microfluidics for developing world settings (Yager et al. 2006).

This review differs from the above by focussing specifically on waterborne pathogens. To the best of the authors' knowledge, no previous review exists, which concentrates specifically upon microfluidic approaches to waterborne pathogen monitoring systems, despite many developments in this field in recent years. Particular areas of focus in this article have been on sample processing applications, a key part of any waterborne pathogen monitoring strategy, as well as on significant developments in optical detection technologies in the last few years. This paper is organised into two main sections. The first will discuss how microfluidics has been applied to the challenge of sample processing within waterborne pathogen monitoring. The second will provide an overview of research advances in the use of microfluidics for waterborne pathogen detection. Finally, the paper concludes by summarising the state-of-the-art approaches, looking forward to analysing the potential of

microfluidics within this application area and making recommendations for future research.

2. Existing Methods of Monitoring

Existing methods of monitoring for waterborne pathogens mainly rely upon the detection of faecal indicators (Gleeson and Gray 1996). This strategy assumes that faecal indicators have contaminated water and will therefore also indicate the presence of any pathogens. The advantage of this approach is that the tests are cheap and easy to perform (2006). However, studies have indicated poor correlation between the detection of indicators and presence of microbial pathogens (Savichtcheva and Okabe 2006, Hunter 1997) and there are also concerns that this approach does not allow a valid identification of the pathogen (Brettar and Höfle 2008).

The alternative to indicator monitoring is the implementation of tests to directly identify particular pathogens. This is extremely challenging due to the low concentrations of pathogens in large water volumes. Furthermore, it is often necessary to determine the species, viability and infectivity of a microorganism to determine its pathogenic potential. Indeed, for some pathogens, identification beyond the species level is required, as in the case of the Vibrio species (Robinson et al. 2010).

Various methods of direct detection exist, including cell culture based methods, immunological methods, microscopy and nucleic acid amplification approaches. Each has advantages and disadvantages and the most suitable choice of monitoring approach is likely to depend upon why monitoring is being undertaken (Bridle 2013).

3. Sample Processing

In some areas, e.g. medical applications such as detection of biomarkers (Mohammed and Desmulliez 2011) or analysis of rare cells (Thomson et al. 2011), the capability of microfluidics to enable analysis of small sample volumes is an attractive feature. However, for environmental applications, such as waterborne pathogen monitoring, the need to process large sample volumes and complex matrices represents a major

challenge for such a technology. System parallelisation is one option to deliver increased device throughput. Avoiding device clogging with complex samples is another potential problem although lab-on-a-chip systems have been proven to handle complex samples, like blood. Many different techniques have been utilised for cell sorting within microfluidics and this review will present their applications to waterborne pathogens, along with other approaches, which have been trialled for waterborne pathogen sample processing on-chip.

Cell sorting in microfluidics is a well-studied and well-reviewed area (Chen et al. 2008, Kersaudy-Kerhoas et al. 2008). Methods can be sorted into those, which either exploit intrinsic properties of the cell or resort to extrinsic 'labels' for cellular differentiation. Additionally, separation can be performed by a variety of means including optical, electric, magnetic or hydrodynamic forces. Examples of such techniques are shown in Figure 1.

Only a small section of the vast literature of microfluidics methods used for cell sorting has concentrated upon sample processing of waterborne pathogens, although research studies in this topic have significantly increased in numbers recently (Table 1). Despite this recent focus, the water sample volumes studied remain small (μL-mL) in relation to the volumes required for environmental applications (L-1000L). Furthermore, the performance of many of the developed devices has only been demonstrated using purified water, a significantly simpler system than real water samples. Centrifugation, which is often employed in traditional methods of waterborne pathogen sample processing (Polaczyk 2008, Lindquist et al. 2007, Stine 2005, EPA 2005), has also been demonstrated on-chip (Madou 2006), though this has not yet been applied to waterborne pathogens. Several on-chip approaches combine some form of microfluidic enrichment with detection. One example of an existing technique used for sample processing and detection that has been miniaturised into an on-chip format, is flow cytometry. This technique is covered later under the optical detection section.

3.1 On-chip Immunological Concentration Methods

Immunological methods of separation, the most common being immunomagnetic separation (IMS) (EPA 2005, Hamza et al. 2012), are routinely applied in traditional methods of waterborne pathogen detection. IMS works by mixing and incubating the immunomagnetic beads and sample, allowing time for binding of the pathogen of interest to the beads. Subsequently, the beads are pulled to one side of the container using a magnet, isolating this pathogen from the rest of the sample, which is removed and discarded. The bead-microorganism complex can be used for direct plating on selective media, for enrichment of a bacterium or for nucleic acid isolation. However, many protocols include dissolution of the bead-pathogen complex, often accomplished by adding acid. The dissociated beads are then removed from the pathogen sample by using the magnet to once again concentrate them at the side of the container, and removing the solution which now contains the isolated pathogens. Both the use of antibodies for selective capture and the use of immunomagnetic beads have been applied within microfluidic systems.

The first demonstration of this approach was by Taguchi and co-workers who investigated the microfluidic trapping of protozoa, for subsequent fluorescence analysis (Taguchi et al. 2005). They developed a micro-well array strategy, consisting of 32x32 microfabricated wells with a 10 or 30µm diameter and a 10µm depth, for oocyst capture. After microfabrication, the micro-wells were selectively coated with streptavidin and anti-*C. parvum*antibodies. For capture experiments, 10 mL of a sample mixture of *C. parvum* oocysts (10^7 oocysts/mL) suspended in PBS was simply deposited onto the array and the whole chip rotated horizontally for 1 h. While the authors claimed this microarray capture process facilitated faster microscopic analysis no data was provided to evidence this, and neither were recovery rates reported.

An alternative strategy employed IMS, using high aspect ratio curved microchannels, and was demonstrated in 2010 by Dharmasiri et al (Dharmarisi et al. 2010). In this case, *E. coli* was isolated from pre-filtered lake water with a 71% recovery rate. A 100 concentration factor was achieved and the device was capable of processing 1mL at a flow rate of around 5µL/min. Another study published in 2010 by Guan et al used antibody-coated microbeads inside the microchannels for the immunoseparation of *E. coli* (Guan et al. 2012). The use of beads was employed to overcome the potential limitations of planar microchannels, by increasing the surface area available for

antibody-pathogen binding. This was illustrated by the high recovery rate obtained, of around 92 to 96%.

Automation and miniaturisation of IMS for *Cryptosporidium* and *Giardia* was developed by Ramadan and colleagues in 2010 (Ramadan et al. 2010). Incubation of the protozoan pathogens with the IMS beads occurs as in standard protocols and then the bead-pathogen complexes are introduced into the flow-through system. The system employs an external permanent magnet, the rotation of which repeatedly captures and releases the magnetic particulate matter as it passes through the channel. Performing multiple capture-release stages away from a wall avoids the problems of aggregation, in which impurities might be trapped, as sometimes observed in IMS. At the end of the flow channel the concentrated sample is captured by another magnet (Figure 2). This system was reported to concentrate samples of 50mL down to 1mL, with efficiencies comparable to the existing methods, for both tap and secondary effluent water. Two different systems were trialled, fabricated from different materials. When comparing channels made of disposable plastic with those made from glass, the latter were found to perform better in tap water. One potential explanation for this finding is that the rougher walls in the plastic cartridge offers sites where pathogens could become trapped, thus reducing the recovery rate. The authors state their work is a step towards creating automated sample processing for protozoa, reducing the number of steps and human intervention required.

One final example of on-chip IMS is the system developed by Agrawal et al in 2012. The 3D circular microfluidic system was fabricated in PDMS with imbedded permanent magnets. The system was designed for multiplex pathogen detection, with the first stage being capture of the bacteria with immunomagnetic nanoparticles (Agrawal et al. 2012). The second stage involved pathogen detection with fluorescent quantum dots. The recovery rate of the concentration stage was not reported.

3.2 Electrical Based Concentration in Microfluidics

The use of dielectrophoresis (DEP) to concentrate pathogen samples on a microfluidic chip was first performed by Goater et al. in 1997, as part of an electrorotation

detection strategy for *Cryptosporidium* oocysts (Goater et al. 1997). Subsequently, in 2005, Gomez–Sjoberg reported DEP based concentration of *E. coli* in a 400pL chamber, achieving concentration factors of 10^4-10^5 (Gomez-Sjoberg et al. 2005). In 2011, Chow and co-workers reported a DEP system for trapping and concentrating *E. coli* (Chow and Du 2011). The system operated at a flow rate of 1μL/min and claimed a 100% trapping efficiency if an appropriate DEP force was chosen. Additionally, different voltages could be applied to distinguish between different species, and viable and non-viable microorganisms. This example illustrates one of the key advantages of electrical separation methods, e.g. the ability to distinguish between pathogens according to their species and viability. For a general review of DEP in microfluidics see the review by Cetin and Li (Cetin and Li 2011), and Jesus-Perez and Lapizco-Encinas (Jesus-Perez and Lapizco-Encinas 2011) for the application of DEP to environmental microorganisms.

An alternative approach to DEP was developed by Cabrera and Yager who utilised an electrokinetic separation approach, at a flow rate of 83nL/s, to isolate bacteria (Cabrera and Yager 2001). This technique could potentially be adapted to waterborne pathogens. The advantages include continuous flow separations with a low power requirement.

Lapizco-Encinas et al developed in 2005 an insulator-based DEP (iDEP) for concentration of *E. coli* (Lapizco-Encinas et al. 2005). iDEP employs insulating structures to generate non-uniformity in an electric field generated by remote electrodes. Lapizco-Encinas and colleagues reported concentration factors on the order of 3000, with recovery efficiencies nearing 100%. The technique was also applied to viruses. In 2009, Cho et al applied iDEP in a plastic chip for the capture of *E.coli* (Cho et al. 2009). The authors reported that a maximal capture efficiency of 66% was achieved at a flow rate 100μL/min. This seems relatively low, especially in comparison to traditional IMS approaches, although it is comparable to recovery rates obtained after membrane filtration and elution.

In 2010, a US patent was granted to Simmons et al. for the use of an iiDEP microfluidic chip to capture *Cryptosporidium* (Simmons et al. 2010). The patent claims that the device could process 1-10mL of water concentrating the sample to

25µL for further study such as immunofluorescence. Potential clogging problems were addressed by the utilisation of an ultrafiltration membrane prior to sample entry into the dielectrophoretic segment. A 2011 thesis reported the design of 3D iDEP systems for trapping of bacteria, reporting stronger trapping forces at lower temperatures, reducing the risk of thermal damage to the trapped cells, thus potentially enabling further downstream analysis (Braff 2011). This work represented the first observation of intra-species differences in membrane surface properties using iDEP. One potential problem with DEP or iDEP as a concentration and isolation technique is their operation in batch mode, which could complicate their integration into continuous flow systems.

Two studies have utilised electrical concentration methods for viruses. Balasubramanian presented a microfluidic system based on electrophoretic transport and electrostatic trapping to study MS-2 virus and Echovirus 11 found in potable water (Balasubramanian et al. 2007). MS-2 virus was captured with 88–99% efficiency and Echovirus showed capture efficiency above 70%. Grom reported the combination of electrohydrodynamic flow and dielectrophoretic forces to trap Hepatitis A virus (Grom et al. 2006). They showed that the microsystem can be useful to accumulate viral particles from relatively large sample volumes.

3.3 On-chip Filtration

Work carried out by Liu and colleagues employed sieves or filters as a means of trapping *Cryptosporidium* oocysts (Zhu et al. 2004, Lay et al. 2008). In one example, a weir was created by interfacing a deep channel (50µm) with a very shallow channel (1 or 2µm) (Zhu et al. 2004). Using positive pressure, a mixture of protozoa in water was injected into the channel, trapping the cells against the wall of the deep channel. The common disadvantage of sieves or filters systems is their rapid clogging, perhaps due to the weir system. However, in further work employing a so-called rain drop bypass filter, Lay et al. significantly reduced this issue (Lay et al. 2008). The design consists of 3 prefilters and a wide composite filter structure, which allows alternative fluidic paths and therefore significantly reduces the pressure and the clogging on the filter. The filters are made of fine arrays of rain drop like shaped pillars, arranged in gaps ranging from 0.2 to 1mm, in the trapping zones and coarse arrays in the bypass

zones. The device was used for bacterial (and protozoal) capture and detection with a limit of detection (LOD) of 10^5 colony forming units per millilitre (cfu/mL). Application of such systems to viral capture would be challenging due to the small size of structures that would be required.

Taguchi and co-workers refined their micro-well array method to increase the capture efficiency. The laser-machined stainless steel micromesh consists of a 10x10 array of 2.7mm diameter cavities to capture single oocysts incorporated into a PDMS microfluidic device (Taguchi et al. 2007). The maximum flow rate tested was 350μL/min, such that 5mL could be processed in under 15 min. When loading a 0.5mL test sample (spiked oocysts in PBS) at a concentration of 36 oocysts/mL a recovery rate of 93% from the mesh was reported, comparable to that achieved by IMS. Batch processing of the sample occurs in the current design; thus while integration into automated systems would be possible, real-time continuous monitoring is not.

4. Use of Microfluidics in Waterborne Pathogen Detection

The use of microfluidics for the detection of waterborne pathogens allows a variety of detection technologies to be implemented. These include optical, electrical and molecular means as well as biosensors. This section reviews the state of the art of these techniques with emphasis on waterborne pathogens, and the data is summarised in Table 2.

4.1 Optical Methods

Some optical detection techniques for waterborne pathogens have already benefited from the advantages of lab-on-a-chip systems. These include optical microscopy of stained oocysts and fluorescence techniques. Other potential systems, such as SERS on chip reviewed by Chen and Choo in 2008 (Chen and Choo 2008), have not yet been applied to waterborne pathogens detection.

In some of the examples of (oo)cyst and bacteria capture given in the previous section, the sample processing module of the device was also utilised for detection, through the addition of fluorescent stains. The systems by Taguchi and Liu (described in section 3.3) represent a microfluidic alternative to the existing IMS and fluorescence detection protocols for (oo)cysts. The method of Taguchi et al has the advantage to include the pre-defined location of the binding of the oocysts and their good adhesion to the substrate during the washing and staining steps (Taguchi et al. 2005). Their second design also allowed the detection to be done in 60 mins compared to 2 to 3 hours for the IMS method (including staining) with automated FITC labelling. Imaging was used for detection. A detection limit of 36 oocysts/mL was reported, which is above the desired ability to detect single oocysts (Taguchi et al. 2007). Zhu et al confirmed that the use of microfluidics enhanced the signal detection, reducing both the time and also claiming that reagent use could be 10-100x more dilute for signals comparable to the traditional glass slide approach for (oo)cyst detection (Zhu et al. 2004). However, fluorescence based detection of bacteria in similar systems appears to be more challenging with Lay et al finding a limit of detection of $1x10^5$ cfu/mL (Lay et al. 2008).

Sandwich immunoassays on-chip have been applied to *E. coli* detection , in either a 0.4mL reaction chamber on-chip (Stokes et al. 2001) or in the case of Li and Su processing a 1mL sample in 2 hours. It was claimed that a microfluidic approach allowed for an improvement of detection sensitivity (to an LOD of 10-100cfu/mL) due to reduced reagent consumption and increased immunoassay kinetics (Li and Su 2006).

A microfluidic flow cytometer with cheap, compact and low-power PIN photodiodes was developed in 2004 (Tung et al. 2004). This device, made from PDMS, hydrodynamically focuses the sample where it is excited and detected using optical fibres and also uses lock-in amplification. While this system has not been applied to waterborne pathogen detection, it did demonstrate the first single-cell fluorescence detection using this type of photodiode. Device performance was characterised with beads and yeast cells, with a sample volume of 5mL/hr. In 2005, Sakamoto et al described a microfluidic flow cytometry set-up for *E. coli* detection capable of analysing six 10μL samples in just 30 mins (Sakamoto et al. 2005). The system was

reported to deliver good agreement with traditional counts in shorter times, and was tested with river water.

Flow cytometry on-chip for *E. coli* was also reported by Yamaguchi in 2011 achieving a good comparison to traditional counting methods, even in pond water (Yamaguchi et al. 2011). The device integrated on-chip mixing of sample and fluorescent stain to avoid pre-treatment steps off-chip and utilised automated image analysis, delivering results in 1 hour. The authors note that using LED light sources could allow for development of a portable system and that integration with sample concentration microfluidics would be an option to reduce the LOD from the reported 10^4 cells/mL.

High throughput flow cytometry on-chip was demonstrated by Oakey et al demonstrated (Oakey et al. 2010) delivering a 1ml/min volumetric throughput. Though the study did not directly target pathogens it was able to discern individual particles of around 10μm diameter, roughly similar in size to *Giardia lamblia.* The system takes advantage of a hydrodynamic inertial focussing mechanism and could characterise around 25,000 particles per second in a relatively low concentration of sample (0.1% w/v). It was not tested with complex media but indicates the potential of a miniaturised detection system, which could perhaps be coupled to a sample processing mechanism to create a fully automated label-free monitoring solution.

Further optical methods include UV/visible spectroscopy and chemiluminescence In 2006, Li and Su produced a microfluidic chip to capture *E. coli* with antibodies for detection by UV/visible spectroscopy (Li and Su 2006). A microfluidic approach using IMS and chemiluminescence detected *E. coli* O157:H7 down to 34 cells in 90 mins, with a sample of 100μL (Varshney et al. 2006). *E. coli* O157:H7 activity has also been monitored in real time, following immunoseparation by microbeads in microchips, with bioluminescence technology (Guan et al. 2012). In 2008, Karsunke and colleagues reported the detection of *Salmonella*, *E. coli* and *Legionella* using chemiluminescence on-chip, with detection limits of $3x10^6$, $1x10^5$ and $3x10^3$ cells/mL, respectively, for an assay which took just 13 mins (Wolter et al. 2008). A system redesign, incorporating six parallel flow through channels enabled easier

calibration and measurement on-chip improving the detection limits for *Salmonella* and *E. coli* though not for *Legionella* (Karsunke et al. 2009). These later experiments were also performed in water, making the results more relevant to water monitoring than the previous PBS experiments.

In 2007, Ymeti et al developed a sensor utilising a Young's interferometer that utilises an off- chip light source and CCD to detect the presence of Herpes Simplex Virus type 1 (HSV-1)(Ymeti et al. 2006). While HSV-1 is not primarily transmitted through water, cutaneous manifestations of infection occur due to its presence in water (Elko et al. 2003). Furthermore, this type of detection mechanism can easily be adapted for the detection of other viruses and other microbial agents. Measurements were taken after flowing buffer through the chip for 30 mins to establish a baseline signal and virus detection occurred over approximately 2.5 hours. The flow rates and sample volume are not reported though the author stipulates that it is suitable for volume of sample measured in micro-litres. Most interesting is the assertion that the LOD is sensitive enough to detect a single binding instance, though only detection of ~700 virus particles is firmly established; this still represents a highly sensitive and easily miniaturised platform. It is important to note however that generally, the higher sensitivity of detection, the more specificity to the target analyte or purity of the sample is necessary to reduce interference from non-target particles in the sample matrix.

Single-cell level detection was reported using a latex immunoaggulination for real-time detection of *E. coli* on a microfluidic chip, reading out the light scattering results using a proximity (i.e. one not integrated on-ship) optical fibre (Han et al. 2008). Single cell detection per microdevice was reported which increased to 4 cfu upon the addition of an extra washing step to determine only viable bacteria. While the volume processed by each device was 100μL, later work by the authors connected the microfluidic system to a larger piped network, sampling a fraction of the water supply and achieving a 10 cfu/mL LOD in less than 5 minutes (Kwon et al. 2010). The same group also applied this technology for the detection of *Cryptosporidium*, via isolation of the *Cryptosporidium* oocyst wall protein (COWP), reporting single oocyst sensitivity, analysing a small volume of sample subsequent to the traditional filtration/concentration processes (Angus et al. 2012). For faster detection, on the

order of 10 mins, filtration and concentration could be skipped though the detection limit in this case was raised to 1-10 oocysts/mL.

In 2011 *E. coli* from lettuce wash was detected using a particle immunoaggulination assay to an LOD of 10 cfu/mL in just 6 mins (You et al. 2011). The drawback of this system was that it only tested 60µL of sample. However, the microfluidic chip was integrated into a handheld portable unit. The same year another approach for *E. coli* detection involved incorporation of single microorganisms into droplets, offering the advantages of fast fluorescent tracer dye accumulation within droplets as well as digital counting (Marcoux et al. 2011). Detection was based on metabolism, showing good agreement with traditional colony counts, achievable within 2 hours for some bacteria, with full quantification possible in less than 10 hours. Although this method was aimed at foodborne pathogens the detection was performed in water, and thus could be directly applied to waterborne pathogen detection.

In 2012, Connelly and co-workers developed a microfluidic system for virus detection, combining pre-concentration and signal amplification via liposomes with different detection methods (Connelly et al. 2012). Liposomes and the virus sample are incubated and then pre-concentrated through electrokinesis towards a nanoporous membrane. The sample is then eluted and captured downstream, where the liposomes are lysed releasing fluorescent signal. The authors note that the LOD of $1x10^5$ pfu/mL is currently quite high, and suggest several improvements, as well as highlighting the suitability of the device for easy-to-use, rapid and portable systems.

Also, in 2012, Agrawal and colleagues used secondary fluorescently labelled antibodies for the fluorescent detection of immunomagnetically captured pathogens, within a circular microfluidic system (Agrawal et al. 2012). The authors stated that the use of the magnetic nanoparticles facilitated the capture of the antigen in a confined space thus enhancing the subsequent fluorescence signal. Cd-Te QDs with different emission wavelengths were conjugated to capture *E. coli* and *S. typhimurium*, respectively. Simultaneous detection was possible in the range of 10^3-10^7 cfu/mL for a 20µL sample.

4.2. Electrical Methods

Despite the relative ease of electrode integration on-chip, there appears to be fewer studies demonstrating electrical methods of waterborne pathogen detection with microfluidics. Existing examples are given in this section.

The Connelly system mentioned in section 4.1 could also be operated using an electrical method for virus detection, with electrochemical compounds encapsulated in liposomes, which can be lysed releasing the compounds for detection. Detection of whole viruses using carbon nanotube thin film field effect devices has been reported by Mandal (Mandal et al. 2012).

Houssin and colleagues reported the detection of *Cryptosporidium* using EIS and could distinguish between viable and non-viable oocysts in 2010 (Houssin et al. 2010). The detection took place on a PDMS chip and was due to ion release in hypo-osmotic conditions, thus requiring oocysts to be resuspended in purified water. A chip consisting of an arrangement of four sensors with 4µm wide interdigitated electrodes was manufactured by optical lithography and metal deposition on a Pyrex substrate. The limit of detection of the device was measured to be 10^4 oocysts/mL in the buffer.

Dielectrophoresis has been applied to the study of both *Cryptosporidium* and *Giardia* (oo)cysts. It has been shown that viable and non-viable oocysts electrorotate at different rates and in opposite directions, depending upon the field strength (Goater et al. 1997, Dalton et al. 2001). Goater et al. designed a system in which travelling wave dielectrophoresis was used to collect oocysts in the centre of a spiral electrode where electrorotation was applied for detection. It was observed that, in the frequency window of 20-600 kHz, viable oocysts rotated faster than non-viable ones, at rates discernible to the human eye or an automated image recognition system. Recently, it has been shown that *Plasmodium falciparum* trophozoites modify the zeta potential of red blood cells, potentially opening the way to for characterization of infectivity by measurement of zeta potential changes (Tokumasu et al. 2012).

4.3. Biosensors

A biosensor is a device that convert biological target recognition into a detectable response; this signal transduction can be performed using optical, electrical or mass-sensitive means. This definition specifically refers to systems where the action of biological recognition triggers the signal transduction (and therefore excludes the previous examples given under the optical and electrical sections).

From a review of the literature on biosensor development for waterborne pathogens it was clear that electrochemical methods of biosensing have been the least applied to waterborne pathogens (Bridle 2013). The same conclusion is true about the integration of this type of sensor into microfluidics for waterborne pathogens. However, impedance based biosensors have been applied to waterborne pathogens within microfluidics. While there are several examples of the use of optical or mass-sensitive transduction for waterborne pathogen detection, this strategy has rarely been integrated on-chip.

A system, developed by Gomez and colleagues, consists of a flow-through system to measure the impedance of pathogenic bacteria, testing for metabolic activity as an indicator of viability (G. They claimed to process low numbers of cells (1-5000) though the sample volume was also very small, being just 30nL. Impedance biosensor chips were also developed for detection of *E. coli* O157:H7 based on the surface immobilization of affinity-purified antibodies onto indium tin oxide (ITO) electrode chips (Ruan et al. 2002). The biosensor can detect the target bacteria with a detection limit of 6×10^3 cells/mL. In 2008, Yang employed electrochemical impedance spectroscopy (EIS) for the detection of *Salmonella*, which were captured, and concentrated, using IMS (Yang 2008).

In 2010, Mannoor et al described a microfluidic impedance system for bacterial detection (Mannoor et al. 2010). In the flow-through set-up the LOD was 10^4 cells/mL, as opposed to 1 bacterium/μL for static operationas shown in Figure 3. In the static set-up the sample was incubated on top of the electrode array for 10-12 mins whereas in continuous flow the sample was passed over the array at a flow rate of 5 μL /min. The authors suggest the difference in LOD between these two approaches, is due to the influence of flow (opposing effects of shear and mixing forces) on the binding kinetics, resulting in reduced binding, as has been reported in other similar

flow through assays. Appropriate system design that take into consideration binding kinetics and flow rates is very important (Squires et al. 2008).

Among optical approaches, surface plasmon resonance (SPR) within microfluidics has been demonstrated, though there is little work with waterborne pathogens. Cell-based SPR is less common than small molecule detection and so SPR lab-on-a-chip devices perhaps hold most promise for detection of the outputs of molecular methods. SPR based biosensors are currently implemented in field-deployable devices sensing of small molecules, proteins, viruses and whole microbes using a 24-channel SPREETA (Sensata) sensor unit (Mairhofer et al. 2009).

4.4. Molecular Methods

The first microfluidic PCR was demonstrated in a 50μL silicon chip in 1993 (Northrup et al. 1993). Since then numerous designs and components for performing molecular methods on-chip have been developed, which are now starting to be incorporated into integrated systems. In addition, many authors have adopted polymeric materials to reduce the turnaround time in device design refinement and to reduce cost. The main advantage of microfluidic PCR is the rapid temperature cycling, which can be obtained due to the reduced thermal mass; in commercial PCR systems this step accounts for over 90% of the operation time. Single cell detection is possible (Whitea et al. 2010).

For PCR there have been two main approaches to on-chip systems, although methods such as oscillatory PCR, convective PCR and continuous flow thermal gradient PCR have been developed (Ahmad and Hashsham 2012). The first is static PCR where the sample volume is held in a chamber and the temperature cycling is performed by means of some kind of heating system. The second is flow-through PCR where the device is designed such that sample travelling in a serpentine channel passes through different temperature zones. The advantage of this approach is faster delivery time of the results (90s to10mins) at the cost of lower detection sensitivity. The high surface area in the serpentine channel, compared to static reactor volume, could be a

significant adsorption factor of reagents. There are several commercially available static PCR systems but as yet no flow-through devices.

. A pocket-sized convective PCR system developed by Agraval et al in 2007 was the cheapest prototype molecular method lab-on-a-chip, costing just $10 for the hardware and a few cents per reaction (Agrawal et al. 2007). The continuous flow thermal gradient is the first demonstration of a real-time flow-through system (Pjescic et al. 2010). Microfluidic systems for isothermal molecular methods have also been developed such as on-chip NASBA (Dimov et al. 2008, Gulliksen et al. 2004, Gulliksen et al. 2005) and LAMP (Ahmad and Hashsham 2012).

Although significant progress has been made in the design and operation of the amplification and detection stages of lab-on-chip systems for molecular methods, only a few systems have presented full-integration with sample processing also performed on-chip but this is an area requiring more development. For more details about the general state-of-the-art regarding microfluidic molecular methods we recommend the 2009 review article by Zhang and Ozdemir (Zhang and Ozdemir 2009) and the more recent 2012 review article by Ahmad and Hashsham (Ahmad and Hashsham 2012). See also Figure 4 for an overview of stages in molecular detection and microfluidic components capable of performing these tasks.

While there have been considerable progress developing microfluidic systems that enable on-chip molecular detection, the number of studies applying these types of technologies to waterborne pathogens is very low. In terms of waterborne viruses we found that RT-PCR on a microfluidic device with integrated amplification and fluorescence detection, which could be performed within 1hour, was demonstrated for rotavirus in 2011 (Li et al. 2011). Work developing a multiplexed PCR system for bacteria was announced in 2006, with promising early results (Weigl et al. 2006). In 2010, Ramalingam and colleagues presented the simultaneous detection of four waterborne bacteria in a PDMS PCR array, which utilised capillary flow for sample loading (Ramalingam et al. 2010).

With regard to protozoa, the two publications that relate to molecular sensing of *Cryptosporidium* in microfluidic systems describe the performance of NASBA off-

chip (Nugen et al. 2009) with only the detection of the mRNAs amplicons performed on-chip. Esch et al. developed a fluorescence based detection assay chip, relying on a sandwich hybridization of the NASBA product between capture probes and reporter probes (Esch et al. 2001). The microfluidic device consists of one channel in a PDMS block bonded to a glass slide with a gold pad at its centre to immobilise the capture probe. The reporter probes were tagged with carboxyfluorescein-filled liposomes giving out better fluorescent intensities than usual fluorophores. This technique gave a LOD of 5 femtomoles of amplicon per test (12.5µL). The overall time for the full analysis was 1-2 hours, including the heat shock and implementation of the NASBA procedure (Esch et al. 2001).

4.5 Commercial Systems

*Crypto*Detect CARD™ is a platform, shown in Figure 5, with on-chip integrated sample preparation features, developed by Rheonix and reportedly capable of detecting *Cryptosporidium* in raw water samples (Rheonix 2011). The technology involves integrated IMS and washing of the oocysts, heat shock, lysis, extraction, purification and detection of RNA amplicons, using fluorescent liposomes. However, the technology is at an early stage and no LOD or recovery rate was communicated.

Early Warning Inc. have combined sample processing and detection into an automated platform for on-line monitoring of pathogens including viruses, bacteria and protozoa (Early Warning Inc 2011). Unlike previous examples, the platform includes a concentrator capable of sampling 10L of water and filtering it to a 10mL sample. The concentrator unit also features hydrodynamic cavitation to disaggregate clumps. The inclusion of this unit also makes the system relatively large (182x139x76 cm) and heavy (around 200kg), which would make it difficult for portable usage in the field. After concentration and filtration, analytes of interest in the sample are subsequently separated using IMS, lysed and pathogen RNA is amplified by NASBA. The detection happens when the hybridization of target RNA amplicons on specific biomolecular probes generates a current via a guanine oxidation process. The company website reports the limit of detection of this device after concentration, filtration and detection on the biosensor chip as 1 cell per 100mL equivalent, sampling a total volume of 10L. Additionally the total load quantification and

viability testing of up to 25 species can be performed on a single chip with a total processing time of less than 3 hours from sampling to results.

5. Conclusions

This paper has reviewed the applications of microfluidics for waterborne pathogen monitoring. Detection to the single-cell level has been demonstrated on-chip, though the range of pathogens studied needs to be expanded. Work with *E. coli* is most common and *Cryptosporidium* is also relatively well studied. Research activities involving other pathogens are significantly more limited. For almost all types of detection approaches demonstrated for waterborne pathogens, there are microfluidic systems for performing these tests. Amongst the optical, electrical and biosensor systems presented, the optical detection option seems to have received the most attention from the lab-on-a-chip community.

The advantages of performing fluorescent detection on-chip are: (1) the reduced sample volume resulting in a lower background noise signal and therefore improved sensitivity and signal to noise ratio; (2) the small sample volume needed and control of flow enhancing binding kinetics and increasing sensitivity; and (3) the reduced consumption of reagents (Rivet et al. 2011). However, there are challenges, especially with the relatively short shelf life of reagents for field devices, and particularly in the production of low-cost, sensitive optics. There has been some progress towards this latter goal with the development of on-chip microscopes and photodiode detectors. Despite this, some authors believe label-free technologies seem more promising for ease of integration with microfluidics (Rivet et al. 2011). However, field devices at fixed locations will require regular operations, such as filter changes, sterile packing and samples transport, to maintain their functionality and integrity as monitoring systems and hence the reagent shelf-life might not be such a problem.

Performing molecular methods on a microfluidic chip exploits many of the advantages of lab-on-a-chip miniaturisation and is likely to be become one of the promising directions for waterborne pathogen detection in microfluidic systems. However, lab-on-a-chip systems have not yet been applied too extensively to the challenge of waterborne pathogen detection. The technology is reaching the stage of

integration although more work needs to be done on incorporating the sample processing module. This challenge is greater for "real" environmental water samples where both the initial sample volumes and the potential number of interferents/inhibitors are high. In general, the smaller the sample volumes or flows used in detection, the greater the burden is on the sample processing for concentration and enrichment. The different detection methods, described above for whole-cell microorganisms can mostly also be applied to molecular detection and so there are many options to perform the on-chip detection. An EU project, entitled "AQUAVALENS: Protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation", which started in the spring of 2013 aims to further the use of molecular methods for monitoring the microbial quality of drinking water with one important element focussing on the delivery of technological platforms, including microfluidics, to perform the testing.

Microfluidics has even been applied to sample processing, though dealing with real water samples is challenging, due to potential problems with device clogging as well as obtaining sufficient throughput. In order to overcome this latter limitation high levels of parallelisation would be required which may complicate the possibility of any portable sized instruments based around these technologies. However, online monitoring of waste water using a microfluidic system has been demonstrated, albeit for phosphate sensing, which is simpler than microorganism analysis (Cleary et al. 2008).

Foodborne pathogen processing and detection in microfluidics seem to have received more attention than waterborne pathogens, though often the pathogens are the same and identical detection procedures are possible. The main difference between two lies in the sample processing, and it is easier to test wash from food samples as opposed to process large volumes of complex environmental water, which might be the explanation for the focus. Additionally, there may be differences in the food and water testing markets, influencing the drive towards, and perhaps also the funding for, the different target areas.

In general for wider exploitation of microfluidic devices integration of optical and electrical detection components would be essential to realise a fully automated system. Either these components need to be included on-chip at low-cost or systems need to be designed where low-cost optical and electrical hardware integrates with a microfluidic chip. Additionally, multiplexed detection systems capable of classifying a range of pathogens from the different kingdoms (virus, bacteria, and protozoa), ideally to beyond the species level would be highly desirable and is perhaps the ultimate goal of any detection system for waterborne pathogens. Some microarray work, e.g. that delivered by the EU project Healthy Water, suggests this is a possibility on-chip. Determination of pathogen viability and nocivity still remains an elusive goal, and off-chip optimisation of these approaches will be required before microfluidics systems can be produced.

Quilliam et al report that increases in the sensitivity and specificity of detection methods for waterborne pathogens are currently being achieved by combining advances in microfluidics technology and analytical chemistry with molecular and immunological methods (Quilliam et al. 2011). Mairhofer, Roppert and Ertl believe the next generation of pathogen sensing developments will be facilitated by advances in lab-on-a-chip devices (Mairhofer et al. 2009). The global market for microfluidic technology is growing at a great pace and was estimated to be worth US$6.2 billion in 2011 (Zhang and Ozdemir 2009). Given their many advantages, and providing some of the above challenges can be met, it can be expected that in the future microfluidics for waterborne pathogens will have an increasing share in that growing market.

Acknowledgements

HB would like to acknowledge The Royal Academy of Engineering/EPSRC for her research fellowship. BM would like to acknowledge his BBSRC Industrial Case Studentship, supported by Scottish Water. Both HB and MD 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”.

References

www.cdc.gov/healthywater/global/wash_statistics.html.
Corso, P.S., Kramer, M.H., Blair, K.A., Addiss, D.G., Davis, J.P. and Haddix, A.C. (2003) Cost of Illness in the 1993 Waterborne Cryptosporidium Outbreak, Milwaukee, Wisconsin. Emerging Infectious Diseases 9(4), 426-431.
Straub, T.M. and Chandler, D.P. (2003) Towards a unified system for detecting waterborne pathogens. J Microbiol Methods 53(2), 185-197.
WHO (2011) World Health Organisation, Guidelines for drinking-water quality.
Seitz, S.R., Leon, J.S., Schwab, K.J., Lyon, G.M., Dowd, M., McDaniels, M., Abdulhafid, G., Fernandez, M.L., Lindesmith, L.C., Baric, R.S. and Moe, C.L. (2011) Norovirus infectivity in humans and persistence in water. Appl Environ Microbiol. 77, 6884-6888.
Baldersson, S. and Karanis, P. (2011) Waterborne transmission of protozoan parasites: Review of worldwide outbreaks e An update 2004e2010. Water Research 45, 6603-6614.
Chen, F., Hunag, K., Qin, S., Zhao, Y. and Pan, C. (2007) Comparison of viability and infectivity of Cryptosporidium parvum oocysts stored in potassium dichromate solution and chlorinated tap water. Veterinary Parasitology 150(1-2), 13-17.
Bridle, H. (2013), Elsevier.
Beebe, D.J., Mensing, G.A. and Walker, G.M. (2002) Physics and applications of microfluidics in biology. Annu Rev Biomed Eng 4, 261-286.
Huh, D., Hamilton, G.A. and Ingber, D.E. (2011) From 3D cell culture to organs-on-chip. Trends in Cell Biology 21(12), 745-754.
Yoon, J.Y. and Kim, B. (2012) Lab-on-a-Chip Pathogen Sensors for Food Safety. Sensors 12, 10713-10741.
Mairhofer, J., Roppert, K. and Ertl, P. (2009) Microfluidic Systems for Pathogen Sensing: A Review. Sensors 9, 4804-4823.
Yager, P., Edwards, T., Fu, E., Helton, K., Nelson, K., Tam, M.R. and Weigl, B.H. (2006) Microfluidic diagnostic technologies for global public health. Nature 422, 412.
Gleeson, C. and Gray, N.F. (1996) The coliform index and waterborne disease: problems of microbial drinking water assessment, Taylor & Francis, London.
(2006) Health Canada.
Savichtcheva, O. and Okabe, S. (2006) Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water Research 40(13), 2463-2476.
Hunter, P.R. (1997) Waterborne disease: epidemiology and ecology. , Wiley, Chichester.
Brettar, I. and Höfle, M.G. (2008) Molecular assessment of bacterial pathogens — a contribution to drinking water safety. Current Opinion Biotechnol 19, 274-280.
Robinson, A.D., Falush, D.E. and Feil, J. (2010) Bacterial population genetics in infectious diseases, Wiley-Blackwell, John Wiley & Sons, Hoboken, New Jersey, USA.
Mohammed, M.-I. and Desmulliez, M.P.Y. (2011) Lab-on-a-chip based immunosensor principles and technologies for the detection of cardiac biomarkers: a review. Lab on a Chip 11, 569-595.
Thomson, D.A.C., Dimitrov, K. and Cooper, M.A. (2011) Amplification free detection of Herpes Simplex Virus DNA. Analyst 136, 1599-1608.
Chen, P., Feng, X. and Liu, B.F. (2008) Microfluidic chips for cell sorting. Frontiers in Bioscience 13, 2464-2483.

792 Kersaudy-Kerhoas, M., Dhariwal, R. and Desmulliez, M.P.Y. (2008) Recent advances
793 in microparticle continuous separation. IET Nanobiotechnology 2(1), 1-13.
794 Polaczyk, A.L., Narayanan, J., Cromeans, T.L., Hahn, D. Roberts, J.M., Amburgey,
795 J.E. and Hill V.R. (2008) Ultrafiltration-based techniques for rapid and simultaneous
796 concentration of multiple microbe classes from 100 L tap water samples. J. Appl.
797 Microbiol. 73, 92-99.
798 Lindquist, H.D., Harris, S., Lucas, S., Hartzel, M., Riner, D., Rochele, P. and Deleon,
799 R. (2007) Using ultrafiltration to concentrate and detect Bacillus anthracis, Bacillus
800 atrophaeus subspecies globigii, and Cryptosporidium parvum in 100-liter water
801 samples. J. Microbiol. Methods. 70, 484-492.
802 Stine, S.W., \Vladich; F.D., Pepper, I.L. and Gerba, C.P. (2005) Development of a
803 Method for the Concentration and Recovery of Microsporidia from Tap water. J.
804 Environ. Sci. Health, Part A 40, 913-925.
805 EPA (2005) Method 1623: Cryptosporidium and Giardia in Water by
806 Filtration/IMS/FA, United States Environmental Protection Agency.
807 Madou, M.Z., J.; Jia, G.; Kido, H.; Kim, J.; Kim, N. (2006) Lab on a CD Ann. Rev.
808 Biomed. Eng. 8, 601-628.
809 Hamza, I.A., Jurzik, L., Überla, K. and Wilhelm, M. (2012) Methods to detect
810 infectious human enteric viruses in environmental water samples. International
811 Journal of Hygiene and Environmental Health 214, 426-436.
812 Taguchi, T., Takeyama, H. and Matsunaga, T. (2005) Immuno-capture of
813 Cryptosporidium parvum using micro-well array. Biosensors and Bioelectronics 20,
814 2276-2282.
815 Dharmarisi, U., Witek, M.A., Adams, A.A., Osiri, J.K., Hupert, M.L., Bianchi, T.S.,
816 Roelke, D.L. and Soper, S.A. (2010) Enrichment and Detection of Escherichia coli
817 O157:H7 from Water Samples Using an Antibody Modified Microfluidic Chip.
818 Analytical Chemistry 82(7), 2844-2849.
819 Guan, X., Zhang, H., Bi, Y., Zhang, L. and Hao, D. (2012) Rapid detection of
820 pathogens using antibody-coated microbeads with bioluminescence in microfluidic
821 chips. Biomed. Microdevices 12, 683-691.
822 Ramadan, Q., Christophe, L., Teo, W., ShuJun, L. and Hua, F.H. (2010) Flow-through
823 immunomagnetic separation system for waterborne pathogen isolation and detection:
824 Application to Giardia and Cryptosporidium cell isolation. Analytica Chimica Acta
825 673(1), 101-108.
826 Agrawal, S., Morarka, A., Bodas, D. and Paknikar, K.M. (2012) Multiplexed
827 Detection of Waterborne Pathogens in Circular Microfluidics. Applied Biochemical
828 Biotechnological.
829 Goater, A.D., Burt, J.P.H. and Pethig, R. (1997) A combined travelling wave
830 dielectrophoresis and electrorotation device: applied to the concentration and viability
831 determination of Cryptosporidium J. Phys. D: Appl Phys 30, L65-L69.
832 Gomez-Sjoberg, R., Morisette, D.T. and Bashir, R. (2005) Impedance microbiology-
833 on-a-chip: Microfluidic bioprocessor for rapid detection of bacterial metabolism.
834 Journal of MEMS 14, 829-838.
835 Chow, K.S. and Du, H. (2011) Dielectrophoretic characterization and trapping of
836 different waterborne pathogen in continuous flow manner. Sensors and Actuators A
837 170, 24-31.
838 Cetin, B. and Li, D. (2011) Dielectrophoresis in microfluidics technology.
839 Electrophoresis 32, 2410-2427.
840 Jesus-Perez, N.M. and Lapizco-Encinas, B.H. (2011) Dielectrophoretic monitoring of
841 microorganisms in environmental applications. Electrophoresis 32, 2331-2357.

842 Cabrera, C.R. and Yager, P. (2001) Continuous concentration of bacteria in a
843 microfluidic flow cell using electrokinetic techniques. Electrophoresis 22, 355-362.
844 Lapizco-Encinas, B.H., Davalos, R.V., Simmons, B.A., Cummings, E.B. and
845 Fintschenko, Y. (2005) An insulator-based (electrodeless) dielectrophoretic
846 concentrator for microbes in water. Journal of Microbiological Methods 62(3), 317-
847 326.
848 Cho, Y.-K., Kim, S., Lee, K., Park, C., Lee, J.-G. and Ko, C. (2009) Bacteria
849 concentration using a membrane type insulator-based dielectrophoresis in a plastic
850 chip Electrophoresis 30, 3153-3159.
851 Simmons, B.A., Hill, V.R., Fintschenko, V. and Cummings, E.B. (2010)
852 Concentration and separaion of bioloigcal organisms by ultrafiltration and
853 dielectrophoresis, Sandia Coporation, U.S.
854 Braff, W.A. (2011) Manipulation of bacteria using a three dimensional insulator based
855 dielectrophoresis, Massachusetts Institute of Technology.
856 Balasubramanian, A.K., Soni, K.A., Beskok, A. and Pillai, S.D. (2007) A microfluidic
857 device for continuous capture and concentration of microorganisms from potable
858 water. Lab Chip 7, 1315-1321.
859 Grom, F., Kentsch, J., Müller, T., Schnelle, T. and Stelzle, M. (2006) Accumulation
860 and trapping of hepatitis A virus particles by electrohydrodynamic flow and
861 dielectrophoresis. Electrophoresis 27, 1386-1393.
862 Zhu, L., Zhang, Q., Feng, H.H., Ang, S., Chauc, F.S. and Liu, W.T. (2004) Filter-
863 based microfluidic device as a platform for immunofluorescent assay of microbial
864 cells. Lab on a Chip 4(4), 337-341.
865 Lay, C., Teo, C.Y., Zhu, L., Peh, X.L., Ji, H.M., Chew, B.R., Murthy, R., Feng, H.H.
866 and Liu, W.T. (2008) Enhanced microfiltration devices configured with
867 hydrodynamic trapping and a rain drop bypass filtering architecture for microbial cells
868 detection. Lab on a Chip 8(5), 830-833.
869 Taguchi, T., Arakaki, A., Takeyama, H., Haraguchi, S., Yoshino, M., Kaneko, M.,
870 Ishimori, Y. and Matsunaga, T. (2007) Detection of Cryptosporidium parvum oocysts
871 using a microfluidic device equipped with the SUS micromesh and FITC-labeled
872 antibody. Biotechnology and Bioengineering 96(2), 272-280.
873 Chen, L. and Choo, J. (2008) Recent advances in surface-enhanced Raman scattering
874 detection technology for microfluidic chips. Electrophoresis 29, 1815-1828.
875 Stokes, D.L., Griffin, G.D. and Vo-Dinh, T. (2001) Detection of E. coli using a
876 microfluidics-based antibody biochip detection system. Fresenius Journal of
877 Analytical Chemistry 369(3-4), 295-301.
878 Li, Y. and Su, X.-L. (2006) MICROFLUIDICS-BASED OPTICAL BIOSENSING
879 METHOD FOR RAPID DETECTION OF ESCHERICHIA COLI O157:H7. Journal
880 of Rapid Methods and Automation in Microbiology 14, **96–109**.
881 Tung, Y.-C., Zhang, M., Lin, C.-T., Kurabayashi, K. and Skerlos, S.J. (2004) PDMS-
882 based opto-fluidic micro flow cytometer with two-color, multi-angle fluorescence
883 detection capability using PIN photodiodes. Sensors and Actuators B: Chemical
884 98(2â€“3), 356-367.
885 Sakamoto, C., Yamaguchi, N. and Nasu, M. (2005) Rapid and Simple Quantification
886 of Bacterial Cells by Using a Microfluidic Device. Appl Environ Microbiol 71(2),
887 1117-1121.
888 Yamaguchi, N., Torii, M., Uebayashi, Y. and Nasu, M. (2011) Rapid, Semiautomated
889 Quantification of Bacterial Cells in Freshwater by Using a Microfluidic Device for
890 On-Chip Staining and Counting. Appl Environ Microbiol 77(4), 1536-1539.

Oakey, J., Applegate, R.W., Arellano, E., Carlo, D.D., Graves, S.W. and Toner, M. (2010) Particle Focusing in Staged Inertial Microfluidic Devices for Flow Cytometry. Analytical Chemistry 82(9), 3862-3867.

Varshney, M., Li, Y., Srinivasan, B., Tung, S., Erf, G.F., Slavik, M.F., Ying, Y. and Fang, W. (2006) A microfluidic filter biochip-based chemiluminescence biosensing method for detection of Escherichia coli O157:H7. Transactions of the ASABE 49(6), 2061-2068.

Wolter, A., Niessner, R. and Seidel, M. (2008) Detection of Escherichia coli O157:H7, Salmonella typhimurium, and Legionella pneumophila in water using a flowthrough chemiluminescence microarray readout system. Analytical Chemistry 80, 5854–5863.

Karsunke, X.Y., Niessner, R. and Seidel, M. (2009) Development of a multichannel flow-through chemiluminescence microarray chip for parallel calibration and detection of pathogenic bacteria. Anal Bioanal Chem 395(6), 1623-1630.

Ymeti, A., Greve, J., Lambeck, P.V., Wink, T., van, H.v., Beumer, Wijn, R.R., Heideman, R.G., Subramaniam, V. and Kanger, J.S. (2006) Fast, Ultrasensitive Virus Detection Using a Young Interferometer Sensor. Nano Letters 7(2), 394-397.

Elko, L., Rosenbach, K. and Sinnott, J. (2003) Cutaneous manifestations of waterborne infections. Current Infectious Disease Reports 5(5), 398-406.

Han, J.-H., Heinze, B.C. and Yoon, J.-Y. (2008) Single cell level detection of Escherichia coli in microfluidic device. Biosensors and Bioelectronics 23(8), 1303-1306.

Kwon, H.J., Dean, Z.S., Angus, S.V. and Yoon, J.Y. (2010) Lab-on-a-Chip for Field Escherichia Coli Assays: Long-Term Stability of Reagents and Automatic Sampling System. Journal of Laboratory Automation 15(3), 216-223.

Angus, S.V., Kwon, H.J. and Yoon, J.Y. (2012) Field-deployable and near real time optical microfluidic biosensors for single-oocyst level detection of Cryptosporidium parvum for field water samples. Journal of Environmental Monitoring 14(12), 3295-3304.

You, D.J., Geshell, K.J. and Yoon, J.Y. (2011) Direct and sensitive detection of foodborne pathogens within fresh produce samples using a field-deployable handheld device. Biosensors & Bioelectronics 28, 399-406.

Marcoux, P.R., Dupoy, M., Mathey, R., Novelli-Rousseau, A., Heran, V., Morales, S., Rivera, F., Joly, P.L., Moy, J.P. and Mallard, F. (2011) Micro-confinement of bacteria into w/o emulsion droplets for rapid detection and enumeration. Colliodals and Surfaces A: Physicochem Eng Aspects 377, 54-62.

Connelly, J.T., Kondapalli, S., Skoupi, M., Parker, J.S.L., Kirby, B.J. and Baeumner, A.J. (2012) Micro-total analysis system for virus detection: microfluidic pre-concentration coupled to liposome-based detection. Anal Bioanal Chem 401, 305-323.

Mandal, H.S., Su, Z.D., Ward, A. and Tang, X.W. (2012) Carbon Nanotube Thin Film Biosensors for Sensitive and Reproducible Whole Virus Detection. Theranostics 2, 251-257.

GOMEZ, R., BASHIR, R., SARIKAYA, A., LADISCH, M.R., STURGIS, J., ROBINSON, J.P., GENG, T., BHUNIA, A.K., APPLE, H.L. and WERELEY, S. (2001) Microfluidic biochip for impedance spectroscopy of biologic species. Biomed. Microdevices 3, 201-209.

Ruan, C.M., Yang, L.J. and Li, Y.B. (2002) Immunobiosensor chips for detection of Escherichia coli O157 : H7 using electrochemical impedance spectroscopy. Analytical Chemistry 74, 4814-4820.

Yang, L. (2008) Electrical impedance spectroscopy for detection of bacterial cells in suspensions using interdigitated microelectrodes. Talanta 74(5), 1621-1629.
Mannoor, M.S., Zhang, S., Link, J. and McAlpine, M.C. (2010) Electrical detection of pathogenic bacteria via immobilized antimicrobial peptides. Proc Natl Acad Sci U S A.
Squires, T.M., Messinger, R.J. and Manalis, S.R. (2008) Making it stick: convection, reaction and diffusion in surface-based biosensors. Nat Biotechnol 26(4), 417-426.
Houssin, T., Folleta, J., Follet, A., Dei-Cas, E. and Senez, V. (2010) Label-free analysis of water-polluting parasite by electrochemical impedance spectroscopy. Biosensors & Bioelectronics 25(5), 1122-1129.
Dalton, C., Goater, A.D., Drysdale, J. and Pethig, R. (2001) Parasite viability by electrorotation. Colloids and Surfaces A: Physicochemical and Engineering Aspects 195(1-3), 263-268.
Tokumasu, F., Ostera, G.R., Amaratunga, C. and Fairhurst, R.M. (2012) Modifications in erythrocyte membrane zeta potential by Plasmodium falciparum infection. Experimental Parasitology 131, 245-251.
Northrup, M.A., Ching, M.T., White, R.M. and Watson, R.T. (1993) Proc. Transducers 93, 924.
Whitea, A.K., VanInsberghea, M., Petriva, O.I., Hamidia, M., Sikorskia, D., Marrae, M.A., Piretc, J., Aparicio, S. and Hansena, C.L. (2010) High-throughput microfluidic single-cell RT-qPCR. Proc Natl Acad Sci U S A.
Ahmad, F. and Hashsham, S.A. (2012) Miniaturized nucleic acid amplification systems for rapid and point-of-care diagnostics: A review. Analytica Chimica Acta 733(0), 1-15.
Agrawal, N., Hassan, Y.A. and Ugaz, V.M. (2007) Angew. Chem. Int. Ed. 46, 4316.
Pjescic, I., Tranter, C., Hindmarsh, P.L. and Crews, N.D. (2010) Biomed.Microdevices 12, 333.
Dimov, I.K., Garcia-Cordero, J.L., O'Grady, J., Poulsen, C.R., Viguier, C., Kent, L., Daly, P., Lincoln, B., Maher, M., O'Kennedy, R., Smith, T.J., Ricco, A.J. and Lee, L.P. (2008) Integrated microfluidic tmRNA purification and real-time NASBA device for molecular diagnostics. Lab on a Chip 8(12), 2071-2078.
Gulliksen, A., Solli, L., Karlsen, F., Rogne, H., Hovig, E., Nordstrom, T. and Sirevag, R. (2004) Real-time nucleic acid sequence-based amplification in nanoliter volumes. Analytical Chemistry 76(1), 9-14.
Gulliksen, A., Solli, L.A., Drese, K.S., Sorensen, O., Karlsen, F., Rogne, H., Hovig, E. and Sirevag, R. (2005) Parallel nanoliter detection of cancer markers using polymer microchips. Lab on a Chip 5(4), 416-420.
Zhang, Y. and Ozdemir, P. (2009) Microfluidic DNA amplification—A review. Analytical Chimica Acta 638, 115-125.
Li, Y., Zhang, C. and Xing, D. (2011) Integrated microfluidic reverse transcription-polymerase chain reaction for rapid detection of food- or waterborne pathogenic rotavirus. Analytical Biochemistry 415(2), 87-96.
Weigl, B.H., Gerdes, J., Tarr, P., Yager, P., Dillman, L., Peck, R., Ramachandran, S., Lemba, M., Kokoris, M., Nabavi, M., Battrell, F., Hoekstra, D., Klein, E.J. and Denno, D.M. (2006) Fully integrated multiplexed lab-on-a-card assay for enteric pathogens. Proc. SPIE 6112, 11.
Ramalingam, N., Rui, Z., Liu, H.-B., Dai, C.-C., Kaushik, R., Ratnaharika, B. and Gong, H.-Q. (2010) Real-time PCR-based microfluidic array chip for simultaneous detection of multiple waterborne pathogens. Sensors and Actuators B: Chemical 145(1), 543-552.

Nugen, S.R., Asiello, P.J., Connelly, J.T. and Baeumner, A.J. (2009) PMMA biosensor for nucleic acids with integrated mixer and electrochemical detection. Biosensors & Bioelectronics 24(8), 2428-2433.
Esch, M.B., Baeumner, A.J. and Durst, R.A. (2001) Detection of Cryptosporidium parvum using oligonucleotide-tagged liposomes in a competitive assay format. Analytical Chemistry 73(13), 3162-3167.
Rivet, C., Lee, H., Hirsch, A., Hamilton, S. and Lu, H. (2011) Microfluidics for medical diagnostics and biosensors. Chemical Engineering Science 66(7), 1490-1507.
Cleary, J., Slater, C., McGraw, C. and Diamond, D. (2008) An Autonomous Microfluidic Sensor for Phosphate: On-Site Analysis of Treated Wastewater. IEEE Sensors JOurnal 8(5), 508-515.
Quilliam, R.S., Prysor Williams, A., Avery, L.M., Malham, S.K. and Jones, D.L. (2011) Unearthing human pathogens at the agricultural–environment interface: A review of current methods for the detection of Escherichia coli O157 in freshwater ecosystems. Agriculture, Ecosystems and Environment 140, 354-360.

Figure Captions

Figure 1:

Schematic illustrating different methods of cellular separation in microfluidic systems. Reproduced with permission from Kersaudy-Kerhoas (2008).

Figure 2

Schematic view of the magnet array system. The microfluidic channel sits on top of the magnet array and as sample passes through the channel several trapping and releasing events occur before the sample is finally captured at the end of the channel. In contrast to the existing procedure, where the sample is pulled to the side of a tube with one magnet, the several steps involved here avoids the problem of aggregation and impurity capture. Figure 2 from Ramadan. Reproduced with permission.

Figure 3

Impedance measurements with a microfluidic device. Figure 5 from [38]. Real-time binding of bacteria to AMP sensors.
(A) Digital photograph of the microfluidic flow cell, indicating the small size of this system, the device set-up and operation.
(B) Optical micrograph of the microfluidic channel with an embedded interdigitated microelectrode array chip, showing the design of the electrodes.
(C) Optical image of the embedded microelectrode array after exposure to 10^7 cfu⁄mL bacterial cells for 30 min. The cells have been captured on the electrodes.
(D) Real-time monitoring of theinteraction of the AMP-functionalized sensor (and an unlabeled control chip) with various concentrations of *E. coli* cells, showing how this device can successfully discriminate between different concentrations of bacteria.

Figure 4

Schematic of stages in molecular detection and the appropriate microfluidic technology. Table 1 from Liu (2009)

Figure 5

Top view: schematic of the Rheonix system, which performs on-chip detection of Cryptosporidium from a 5mL starting sample. The sample firstly enters a large chamber where IMS takes places. Next the sample moves to a chamber for heat-shock to induce viable oocysts to activate the heat shock protein gene that is subsequently used for detection. From there the sample passes into a chamber where controlled interactions with the chemicals required for lysis and purification occurs before the RNA is isolated. Finally the sample flows along the channel at the bottom left of the chip before entering the lateral flow assay component where detection takes place. Bottom view: optical image of the microfluidic indicating the various stages and showing the size of the device relative to a US dollar coin.

Microorganism		Approach	Example	Volume Processed	Time Required	Water Sample	Concentration Achieved	Recovery Rate	Reference
Viruses	*Tobacco Mosaic Virus*	Electrical	iDEP	20 μL	2 min	DI water	Unreported	Unreported	Lapizco-Encinas (2005)
	Hepatitis A	Electrical	DEP	Unreported	9 mins	1:5 PBS/Water	10^7	Accumulated, Unrecovered	Grom (2006)
	MS-2	Electrical	DEP	2 mL	2 mL/hr	Potable bottled water	14.2 x * *maximum reported with salmonella	90-99%	Balasubramanian (2007)
	Echovirus 11	Electrical	DEP	2 mL	2 mL/hr	Potable bottled water	14.2 x * *maximum reported with salmonella	70%	Balasubramanian (2007)
Bacteria	*Lysteria*	Electrical	DEP	40 μL	2 μL /min	HLB,LB and DI Water	10^{4-5}	at least 10%	Gomez-Sjoberg (2005)
	E. coli	Electrical	iDEP	20 μL	2 min	DI water	3200x	Nearing 100%	Lapizco-Encinas (2005)
	E Coli	Filtration	Raindrop bypass microfilter	100 μL	10 μL/min	PBS	Unreported	Unreported	Lay (2008)
	E. coli	Electrical	iDEP	Unknown	100μL/min	PBS + Wash buffer	Unreported	66%	Cho (2009)
	E.coli	IMS	High aspect ratio curved channels coated in antibody	1 mL	5 μL/min	Pre-filtered lake water	10^2	71%	Dharmasiri (2010)
	E. coli	IMS	Antibody coated	1 μL	5 μL/min	PBS	Unreported	92-96%	Guan (2010)

			microbeads in channels						
	E. coli and *E. faecalis*	Electrical	DEP	60 µL	1µL/min	Nanopure water	Unreported	>90%	Chow (2011)
	E.Coli and *Salmonella Typhimurium*	IMS	3D circular system with immunomagnetic nanoparticles	20 µL	10 µL /min	PBS	10^4	Not reported (for sample processing stage)	Agrawal (2012)
Protozoa	*Cryptosporidium*	Electrical	DEP/travelling wave	300 µL	Mins	PBS	Unreported	90%	Goater (1997)
	Cryptosporidium and *Giardia*	Filtration	Weir filters	1-5 µL	20 µL/min	PBS	Unreported	Up to 100%, depending on weir size	Zhu (2004)
	Cryptosporidium	IMS	Antibody capture in microfabricated wells	10 mL	1hr	PBS	Unreported	Unreported	Taguchi (2005)
	Cryptosporidium	Filtration	SUS micromesh	500 µL	60 mins	Tap Water	Unreported	93%	Taguchi (2007)
	Cryptosporidium and *Giardia*	Filtration	Raindrop bypass microfilter	Unreported	Unreported	PBS	Unreported	Unreported	Lay (2008)
	Cryptosporidium; Giardia	IMS	Use of an in-channel micromesh for oocyst capture	5 mL	350 µL/min 5mL in 15mins	Tap, effluent and DI water	5x (extrapolated)	93%	Ramandan (2010)
	Cryptosporidium	Electrical	iDEP	1-10mL	Unreported	Water	To 25 µL	Unreported	Simmons (2010)

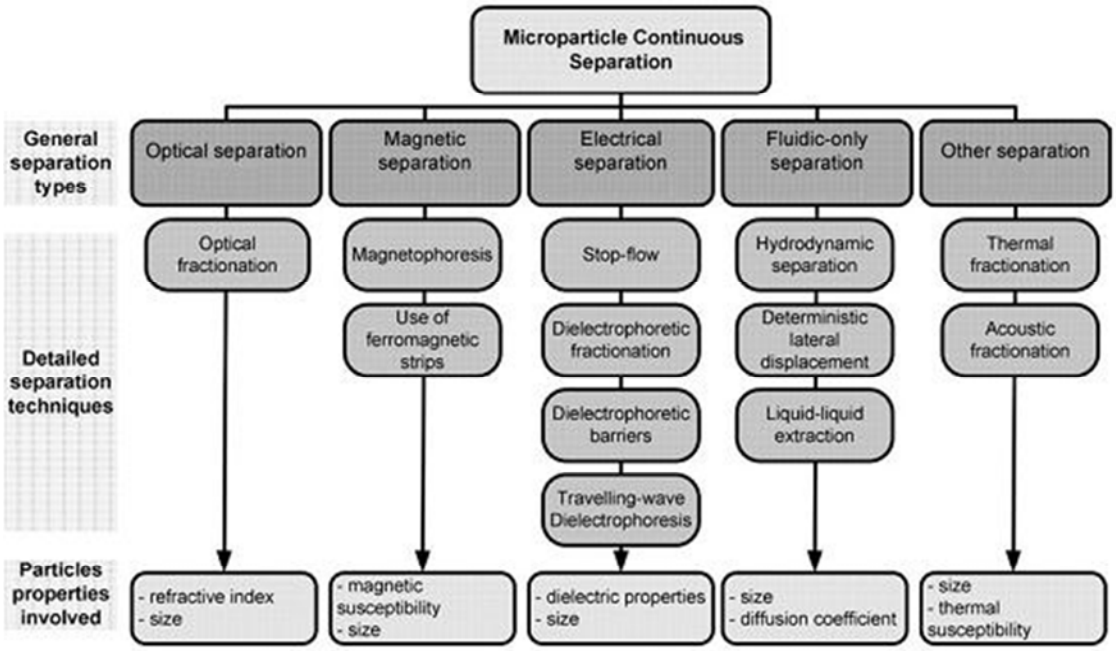
Table 1: Microfluidics Applications in Sample Processing for Waterborne Pathogens

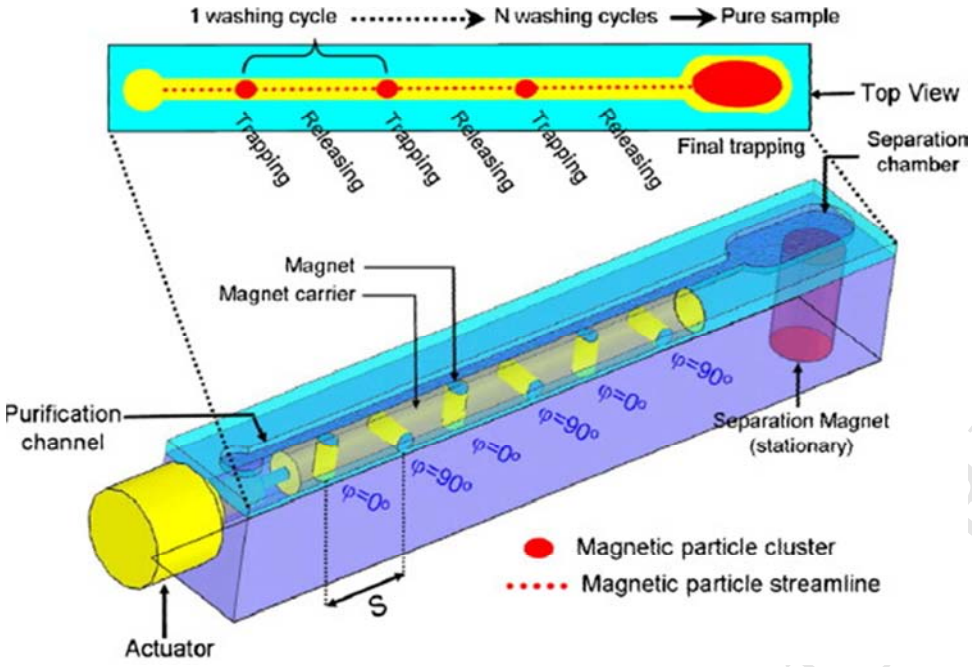
	Microorganism	Approach	Example	Volume Processed	Time Required	Water Sample	Limit of detection	Reference
Viruses	*Herpes Simplex Virus- Type 1*	Optical	Youngs Interferometer	~ µL	2.5 hrs	PBS	700 pfu	Ymeti (2006)
	Rotavirus	Molecular	RT-PCR (fluorescence detection)		1 hr			Li (2011)
	Feline Calicivirus	Optical or electrical detection	With liposome amplification	60 µL	2.5 hrs	PBS	$1x10^5$ pfu/mL	Connelly (2012)
	M13 Phage	Electrical	Impedance	~ nL	6 hrs	PBS	550 pfu	Mandal (2012)
Bacteria	*E coli*	Optical	Sandwich immunoassay	0.4 mL	1 hr	PBS +/- milk +/- BSA	20 cfu	Stokes (2001)
	E coli	Optical	Sandwich immunoassay	1 mL	2 hrs	PBS	10-100 cfu/mL	Li (2005)
	E coli	Optical	Flow cytometry	60 µL	30 mins	River water	Comparable to fluorescence microscopy ~1	Sakamoto (2005)
	E coli	Optical	UV-vis	1 mL	2hrs	PBS	10-100 cfu/mL	Li (2006)
	E coli O157:H7	Optical	Chemiluminescence	100 µL	90 mins	Food sample	34 cells	Varshney (2006)
	E.coli	Optical	Fluorescence	100 µL	20 mins	PBS	$1x10^5$ cfu/mL	Lay (2008)
	E coli, Salmonella and Legionella	Optical	Chemiluminescence	1.2 mL	13 mins	PBS	$1x10^5$, $3x10^6$, $3x10^3$ cells/mL	Wolter (2008)

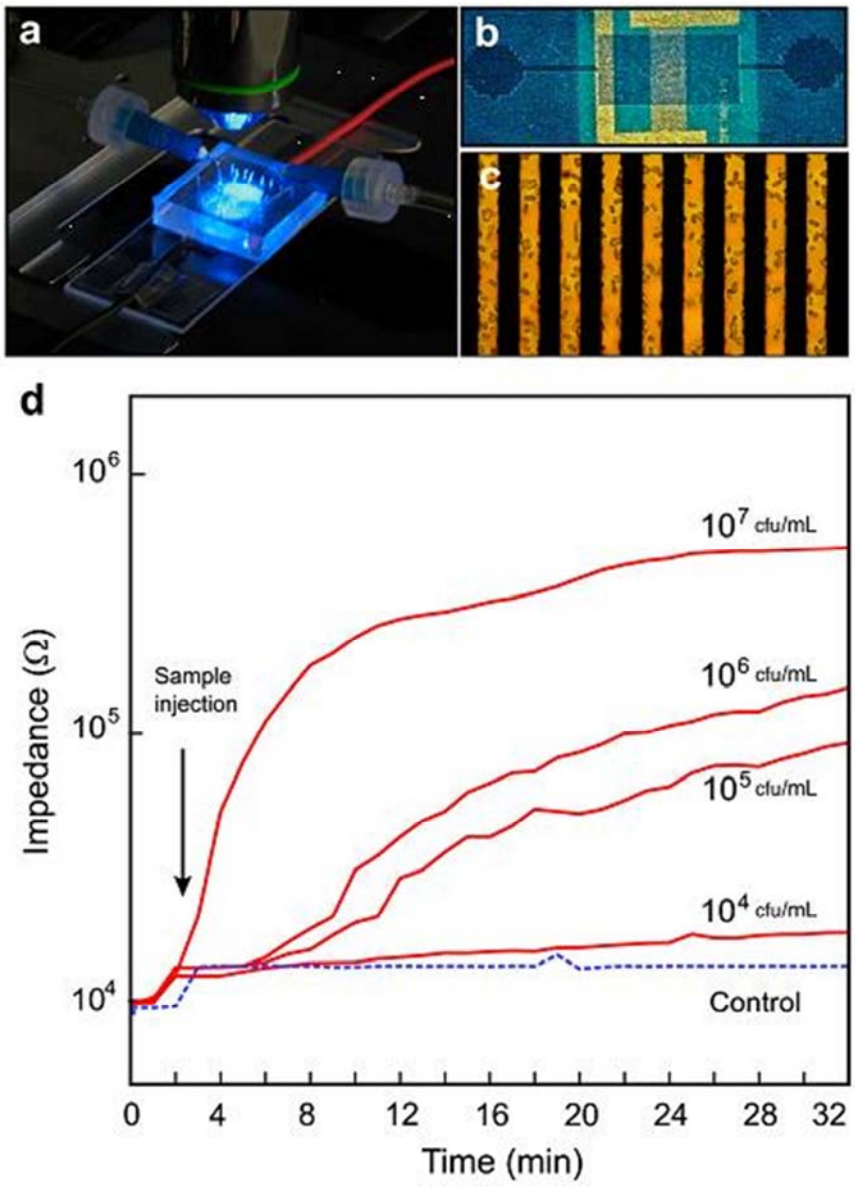
							respectively	
	E coli	Optical	Immunoaggulutination, light scattering	100 µL	5 mins	PBS	Single cells or 4 cfu/mL (if viable only)	Han (2008)
	E coli, Salmonella and Legionella	Optical	Chemiluminescence	1.2 mL	13 mins	PBS	$1x10^5$, $3x10^6$, $3x10^3$ cells/mL respectively	Wolter (2008)
	E coli, Salmonella and Legionella	Optical	Chemiluminescence	0.64 mL	18 mins	Millipore water	$1.8x10^4$, $7.9x10^4$ and $2.0x10^7$ respectively	Karsunke (2009)
	E coli	Optical	Immunoaggulutination, light scattering	100 µL	5 mins	Sampling piped network	10 cfu/mL connected to sample pipe	Kwon (2010)
	E coli	Optical	Flow cytometry	35 µL	~45mins	Pond water	10^4 cells/mL	Yamaguchi (2011)
	E coli	Optical	Particle immunoaggultination	60 µL	6 mins	Lettuce wash	10 cfu/mL	You (2011)
	E coli	Optical	Droplet counting, metabolism	<1 µL	2 hrs	Water (pL droplets in oil)	Single cells	Marcoux (2011)
	E coli O157:H7	Optical	Bioluminescence	1 µL	20 mins	PBS	3.2 cfu/µL	Guan (2012)
	E coli and S typhimurium	Optical	Fluorescence	50 µL	Not reported	PBS	8.8 and 9.3 10^5 cfu/mL, respectively, simultaneous detection	Agrawal (2012)

	L. innocua	Electrical	Impedance	30 nL	15 mins	Tris-Gly Buffer	10 cells	Gomez (2001)
	E.coli	Electrical	Impedance	100 µL	>1hr	Modified PBS	$6x10^3$ cfu/mL	Ruan (2002)
	Salmonella	Electrical	Impedance	20 µL	25 mins	DI Water	$3.45x10^6$ cfu/mL	Yang (2007)
	E coli	Electrical	Impedance	5 µL/min	32 mins	PBS	10^4 cells/mL	Manoor (2010)
	Shigella dysenteriae serotype 1, Shigella toxin-producing Escherichia coli, E. coli 0157, *Campylobacter jejuni,* and *Salmonella* and *Shigella* species	Molecular	Immunocapture of pathogens plus on-chip nucleic extraction and detection	Unknown	Unknown	Unknown	Unknown	Weigl (2006)
	A. hydrophilia, K. pneumoniae, S. aureus and P. aeruginosa and E. coli	Molecular	On-chip PCR	Not reported	Not reported	Not reported	51 cfu/mL	Ramalingam (2010)
Protozoa	*Cryptosporidium* and *Giardia*	Optical	Fluorescence	5 µL	Max 12 mins	PBS	Not reported; images show single (oo)cyst	Zhu (2004)
	Cryptosporidium	Optical	Fluorescence	10 µL	Not reported	PBS	Not reported, images show	Taguchi (2005)

							single (oo)cysts	
	Cryptosporidium	Optical	Fluorescence	100-500 µL	1 hr	Tap water and Milli-Q water	36 oocysts/mL	Taguchi (2007)
	Cryptosporidium	Optical	Immunoagglutination	15 µL of 1000L concentrate	10 mins (not including concentration)	Tap water	Single oocyst with pre-concentration; 1-10oocysts/mL without	Angus (2012)
	Cryptosporidium	Electrical	Impedance	110 µL	~22 mins	WFI	$<1x10^4$ oocysts/mL	Houssin (2010)
	Cryptosporidium	Molecular	NASBA (off-chip) and on-chip fluorescence detection	12.5 µL of NASBA amplicon	1-2 hrs	Not reported	5 fmol of amplicon/ 0.4 fmol/µL	Esch (2001)
	Cryptosporidium	Molecular	NASBA (off-chip) and on-chip electrochemical detection	1 µL of NASBA amplicon	~30 mins (not including lysis time etc)	Nuclease free water	1 oocyst	Nugen (2009)






a
b
c
d
Impedance (Ω)
10^6
10^5
10^4
Sample injection
10^7 cfu/mL
10^6 cfu/mL
10^5 cfu/mL
10^4 cfu/mL
Control
0
4
8
12
16
20
24
28
32
Time (min)